Corneal Confocal Microscopy Detects Small-Fiber Neuropathy in Burning Mouth Syndrome: A Cross-Sectional Study

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Aims: To assess the utility of corneal confocal microscopy in identifying small fiber damage in patients with burning mouth syndrome (BMS). **Methods:** A prospective cross-sectional cohort study was conducted at two United Kingdom dental hospitals between 2014 and 2017. A total of 17 consecutive patients with idiopathic BMS aged between 18 and 85 years and 14 healthy age-matched control subjects were enrolled in this study. Corneal subbasal nerve plexus measures were quantified in images acquired using a laser-scanning in vivo corneal confocal microscope. The main outcome measures were corneal nerve fiber density, nerve branch density, nerve fiber length, and Langerhans cell density. **Results:** Of the 17 patients with BMS, 15 (88%) were women, and the mean (standard deviation) age of the sample was 61.7 (6.5) years. Of the healthy controls, 7 (50%) were women, and the mean (standard deviation) age was 59.3 (8.68) years. Corneal nerve fiber density (no./mm^2) (BMS: 29.27 ± 6.22 vs controls: 36.19 ± 5.9; median difference = 6.71; 95% CI: 1.56 to 11.56; P = .007) and corneal nerve fiber length (mm/mm^2) (BMS: 21.06 ± 4.77 vs controls: 25.39 ± 3.91; median difference = 4.5; 95% CI: 1.22 to 6.81; P = .007) were significantly lower in BMS patients compared to controls, and Langerhans cell density (no./mm^2) (BMS: 74.04 ± 83.37 vs controls: 29.17 ± 45.14; median difference = –21.27; 95% CI: –65.35 to –2.91; P = .02) was significantly higher. **Conclusion:** Using a rapid noninvasive ophthalmic imaging technique, this study provides further evidence for small fiber damage in BMS and has potential utility for monitoring disease progression and/or response. Furthermore, this technique shows a hitherto undocumented increased density of immune cells in this group of patients. *J Oral Facial Pain Headache 2019;33:337–341. doi: 10.11607/ofph.2338*

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Idiopathic burning mouth syndrome (BMS) is a debilitating painful condition of the oral cavity characterized by a burning sensation of the tongue, palate, or buccal mucosa.[1] This condition has a major impact on quality of life[2,3] and affects 0.7% to 3.7% of the general population.[4,5]

When diagnosing BMS, systemic causes such as Sjögren's syndrome should be excluded, and the oral mucosa should be normal upon inspection.[6] The underlying etiology of BMS is complex and poorly understood,[7] with abnormalities extending from the altered expression of vanilloid and cannabinoid receptors on the epithelium[8] to peripheral nerve[9] and central functional and structural alterations in the hippocampus and prefrontal cortex.[10] Altered immune and endocrine function has also been implicated in the etiology of BMS.[11,12]

The management of BMS is very difficult in relation to accurate diagnosis, especially as it is often misdiagnosed as Sjögren's syndrome.[13] A wide array of suboptimally effective therapies have been used, including antidepressants, alpha-lipoic acid, anti-inflammatory agents, and nonpharmacologic therapies.[14–16] The complex etiology of BMS and the existence of specific subtypes with differing contributions of peripheral and central neuropathic pain may explain the limited therapeutic response.[14,17]

The role of small fiber pathology was explored in an early tongue biopsy study that revealed a significant decrease in epithelial nerve

density and active axonal degeneration in the subpapillary nerve plexus in patients with BMS.[18] A more recent study observed a loss of epidermal nerve fibers, but no difference in subepithelial nerve fiber density.[9] In a further study, there was an overall loss of epidermal nerve fibers, but with an increase in transient receptor potential vanilloid 1 (TRPV1)– and nerve growth factor (NGF)–expressing pain nerve fibers.[19] Furthermore, in a recent study, mechanical sensitivity thresholds were preserved, indicating preferential small fiber involvement in BMS.[20]

A tongue biopsy may be useful for identifying small fiber damage and exploring the underlying etiology of BMS; however, its invasive nature limits its usefulness. The present authors have pioneered the technique of corneal confocal microscopy (CCM) for rapid noninvasive imaging of the corneal subbasal plexus, which is made up of sensory nerves derived from the trigeminal nerve.[21] It has been shown that CCM is a reproducible and repeatable technique[22] for identifying small fiber damage in diabetic neuropathy[23–25] and a range of other peripheral neuropathies.[26–29] Increased Langerhans cell (LC) density in relation to corneal nerve loss in diabetic neuropathy,[30] chronic inflammatory demyelinating polyneuropathy,[26] and multiple sclerosis[31] have also been shown, in addition to corneal nerve fiber loss in patients with multiple sclerosis[31] and Parkinson disease.[32]

The aim of this study was to investigate whether CCM can detect an abnormality in corneal small nerve fibers and LC density in patients with BMS compared to age-matched controls.

Materials and Methods

Study Subjects

This was a prospective cross-sectional cohort study conducted at two tertiary referral dental hospitals in the United Kingdom between June 2014 and 2017.

A total of 17 consecutive patients with BMS who were able to attend for further investigations were studied and compared to 14 age-matched healthy control subjects selected from hospital and university staff without any cause of neuropathy. The study was approved by the NHS Health Research Authority, National Research Ethics Service reference 14/NW/0004, and written informed consent was obtained from all participants. This research adhered to the tenets of the Declaration of Helsinki.

Eligibility

Patients with a definite clinical history of primary BMS for a duration of at least 6 months and aged between 18 and 85 years were invited for the study. The diagnostic criteria were based on the International Classification of Headache Disorders (ICHD-3 beta) and defined as an intraoral burning or dysesthetic sensation recurring daily for more than 2 hours per day over more than 3 months without clinically evident causative lesions. The diagnosis of BMS included a clinical investigation of the oral cavity in order to exclude local causes and a laboratory analysis to eliminate any systemic cause of the burning or sore mouth.[13] Subjects with a known history of corneal abnormality, trauma, or surgery; wearing contact lenses; any other cause of neuropathy; and/or burning mouth symptoms attributed to any other underlying cause such as candidiasis, trauma, or thermal or chemical burns were excluded from the study.

Corneal Confocal Microscopy

All participants underwent CCM using a Heidelberg Retinal Tomograph III with Rostock Cornea Module (HRT III RCM) (Heidelberg Engineering). The examination took 5 to 10 minutes per patient and was performed by highly experienced optometrists (M.F.). Six images (three per eye) from the central corneal subbasal nerve plexus were selected, following a previously published protocol.[22]

Image Analysis

An experienced examiner (M.F.) analyzed all the images manually using CCMetrics (MA Dabbah; Imaging Science and Biomedical Engineering) while being masked from the diagnoses. The measurements that were performed were: corneal nerve fiber density (CNFD), indicating the number of major nerves/mm^2 of corneal tissue; corneal nerve fiber length (CNFL), indicating the length of nerves/mm^2 of corneal tissue; corneal nerve branch density (CNBD), indicating the number of nerve branches/mm^2 of corneal tissue; and corneal nerve fiber tortuosity (CNFT), indicating the degree of nonlinearity of the nerve fibers. LCs were identified from their size and morphology as highly bright dendritic structures, and the density (no./mm^2) was derived by counting the total number of LCs in the area of the cornea using the nerve branch density (NBD) feature of the CCMetrics software.[26]

Statistical Analyses

IBM SPSS v 22 for Windows and Stata v 15 were used to compute the results. Analyses included descriptive and frequency statistics. All data are presented as mean ± standard deviation (SD). All data were tested for normality using Shapiro-Wilk test and Q-Q plots. Two-tailed independent sample *t* tests (for parametric variables) and Mann-Whitney *U* test (for nonparametric variables) were used to compare means between the two groups. When appropriate, 95% confidence intervals (CI) were expressed.

Sample Size

Based on previously published results in patients with Charcot-Marie-Tooth disorder,[27] it was estimated that a minimum of 12 patients and 12 controls were required to detect a difference in CCM parameters with a significance level of .05 and power of 0.80 based on a two-tailed independent sample *t* test per group.

Results

Demographics

A total of 17 patients with BMS (15 women, 2 men) were compared to 14 healthy, age-matched control subjects (7 women, 7 men). There was no difference in age between BMS patients and controls (BMS: 61.7 ± 6.5 years vs controls: 59.3 ± 8.7 years; mean difference = −2.45 years; 95% CI: −8.46 to 3.55; $P = .4$).

Corneal Nerve Fibers

CNFD (no./mm^2) (BMS: 29.27 ± 6.22 vs controls: 36.19 ± 5.9; median difference = 6.71; 95% CI: 1.56 to 11.56; $P = .007$) and CNFL (mm/mm^2) (BMS: 21.06 ± 4.77 vs controls: 25.39 ± 3.91; median difference = 4.52; 95% CI: 1.22 to 6.81; $P = .007$) were significantly lower in BMS patients compared to controls. There was no difference in CNBD (no./mm^2) (BMS: 74.83 ± 27.43 vs controls: 76.48 ± 23; median difference = 2.86; 95% CI: −19.27 to 21.66; $P = .7$) or CNFT (BMS: 14.42 ± 2.95 vs controls: 16.41 ± 2.7;mean difference = 2.42; 95% CI: −0.24 to 4.58; $P = .06$) between BMS patients and controls (Table 1, Figs 1 and 2).

LC Density

LC density was significantly increased in patients with BMS compared to controls (no./mm^2) (BMS: 74.04 ± 83.37 vs controls: 29.17 ± 45.14; median difference = −21.27; 95% CI: −65.35 to −2.91; $P = .02$) (Figs 1 and 2, Table 1).

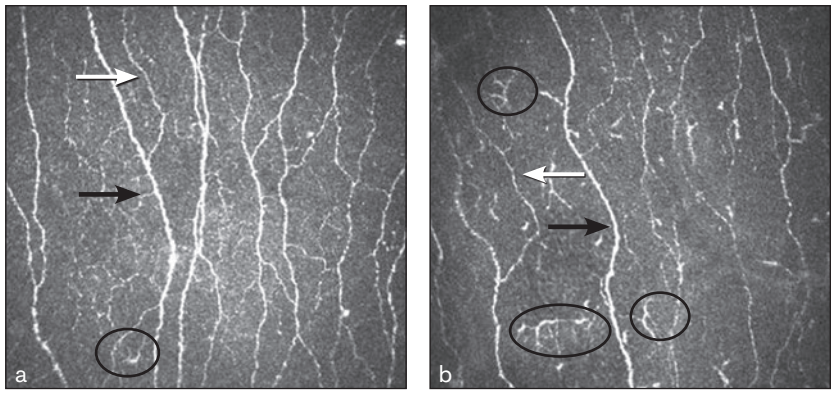

Fig 1 Corneal confocal microscopy images of the central subbasal nerve plexus from **(a)** a healthy control subject and **(b)** a patient with burning mouth syndrome. Black arrows indicate main nerves, white arrows indicate branches, and circles indicate Langerhans cells.

Table 1 Corneal Confocal Microscopy Measurements in BMS Patients and Controls

	BMS (n = 17)	Controls (n = 14)	*P* value
Age (y)	61.76 ± 6.5	59.3 ± 8.68	.4
CNFD (no./mm^2)	29.27 ± 6.22	36.19 ± 5.9	.007
CNBD (no./mm^2)	74.83 ± 27.43	76.48 ± 23.15	.7
CNFL (mm/mm^2)	21.06 ± 4.77	25.39 ± 3.91	.007
CNFT (TC)	14.42 ± 2.95	16.41 ± 2.79	.06
LC density (no./mm^2)	74.04 ± 83.37	29.17 ± 45.14	.02

All data are presented as mean ± standard deviation. CNFD = corneal nerve fiber density; CNBD = corneal nerve branch density; CNFL = corneal nerve fiber length; CNFT = corneal nerve fiber tortuosity; TC = tortuosity coefficient; LC = Langerhans cells.

Discussion

CCM has identified corneal small-fiber damage in patients with BMS. This confirms the presence of a small-fiber neuropathy in patients with BMS, which could previously only be shown through a reduction in epidermal nerve fiber density in tongue biopsies.[9,18,19]

The key advantage with CCM is that it is a rapid noninvasive imaging method that accurately and reproducibly[25,33] quantifies small fiber damage in a range of peripheral neuropathies.[25,34,35] Indeed, it has been previously shown that CCM has a diagnostic utility comparable to intra-epithelial nerve fiber density in skin biopsies for patients with diabetic neuropathy.[33,35]

It has also been shown that CCM can predict the development of clinical neuropathy[36,37] and detect early nerve fiber repair after therapeutic intervention[34,38]; therefore, it is hoped that CCM may be able to detect the response to treatment of small nerve fibers in BMS patients as well. As CCM allows the detection of small fiber damage in BMS patients, it may also help to identify BMS patients with a greater abnormality in peripheral rather than central pain pathways.[14,39]

Furthermore, a significant increase in corneal LC density in BMS patients was also shown, which is suggestive of immune alterations in BMS. Two previous studies have suggested immune alterations in BMS patients with a reduction in CD8 cells and altered CD4/CD8 ratios.[12,40]

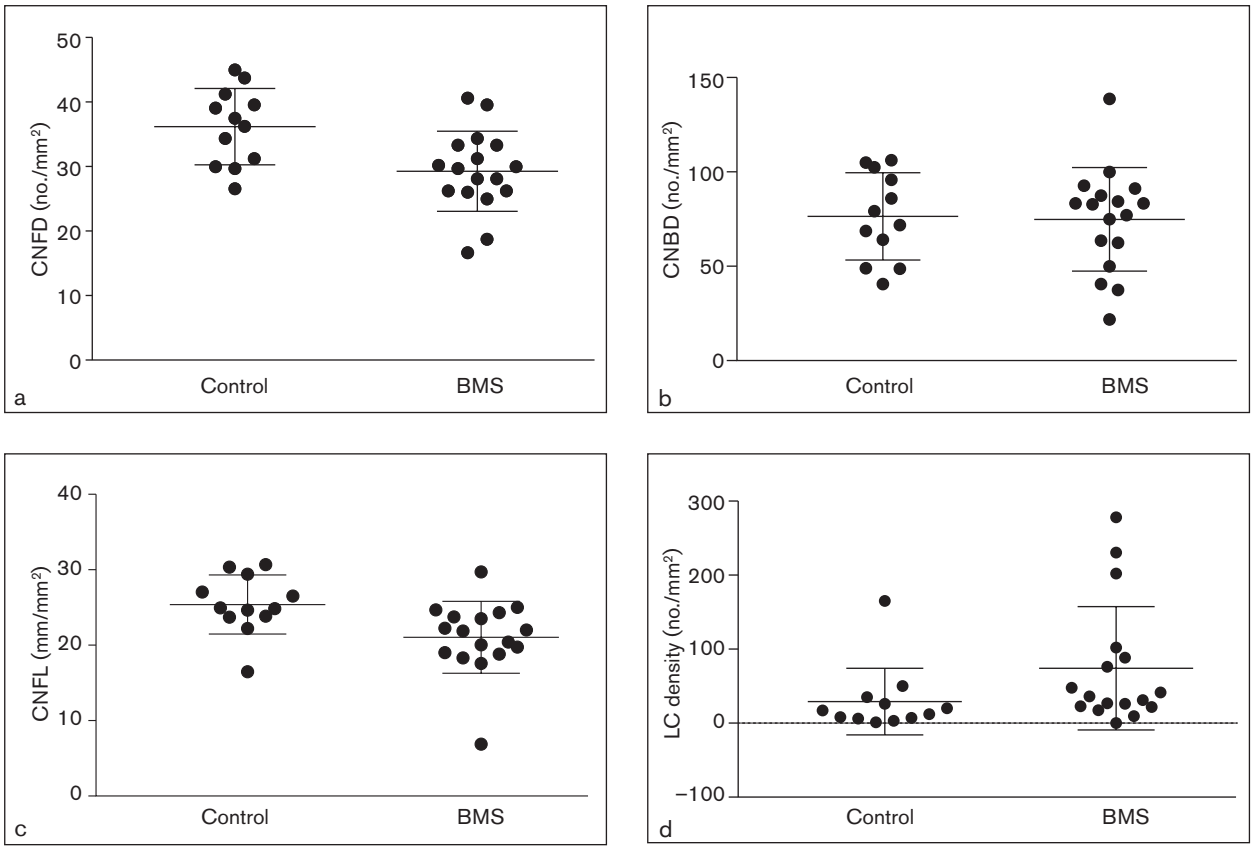

Fig 2 **(a)** Corneal nerve fiber density (CNFD), **(b)** corneal nerve branch density (CNBD), **(c)** corneal nerve fiber length (CNFL), and **(d)** Langerhans cell (LC) density in BMS patients and age-matched control subjects. Bars indicate mean and one standard deviation.

While LC density has not been assessed directly in biopsies from BMS patients, TRPV1 receptors are expressed on LCs, and TRPV1 immunoreactivity has been shown to be increased in tongue biopsies of BMS patients.[19] In relation to a mechanistic link to nerve degeneration, increased LC density has been associated with a reduced density of intra-epidermal nerve fibers in patients with painful diabetic neuropathy.[41]

Conclusions

CCM is a fast, noninvasive imaging method for quantifying small nerve fiber damage in patients with BMS. Further studies utilizing CCM are needed to investigate its utility in differentiating disease subtypes and monitoring disease progression and/or response to treatment.

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