Understanding of Burning Mouth Syndrome Based on Psychological Aspects

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Burning mouth syndrome (BMS) is a chronic pain condition characterised by a persistent burning sensation in clinically normal oral mucosa. BMS most commonly occurs in middle-aged and elderly women. Various local and systemic factors can cause oral burning symptoms. When all possible local and systemic factors are excluded, burning mouth symptoms can be diagnosed as BMS. Psychophysical tests and histopathological data suggest the involvement of peripheral and central neuropathic mechanisms in BMS etiopathogenesis. Psychological problems are frequently observed in BMS patients. Several mechanisms, including increased parafunctional habits, steroid dysregulation, central disinhibition due to taste dysfunction, and low dopamine levels in the brain, have been proposed as an explanation for the role of psychological factors in BMS pathophysiology. However, the causal relationship between BMS and psychological problems remains controversial. Given the neuropathic nature of BMS, treatment for it is similar to other neuropathic pain conditions. Although various treatment modalities, including pharmacological intervention, behavioural therapy and psychotherapy, have been proposed, there is no definitive treatment always effective for the majority of BMS patients. In conclusion, for better understanding of the relationship between BMS and psychological factors, well-designed prospective studies are needed. In addition, the evaluation and treatment of psychological problems are essential for successful management of BMS patients.

Key words: burning mouth syndrome, pathophysiology, psychological factors

be between 0.7% and 4.6% of the general population\textsuperscript{2,3}. These epidemiological data may include patients with “burning mouth symptoms.” In a recent population-based study using stringent BMS criteria, BMS prevalence was estimated to be 0.1%\textsuperscript{6}, which is much lower than results of previous studies. In general, BMS is more common in women than in men, and it appears to be most prevalent in peri- or post-menopausal women\textsuperscript{7}. After the age of 50, BMS incidence drastically increases (under 50 years: 3.3 per 100,000 person-years vs 50 to 59 years: 22.8 per 100,000 person-years), and the maximal incidence rate was found among people aged 70 to 79 (46.9 per 100,000 person-years)\textsuperscript{8}.

The main symptom of BMS is oral burning pain. Patients often describe pain as “pricking”, “tingling”, “numbness”, and “itching” instead of “burning”\textsuperscript{9}. Burning pain usually occurs spontaneously without any causative factors, and persists for several months to years. Pain ranges from moderate to severe, similar to toothache\textsuperscript{10,11}, and its intensity is usually lowest in the morning and gradually increases during the day\textsuperscript{9,12}. Spontaneous remission of the burning pain is rare\textsuperscript{13}. The burning pain usually occurs bilaterally, and the most frequently affected area in the oral cavity is the tongue, especially the tip and the anterior two-thirds\textsuperscript{14-16}. Other areas, such as the lower lip mucosa and the anterior hard palate, are also frequently affected. In addition, BMS patients often have dry mouth and dysgeusia\textsuperscript{2}. Therefore, these three symptoms are referred to as the BMS symptom triad.

Although several articles have been published on various aspects of BMS, there is a lack of consensus regarding the contributing factors related to BMS pathophysiology. In addition, BMS patients often have psychological problems\textsuperscript{17-20}, which may be closely associated with poor prognosis\textsuperscript{2,21-23}. However, the relationship between BMS and psychological problems remains unclear. The aim of this review is to summarise current knowledge on the pathophysiology and management of this syndrome, focusing especially on the complex interactions between psychological problems and BMS.

Etiopathogenesis of BMS

Knowledge of BMS etiopathogenesis has increased considerably in recent years. At present, complex interactions between various etiological factors appear to be associated with the development of oral burning sensations (burning mouth symptoms, not “true” BMS). However, in many cases of burning mouth symptoms, no particular causative factors can be identified, and peripheral and/or central neuropathic changes have been suggested\textsuperscript{5,24,25}.

Local and systemic factors related to burning mouth symptoms

Previous studies have uncovered various local factors associated with burning mouth symptoms. Local factors, including biological and mechanical factors, can directly irritate the oral mucosal tissues and produce oral burning sensations. In these cases, treating the factors can alleviate oral burning mouth symptoms.

Even after identifying a clinically normal oral mucosa through a comprehensive oral examination, fungal infections and allergic reactions should be considered as important causes of oral burning sensations. It has been reported that fungal infection can be found in patients diagnosed with BMS\textsuperscript{2}, and some patients with burning pain in normal oral mucosa experience a reduction of symptoms after antifungal therapy. One study categorised the participants according to pain intensity during meals and at rest, and compared the clinical features and response to antifungal agents\textsuperscript{26}. The study concluded that although the clinical features were similar for all participants, only the group of patients who complained of greater pain while eating showed a good response to antifungal therapy. BMS and atrophic candidiasis frequently exist as comorbidities. Thus, the possibility of fungal infection should be carefully examined.

In the case of allergic reactions, although no significant association has been identified between BMS and a positive patch test reaction\textsuperscript{27,28}, earlier studies found that some patients with burning mouth symptoms had local hypersensitivity reactions to dentures and dental filling materials\textsuperscript{29,30}, and in some cases\textsuperscript{31,32}, remission of the burning sensation after removal of the allergen was observed. Therefore, it is necessary to consider the possibility of allergic reaction as the cause in those patients complaining of oral burning pain after dental treatment.

Microtrauma resulting from mechanical irritation is regarded as a causative factor for burning mouth symptoms. Causes of mechanical irritation include ill-fitting prostheses and oral parafunctional habits. In particular, there is strong evidence supporting oral parafunctional habits as a cause of oral burning pain\textsuperscript{2,33}. Oral parafunctional habits, including clenching, bruxing, and tongue thrusting, have been frequently reported in BMS patients\textsuperscript{34,35}. These habits might lead to traumatic inflammation, which can result in a burning sensation\textsuperscript{36}. It has also been reported that habit control and the use of oral lubricants can decrease the burning sensations\textsuperscript{37}. 

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Previous studies have suggested that BMS may be caused by decreased salivary output\textsuperscript{14,34,38}. Saliva plays an important role in the protection and lubrication of the oral mucosa\textsuperscript{39}. Thus, reduction of salivary output can result in decreased oral lubrication and increased friction between oral mucosal tissues, which can ultimately lead to microtrauma. In fact, BMS patients often have salivary gland dysfunction\textsuperscript{34,40}. In addition, medical interventions and systemic diseases, which are known to induce the reduction of salivary flow rate, are associated with increased incidence of BMS\textsuperscript{2}. However, salivary flow rates for BMS patients were not consistently different from those of control subjects, and salivary gland stimulation with a sialogogue was not effective for BMS patients\textsuperscript{38}. Recent evidence seems to indicate that both the quantity (objective reduction of salivary output) and quality of saliva (change in salivary components related to protection and lubrication) are important factors that influence BMS incidence.

Systemic factors have also been described as possible causes of burning mouth symptoms. These include nutritional deficiencies (low serum levels of vitamin B12, folic acid, ferritin, zinc, and magnesium), systemic diseases (anaemia, diabetes, thyroid diseases, and immunological diseases), and medications (antihistamines, neuroleptics, antihypertensives, and benzodiazepines)\textsuperscript{2,41-43}. Many peri- and post-menopausal women complain of burning mouth symptoms\textsuperscript{44,45}, and the majority of BMS patients are peri- or post-menopausal women\textsuperscript{7}. Thus, hormonal changes in peri- or post-menopausal stages are considered to be a key factor in the development and progression of BMS. However, hormone replacement therapy was not effective in relieving pain in many BMS patients\textsuperscript{44,46}, and a definite relationship between BMS and hormonal changes has not yet been established.

**Neuropathic alterations in BMS pathophysiology**

BMS can be classified as either true or primary when all possible local and systemic factors are excluded. Several features of primary BMS suggest the involvement of neuropathic pain mechanisms in the pathophysiology of this condition. Firstly, a burning sensation, the main symptom of BMS, is a typical characteristic of many chronic neuropathic pain syndromes\textsuperscript{2,9}. Secondly, previous clinical and animal studies regarding neuropathic pain reported that the pain worsens over the course of the day\textsuperscript{47-49} for the vast majority of BMS patients\textsuperscript{9,12}. Finally, taste and sensory dysfunction\textsuperscript{50}, commonly observed in BMS patients, imply the possibility of alterations in the peripheral and central nervous systems.

Grushka et al\textsuperscript{50} first applied a psychophysical test to investigate the tactile and sensory functions of the oral mucosa in BMS patients. In this study, pain tolerance for the BMS subjects was found to be significantly lower at the tip of the tongue. Other earlier studies also reported similar results\textsuperscript{51,52}. In studies using objective electrophysiological tests\textsuperscript{5,53}, higher stimulus intensities were needed to evoke the R1 component of the blink reflex in BMS patients than in the controls\textsuperscript{5,53}, and the majority of BMS patients showed signs of hypoesthesia in quantitative sensory tests (QST)\textsuperscript{5}.

A more recent study that applied strict diagnostic criteria for primary BMS and meticulous clinical and neurophysiological examinations to exclude patients with subclinical trigeminal neuropathy reaffirmed that BMS patients show negative sensory signs (hypoesthesia and hypoalgesia) in QST\textsuperscript{54}. Furthermore, Grémeau-Richard et al\textsuperscript{55} reported that some BMS patients experience a reduction of the burning pain following a lingual nerve block. Taken together, the above data suggest that peripheral neuropathy in the oral mucosal areas may be the underlying cause of the burning sensation, and several previous studies reporting a significant reduction of intraepithelial small diameter nerve fibre density lend support to this possibility\textsuperscript{54,56,57}.

Neuropathic alterations associated with BMS are not always confined to the peripheral nervous system. The electrophysiological findings on BMS patient groups show considerable heterogeneity\textsuperscript{5,53}. Some BMS patients showed negative sensory signs, whereas others showed positive sensory signs (warm allodynia, decreased heat pain tolerance). Furthermore, BMS patients showed generalised sensory abnormalities that were not restricted to the intraoral mucosa\textsuperscript{5,53}. Although lingual nerve block was shown to decrease burning pain in some BMS patients, on others it had no effect\textsuperscript{55}. These findings indicate that in BMS, neuropathic alterations can occur in the multilevel of neuraxis.

Some BMS patients have been reported to show sensory signs in the form of deficient habituation of R2 components and low thresholds for the R3 components of the blink reflex test\textsuperscript{5,53,55,58}. These facts imply that the dysfunction is located higher within the nervous system\textsuperscript{5}. Deficient habituation of the blink reflex is also a common finding in Parkinson’s disease\textsuperscript{59}, and appears to be due to a deficient dopaminergic striatal influence on the brainstem nuclei\textsuperscript{60}. Likewise, several studies using fluorodopa-positron emission tomography on BMS patients demonstrated a decreased level of endogenous dopamine in the putamen\textsuperscript{61,62}. In one MRI study\textsuperscript{63}, BMS patients showed less volumetric activation to painful stimuli in the brain than control subjects,
and the brain activity patterns in BMS patients were similar to those in other types of neuropathic patients.

On the basis of existing evidence, BMS is thought to occur through neuropathic mechanisms at the peripheral and the central nervous system levels, or both. However, the cause of neuropathic changes is still unknown, although hypotheses based on repeated epithelial nerve fibre trauma\textsuperscript{2,64} or neuroactive steroid depletion\textsuperscript{65} have been proposed.

**Psychological features of BMS patients**

Over the past decades, psychological problems have been commonly reported in BMS patients, and many studies have been conducted to clarify the relationship between BMS and psychological problems. In 1987, an objective and standardised personality test, the Minnesota Multiphasic Personality Inventory, was first employed to investigate the psychological characteristics of BMS patients\textsuperscript{66}. Since then, several studies using various psychometric tests (Table 1) have been conducted, and much evidence for psychiatric comorbidities in BMS has been accumulated\textsuperscript{15,17-20,67,68}. On the basis of the results of previous studies, patients with BMS, similar to other patients with chronic pain, have a higher risk of psychological distress than healthy individuals—the most common psychological problems in BMS patients being anxiety, depression, cancer phobia, and hypochondriasis. A recent systematic review of psychiatric aspects of BMS reaffirmed the high prevalence of anxiety and depression in BMS patients\textsuperscript{69}. One study reported that a large proportion of BMS patients had a history of hospitalisation with previous psychiatric illness, or were currently receiving psychiatric treatments\textsuperscript{15,70}. Interestingly, some authors found that BMS patients were more likely to have experienced stressful life events recently or early in life than normal controls\textsuperscript{17,18,70}. This fact may explain some of the relationships between BMS and psychological problems.

Many studies have examined the psychological problems in BMS patients in terms of personality using various diagnostic tools (Table 1). Regarding personality characteristics, BMS patients were found to be significantly different from control subjects\textsuperscript{71-73}, and showed higher levels of neuroticism\textsuperscript{72-75} and lower levels of novelty seeking\textsuperscript{76}. Pain catastrophizing defined as an exaggerated negative orientation towards actual or anticipated pain experiences\textsuperscript{77}, was also found to be significantly higher in BMS patients than in normal controls\textsuperscript{68}. These aberrant personality traits in BMS patients have been observed in other chronic pain patients\textsuperscript{78}.

**Pathophysiology of BMS based on psychological factors**

The high comorbidity rates of psychiatric conditions in BMS suggest that psychological problems are important in its pathophysiology, and several hypotheses

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<th>Table 1</th>
<th>Diagnostic tools used to examine psychological problems in BMS patients.</th>
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<td>Mini International Neuropsychiatric Interview-PLUS (MINI-PLUS)</td>
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<td>Personality traits</td>
<td>Diagnostic and Statistical Manual of Mental Disorder, 4th edition (DSM-IV)</td>
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<td>NEO Personality Inventory (NEO PI-R)</td>
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have been proposed to explain the role of psychological problems in the occurrence and development of BMS (Fig 1).

The relationship between BMS and psychological factors may be related to oral parafunctional habits. The etiology of oral parafunctional habits, such as bruxism, is probably multifactorial, and psychological problems are also considered to be among causative factors. In BMS patients, oral parafunctional habits and anxiety are significantly related. Thus, psychological problems can cause oral parafunctional habits, which can ultimately lead to neuropathic changes in the oral mucosa through small nerve fiber damage. Psychological problems and related medications are causative factors of dry mouth, which can further aggravate the occurrence of microtrauma in the oral mucosa caused by oral parafunctional habits.

An interesting hypothesis has been published regarding the link between psychological distress and hormonal changes during the menopause, which are common features of BMS. It is well known that psychological distress can induce hypothalamic-pituitary-adrenal axis dysfunction and steroid dysregulation. Previous studies have reported elevated cortisol levels in patients with anxiety, major depression and chronic stress.

In a comparative study of BMS patients and normal controls, BMS patients showed higher anxiety scores and cortisol levels. Steroid dysregulation induced by psychological distress could result in neurodegenerative changes in the oral mucosal tissues, and the depletion of neuroactive steroids because of menopausal hormonal changes could further promote these changes. This phenomenon could lead to oral burning pain and other symptoms of BMS.

Psychological problems can also cause oral burning sensations as a result of taste disturbances, which are one of the symptom triads of BMS and a common clinical characteristic in BMS patients. Previous studies have reported an association between taste and pain perception. Applying sucrose to the tongue decreased the burning sensation induced by capsaicin, and conversely, interruption of taste signal transmission (chorda tympani nerve block or topical anaesthesia of the mouth) intensified the oral burning sensation.

These findings suggested that taste stimulation may result in centrally mediated inhibition of the trigeminal nociceptive pathway, and central disinhibition due to taste dysfunction may increase oral pain perception. In addition, taste disturbance was strongly associated with psychological distress, and cortisol levels may affect taste perception. Therefore, psychological distress could alter taste perception and consequently exacerbate oral burning sensations, as well as other oral symptoms of BMS.

Changes in the central nervous system also suggest a connection between psychological problems and BMS. Low dopamine levels in the brain are frequently found in patients with depressive illness, and they are also associated with BMS. Low dopamine levels in the brain may increase the vulnerability of some individuals to BMS incidence. This possibility is supported by the fact that BMS patients who have burning pain related to central nervous system etiologies frequently have anxiety and depression.

Relationships between psychological factors and BMS

Psychological factors and BMS, the chicken or egg problem

Because psychological problems are common in BMS patients, and are considered to play an important role in its pathophysiology, many authors have suggested that BMS may be a somatoform disorder or a psychogenic problem. Previous studies demonstrated that stressful life events precede BMS incidence, and the majority of BMS patients have psychiatric disorders or a history of psychiatric treatment before BMS onset. Anxiety and depression have been identified as major risk factors in previous studies based on
regression analysis\textsuperscript{18,19}. The fact that the majority of BMS patients have unexplained extraoral comorbidities also suggests that BMS may be a somatoform disorder\textsuperscript{95}.

However, there are studies that refute the suggestion that psychological factors cause BMS. Contradictory results exist on the prevalence of psychological problems among BMS patients. One study reported that only 21\% BMS patients have severe psychological distress\textsuperscript{97}, and another reported that only one-third of patients have an underlying psychiatric diagnosis\textsuperscript{98}. It has also been reported that personality profiles do not differ significantly between BMS patients and controls\textsuperscript{99}. These findings suggest that the presence of psychological problems may not be a common feature in BMS patients, and that BMS can occur in the absence of psychological problems\textsuperscript{100}. In a study examining 69 variables as potential risk factors for BMS, only three neurological variables were significant in BMS cases, and no psychological variables were relevant\textsuperscript{101}. Because psychologically distressed patients tend to seek treatment, and many studies usually involve patients seeking treatment, the prevalence of psychological problems in BMS patients may be overestimated\textsuperscript{102}.

Psychological dysfunctions are also common in other chronic pain disorders, such as atypical facial pain and temporomandibular disorders\textsuperscript{2,103}, and the psychological characteristics of BMS patients are similar to those found in other chronic pain patients\textsuperscript{66}.

Therefore, some researchers claim that psychological problems in BMS patients are a secondary to pain\textsuperscript{51,66}. Similar to the other chronic pain disorders, a prolonged period of pain and a long history of repetitive unsuccessful treatments may relate to the onset of psychological problems\textsuperscript{71}. However, some previous studies found that symptom severity and duration were not associated with psychological problems\textsuperscript{2,15,104}. Therefore, it appears that the relationship between BMS and psychological problems is more complicated than a simple causal relationship.

\textbf{Psychological problems as an aggravating factor}

Based on current knowledge, it is difficult to determine whether psychological problems are the main cause or they are just a secondary effect. They do, however, appear to be aggravating factors in BMS symptoms, and BMS patients with psychological problems tend to suffer from greater levels of pain\textsuperscript{23}. In a study investigating the relationship between the catastrophizing trait and BMS symptoms, the catastrophizing score was significantly correlated with symptom intensity\textsuperscript{104}. Conversely, there are other studies showing that pain intensity and psychological problems are not associated\textsuperscript{7,15}. These contradictory results can be explained by the fact that BMS is a multifactorial disorder; hence, various factors – including psychological factors – can affect BMS symptoms.

BMS patients with psychological problems do not respond well to treatment\textsuperscript{22,23}, and a bad prognostic index may be associated with hypochondria and other phobias in BMS patients\textsuperscript{2}. Thus, it is important to evaluate and manage psychological problems in BMS patients, especially in those with a poor prognosis.

\textbf{Management of BMS}

Given the chronic nature of BMS and the decreased quality of life seen in BMS patients, it is necessary to identify effective treatment modalities. Nevertheless, there is no definitive clinical guideline for BMS management because of the complexity of the pathophysiology. Although a variety of medications, behavioural approaches, psychotherapy and many other modalities have been proposed for BMS (Table 2), its treatment remains unsatisfactory.

In retrospective studies, symptomatic improvement was observed in less than half of the patients during long-term follow-up.

Complete remission is rare, and has been reported to be only 3\%\textsuperscript{13,105}. Thus, BMS management is clinically challenging. BMS treatment is supportive in nature, and is aimed at alleviating symptoms and improving the quality of life. For effective management, a multidisciplinary approach combining careful assessment of a patient’s condition and various treatment modalities is needed.

\textit{Initial treatment}

Before treatment begins it is important to communicate information regarding the nature of BMS and to reassure the patient. Explanation should be provided that BMS is a chronic pain disorder unrelated to intraoral problems (oral mucosal lesions, prosthesis, etc), that BMS etiology is not still fully understood, there is no definite treatment, and it is necessary to relieve symptoms through possible treatments.

Regarding the effects of cancer phobia on BMS symptoms, it should be emphasised that BMS is not a life-threatening disease and is not associated with malignant conditions. Lack of objective information regarding the disease is a major cause of concern in chronic pain patients\textsuperscript{106} and providing them with the right information can eliminate the negative thinking and behavioural patterns associated with the symptoms\textsuperscript{107}. 

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Reassurance can help control psychological problems, including anxiety due to chronic pain, as well as contribute to symptom reduction. Reassurance alone has been proven to significantly decrease pain intensity and increase the quality of life for patients. This method is particularly relevant in BMS treatment, for which there are no definitive treatments.

Control of parafunctional habits and use of topical lubricants may also be considered as an initial treatment procedure. Parafunctional habits and repeated oral mucosal microtrauma can cause neuropathic changes through the damage of peripheral small nerve endings. It has been shown that the burning sensation and other symptoms can be significantly decreased with these simple initial treatments. Topical lubricant application with oral habit control is considered an effective initial treatment strategy because it is easy to administer and has no side effects. Pharmacological treatments can be reserved for BMS patients who do not respond to this simple treatment protocol.

Medications

As BMS is considered to have a neuropathic origin, the medications used to treat BMS are similar to those used in other neuropathic pain conditions. Although several medications have been employed for managing BMS (Table 2), most of them are unsupported by controlled studies, and for the majority of patients there is no consistently effective medication. A recently published systematic review on BMS therapy reported the following medications to be effective: clonazepam, alpha-lipoic acid, gabapentin, and capsaicin.

Clonazepam, a benzodiazepine, is an anticonvulsant and is thought to contribute to the reduction of BMS symptoms because of its role as a GABA receptor agonist and to contribute to decreased anxiety levels. It is usually administered orally, although recent studies have focused on topical administration to achieve immediate pharmacological effects and fewer adverse events. Various previous studies have consistently reported the beneficial effects of clonazepam. According to a recent meta-analysis, clonazepam therapy is effective for BMS management, irrespective of treatment duration, administration mode, or dosage.

Alpha-lipoic acid, which is an antioxidant and potent nerve regeneration agent, has been used in the treatment of diabetic neuropathy. Although many authors have conducted comparative studies investigating the effects of alpha-lipoic acid and placebo on pain reduction, the results have been inconsistent, and evidence for significant improvement is lacking. Nonetheless, because no significant side effects have been reported, alpha-lipoic acid is at least useful as a first line treatment for BMS management. One randomised controlled trial demonstrated that alpha-lipoic acid, gabapentin, and a combination of both medications are more effective in decreasing pain than placebo controls, with combination therapy showing the best results. However, most studies on the efficacy of gabapentin were poorly designed. Well-designed gabapentin trials for BMS management are needed when considering the role of gabapentin, which has been widely used in the treatment of general neuropathic diseases. Capsaicin regulates oral symptoms by inducing desensitisation of nociceptors. It often exhibits side effects such as temporary pain exacerbation and gastrointestinal disturbance, and therefore proper administration methods should be established.

Cognitive psychotherapy

Cognitive psychotherapy, which is based on the concept that the way we think about things (cognitive structure) affects how we feel emotionally, is focused on replacing dysfunctional cognitive structures. Cognitive psychotherapy is used in the treatment of various psychological disorders, including depression, anxiety, phobias and chronic pain disorders. Considering the relationship...
between BMS and psychological factors, cognitive psychotherapy has been proposed as a treatment for BMS. Two studies reported a significant improvement in BMS symptoms following cognitive psychotherapy \(^{71,119}\), suggesting that BMS has a psychological origin.

Several authors have attempted to confirm the efficacy of a combination of psychotherapy and pharmacological interventions. A case report on BMS patients successfully treated using a combination of psychotherapy and the antidepressant sertraline has been published\(^ {120}\). Another study compared the effects of psychotherapy alone, alpha-lipoic acid alone, and combination therapy (psychotherapy plus alpha-lipoic acid). The results showed that the combination therapy was more effective than the individual treatments\(^ {121}\). Considering the excellent results of combination therapies, further studies on combination therapies are needed.

Conclusion

To date, accumulated research results indicate that psychological problems are frequently observed in BMS patients and play an important role in symptom development and aggravation.

Nevertheless, the detailed mechanisms underlying the relationship between psychological problems and BMS are difficult to establish, and well-designed prospective studies are needed to understand this relationship. For successful management of BMS symptoms in patients, their psychological status should be evaluated, and if psychological factors are found, they should be appropriately managed.

Conflicts of interest

The authors reported no conflicts of interest related to this study.

Author contribution

Dr Moon-Jong KIM collected the literature and prepared the manuscript; Dr Hong-Seop KHO supervised the procedures, revised and approved the manuscript.

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