Melatonin Treatment in Patients with Burning Mouth Syndrome: A Triple-Blind, Placebo-Controlled, Crossover Randomized Clinical Trial

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Aims: To evaluate the efficacy of melatonin compared to placebo in reducing pain associated with burning mouth syndrome (BMS), as well as side effects of treatment and effects on sleep quality, anxiety, and serum and salivary melatonin levels. **Methods:** In this triple-blind, randomized clinical trial, 20 BMS patients (mean age ± standard deviation: 64.4 ± 11.5 years; range: 35 to 82 years) were enrolled to receive melatonin (12 mg/day) or placebo for 8 weeks in a crossover design. After treatment, changes in pain from baseline were ascertained by patient self-assessment with a verbal category scale and a visual analog scale. Secondary outcomes included evaluation of changes in sleep quality and anxiety. Data were subjected to analysis of variance (ANOVA), Fisher exact test, paired *t* test, Wilcoxon signed rank test, or chi-square test, as appropriate. **Results:** Melatonin was not superior to placebo in reducing pain. Melatonin significantly improved anxiety scores, though without strong clinical relevance. Independent of treatment, sleep quality did not significantly change during the trial, although melatonin slightly increased the number of hours slept. After active treatment, the mean ± standard error serum melatonin level peaked at 1,520 ± 646 pg/mL. A generally safe pharmacologic profile of melatonin was observed, and the placebo and melatonin treatments resulted in similar adverse effects. **Conclusion:** Within the limitations of this study, melatonin did not exhibit higher efficacy than placebo in relieving pain in BMS patients. *J Oral Facial Pain Headache 2018;32:178–188. doi: 10.11607/ofph.1913*

Keywords: *anxiety, indoleamine, orofacial pain, sleep, stomatodynia*

Burning mouth syndrome (BMS) is a spontaneous, painful burning sensation that has been recently categorized as a neuropathic pain and for which no dental or medical cause can be found.[1] Circadian variation of symptoms often occurs, as pain typically increases as the day progresses.[2] This intense pain significantly reduces the patients' quality of life.[3] The prevalence of BMS ranges from 0.7% to 7% of the general population, increasing to 12% to 18% in postmenopausal women.[4] It is more frequent among anxious and depressed patients, suggesting that psychological factors play a role.[5,6]

The etiology of BMS is unknown, although recent findings suggest alterations of the peripheral and central nervous systems.[4,7] An imbalanced antioxidant status[8,9] and an impaired inflammatory response[10] have been reported, suggesting an influence of these factors on its pathogenesis. One recent retrospective cohort study and three case-control studies have reported associations between sleep disorders and BMS.[11–14] Compared with controls, BMS patients showed poorer sleep quality and increased frequency of sleep disorders[15]; conversely, patients with sleep disorders showed a higher frequency of BMS.[15] These findings suggest that sleep disturbance could be a risk factor for BMS and a promising therapeutic target. Therapy for BMS is still largely empirical due to the lack of strong scientific evidence.[16–18] Many agents, from salivary substitutes to anxiolytics, antidepressants, and anticonvulsants, have been proposed for coping with BMS and improving symptoms, but the results have been disappointing.[16,17,19] Some recent attention has been focused on clonazepam, a benzodiazepine agonist of gamma-aminobutyric acid (GABA-A) receptors.[20,21]

Melatonin is a pleiotropic molecule that possesses multiple mechanisms of action against chronic pain.[22] It is also an indolamine involved in the regulation of several chronobiologic processes, such as the circadian and circannual rhythms, the sleep-wake cycle, and recovery from jet lag.[23–26] Moreover, it is an immunomodulating, antioxidant, anti-inflammatory, and neuroprotective agent.[23,27,28] Thus, melatonin has been proposed to improve sleep and antioxidant status in patients affected by neurodegenerative disorders, including Parkinson and Alzheimer diseases[29] and multiple sclerosis,[30] as well as in postmenopausal women.[31] The effect of melatonin in improving mood and anxiety and in promoting analgesia supports its potential for therapy of chronic pain, as shown for fibromyalgia[32] and temporomandibular disorders (TMD).[22,33] The anti-nociceptive effect of melatonin involves activation of GABA-A-benzodiazepine receptors (similar to clonazepam) and increased endogenous β-endorphin release in the central nervous system.[34]

Therefore, the aim of this clinical trial was to evaluate the efficacy of melatonin compared to placebo in reducing BMS-related pain. Sleep quality, anxiety, side effects, and serum and salivary melatonin levels were also evaluated.

Materials and Methods

Study Design

A placebo-controlled, crossover randomized clinical trial was carried out at the Unità Complessa Odontostomatologia II (Ospedale San Paolo, Università degli Studi di Milano), where the interventions were performed and data were collected and analyzed. The trial was triple-blinded; ie, the participants, care providers, and researchers assessing outcomes were blinded after assignment to interventions.

Patient Recruitment

Patients were enrolled at the Unità Complessa Odontostomatologia II from October 2013 to July 2015 after approval by the Ethics Committee of the hospital (reference: BMS2013), in accordance with the ethical principles of the World Medical Association Declaration of Helsinki. Written informed consent was obtained from each patient. The trial was registered at www.clinicaltrials.gov (reference number: NCT02580734), and the CONSORT statement for randomized clinical trials was applied (http://www.consort-statement.org/consort-2010). Consistent with Gremeau-Richard et al,[21,35] the inclusion criteria were as follows: the presence of burning or stinging chronic oral pain, with or without xerostomia or dysgeusia, in patients with a normal oral mucosa upon clinical examination and without hyposalivation (salivary flow rate was assessed by the spitting method[36]); and presence of pain for more than 4 months with no trigger paroxysms and not following a specific nerve trajectory. In addition, any organic condition associated with an oral burning sensation was ruled out in all patients by laboratory tests (full blood cell count and serum levels of iron, ferritin, folate, vitamin B12, glucose, and zinc).[35] Patients who were taking or had previously taken anxiolytics to induce sleep, antidepressants, anticonvulsants, or psychotropic drugs or who were receiving psychological therapy for anxiety and depression were included, as the current study was designed to be as similar as possible to the clinical condition, and BMS patients are often anxious and/or depressed, for which they require drug treatment.

The exclusion criteria were: previous or current therapy with melatonin, serotonin, or other analogs in the last month; previous or current therapy with phytotherapeutics or dietary supplements with melatonin, serotonin, and tryptophan in the last month; documented specific allergy or hypersensitivity to melatonin; working at night; being treated with anticoagulants because of a potential pharmacologic interaction[23]; being pregnant or lactating; or being less than 18 years old.

Intervention

At baseline, the personal and clinical data of the participants were recorded and the inclusion/exclusion criteria reviewed. Each patient received two sequential 8-week treatments of melatonin or placebo compresses separated by a washout period of 4 weeks. The clinical trial had a 20-week duration. Participants were randomly assigned to treatment sequence A (melatonin followed by placebo) or B (placebo followed by melatonin) (Fig 1). During treatment and data analysis, the physicians, patients, and laboratory investigators were blind to the medication assignment. A simple randomization method was applied with an online tool (http://graphpad.com/quickcalcs/%20randomise1.cfm). Allocation concealment was ensured because the person who generated the randomization list and assigned the individuals to the two treatment sequences was not involved in evaluating patient eligibility for the study.

Active compresses (Tranquillus, Functional Point Srl) contained 3 mg of N-acetyl-5-methoxytryptamine (melatonin). Melatonin and placebo compresses had the same color, shape, and taste and were distributed in identical containers without any labels. They had identical inert excipients (microcrystalline cellulose, calcium phosphate, inulin, talc, and magnesium stearate). Patients were instructed to take four compresses a day for 8 weeks at around 8:30 am, 12:30 pm,

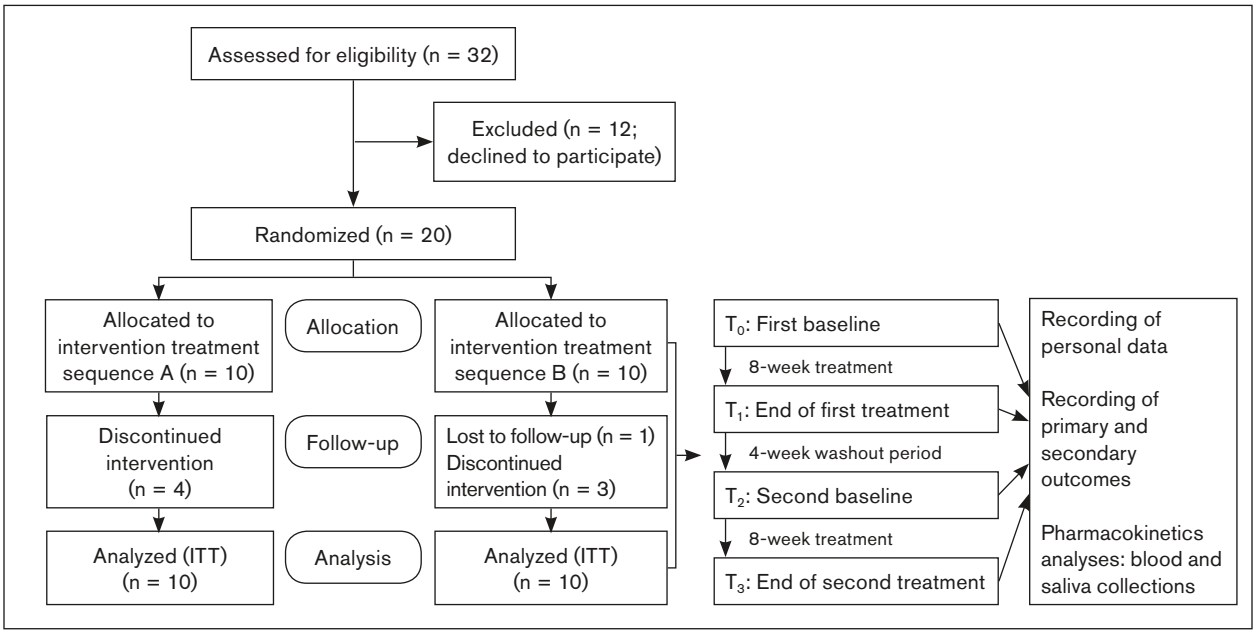

Fig 1 Study flowchart. This crossover randomized clinical trial involved four clinic visits at the following time points: T_0 (baseline visit before starting first treatment); T_1 (visit at end of first 8 weeks of treatment); T_2 (baseline of second 8 weeks of treatment after washout period of 4 weeks); and T_3 (end of second treatment). Primary (change in pain score) and secondary (sleep quality and anxiety) outcomes were recorded and blood and saliva samples collected at all time points.

3:30 pm, and 7:30 pm. The total dose of melatonin was 12 mg/day, based on previous studies.[26,32,37,38] Four experimental time points were recorded: T_0, baseline visit before starting the first treatment; T_1, visit at the end of the first 8 weeks of treatment; T_2, baseline visit of the second treatment (after a washout period of 4 weeks); and T_3, visit at the end of the second 8 weeks of treatment (Fig 1). Each patient was examined at the same time of day at all four time points (in general, visits were scheduled between 8:30 am and 2:00 pm). At each time point, clinical data for the primary and secondary outcomes were recorded, and the same investigator who provided the compresses performed a clinical examination of the oral mucosa. Side effects were recorded, and blood was drawn to measure serum and salivary melatonin levels. Treatment compliance was self-reported with a questionnaire and assessed by counting the number of compresses left in the container at the end of each treatment.

Primary Outcome: Pain Evaluation

The primary outcome was the self-reported perception of pain during the trial.

Global Impression of Pain Change. Any change in pain intensity was recorded by the patients at T_1 and T_3. The patients were asked to verbally express their feelings about the treatment by using the following five-point categorical scale: worse, no change, mild improvement, moderate improvement, and strong improvement (adapted from Farrar et al[39]).

Visual Analog Scale. The visual analog scale (VAS) consisted of a 10-cm horizontal line marked from 0 (no pain) to 10 (most severe pain experienced) on which the patient was requested to record the level of pain felt at the end of the 8-week treatment.[40,41] Changes in BMS symptoms were calculated as:

$$\Delta\text{VAS} = \text{baseline value} - \text{posttreatment value}$$

Number of Oral Sites Involved. The number of oral sites affected by the burning sensation was recorded, and percentages were calculated as the ratio of the number of sites measured to the total number of oral sites considered (n = 15).[41] Changes in oral sites affected were calculated as:

$$\Delta\%\ \text{oral sites} = \text{baseline \% value} - \text{posttreatment \% value}$$

Secondary Outcomes: Sleep Disturbances and Anxiety

Sleep Quality. The Medical Outcomes Survey (MOS) sleep scale questionnaire was administered. Items on the questionnaire were used to calculate various subscales, which included raw sleep quantity (SLPQRAW), which refers to hours of sleep per night in the previous week; sleep disturbance (SLPD4), which assesses trouble falling asleep and non-quiet sleep (which assesses wakefulness during sleep time); snoring (SLPSNR1); shortness

of breath (SLPSOB1), which evaluates waking up with shortness of breath or headache; sleep adequacy (SLPA2), which evaluates whether sleep was sufficient to feel rested upon waking in the morning; and daytime somnolence (SLPS3), which assesses drowsiness during the day, trouble staying awake during the day, or the need to take naps. The sleep problems index II (SLP9), which summarizes sleep disturbances, was calculated by combining these items. The SLP9 refers to sleep adequacy, respiratory impairment, somnolence, time to fall asleep, sleep quietness, and drowsiness during the day.[13] For the SLP9 and all subscales except for SLPQRAW, for which lower scores indicate worse sleep, higher scores indicate more severe sleep problems.

Anxiety. The severity of anxiety symptoms was evaluated using the clinician-rated Hamilton Rating Scale for Anxiety (HAM-A)[6,12]: < 17 indicates mild anxiety; 18–24 indicates mild to moderate anxiety; and 24–30 indicates moderate to severe anxiety.[42] Changes in HAM-A scores were calculated as follows:

$$\Delta\text{HAM-A} = \text{baseline value} - \text{posttreatment value}$$

Side Effects

Diurnal Sleepiness. Somnolence during the day was measured using the Epworth Sleepiness Scale (ESS). Respondents were asked to rate on a 4-point scale (0 to 3) their typical probability of dozing or falling asleep while engaged in eight activities. The ESS score (the sum of the eight item scores, which range from 0 [never] to 3 [always]) can range from 0 to 24; the higher the ESS score, the higher the average sleep propensity of a person in daily life; ie, his/her daytime sleepiness.[43] An ESS score of 10 has been proposed as a threshold for normality. The change in ESS score before and after each treatment was calculated as follows:

$$\Delta\text{ESS} = \text{baseline value} - \text{posttreatment value}$$

Table 1 Quantification of Melatonin (MLT) in Samples Using Multiple Reaction Monitoring With Optimized Fragmentation Mass-to-Charge Ratio (M/Z)

Q1 mass (Da)	Q3 mass (Da)	Dwell (ms)	Parameter
233.1 (MLT)	174.1	80	Declustering potential: 55 V Collision energy: 20 V CXP: 10 V
233.1 (MLT)	159.0	80	Declustering potential: 56 V Collision energy: 33 V CXP: 10 V
233.1 (MLT)	130.1	80	Declustering potential: 55 V Collision energy: 55 V CXP: 14 V
237.4 (IS)	178.1	80	Declustering potential: 100 V Collision energy: 29 V CXP: 5 V
237.4 (IS)	220.1	80	Declustering potential: 100 V Collision energy: 15 V CXP: 26 V

Q = quadrupole; CXP = cell exit potential; IS = internal standard.

Other Side Effects. Any other side effect was recorded using a questionnaire about daily somnolence, dizziness, nausea and vomiting, impaired concentration, and appetite alteration.

Oral Bioavailability: Serum and Salivary Melatonin Levels

Serum and salivary samples were collected to measure melatonin levels. Saliva was collected using the spitting method.[36,44] Serum melatonin levels were determined in blinded samples by using a high-performance liquid chromatograph (Agilent 1290 Infinity Autosampler G4220B) coupled with a triple-quadrupole mass spectrometer (ABSciex QTrap 5500) (HPLC-MS/MS) using [2H4]-N-acetyl-5-methoxytryptamine (98.3%) as the internal standard. After pre-analytical processing based on liquid-phase extraction, samples were injected in a Kinetex 2.6-µm XB-C18 100A column (Phenomenex) (100 × 2.10 mm) at 30°C. The flow rate was 0.45 mL/minute, and the samples were eluted using the following gradient of mobile phase A (2 M ammonium formate and 0.1% formic acid) and B (acetonitrile): 90% A for 2 minutes, 30% to 70% A for 1 minute, 70% to 15% A for 1 minute, 90% B for 2 minutes, and 90% to 10% B for 2 minutes. The total run time was 10.5 minutes. The multiple reaction monitoring (MRM) technique was used to quantify melatonin levels with the optimized fragmentation mass-to-charge ratio (m/z) (Table 1). The limit of quantification was 5 pg/mL. Salivary melatonin level was measured by enzyme-linked immunosorbent assay (ELISA) (BTB-E1013Hu, Human Melatonin, MT ELISA Kit, Li StarFISH) following the manufacturer's instructions.

Statistical Analyses

The sample size (n = 20 patients) was calculated according to effect size and standard deviations (SD) derived from previous studies,[36–38] with a power of 80% and a type I error of 0.05, considering a crossover design and a 20% dropout rate. An intention-to-treat analysis (ITT) was performed to evaluate primary and secondary outcomes. The data of dropouts were included in the calculations; the last observation carried forward (LOCF)

Table 2 Sociodemographic Data of Included Patients at Baseline (N = 20)

Gender, n (%)	Age, mean ± SD (y)	Education, n (%)	Civil status, n (%)	No. of offspring, mean ± SD	History of depression, n (%)	History of insomnia, n (%)	Current pharmacologic therapies, n (%)
M: 4 (20)	64.4 ± 11.6	Primary school: 15 (75)	Unmarried: 0 (0)	1.6	Yes: 9 (45)	Yes: 9 (45)	Anti-cholesterol drugs: 3 (15)
F[a]: 16 (80)		High school: 5 (25)	Married: 17 (85)		No: 11 (55)	No: 11 (55)	Gastro-protective drugs: 5 (25)
		University: 0 (0)	Widow/er: 3 (15)				Anti-platelet drugs: 3 (15)
							Anti-hypertensive/ anti-arrhythmic drugs: 10 (50)
							Anxiolytics, antipsychotics, and antidepressants: 7 (35)
							Bisphosphonates: 2 (10)
							Other (hypoglycemic, immunosuppressant, anti-diarrheic) drugs: 3 (15)

[a]All women were in menopause (mean ± SD age at menopause: 50.6 ± 2.0 years). SD = standard deviation.

approach, in which the last available data are assumed to be the same at all further time points, was used.[48] The LOCF assumption was justified, as the average unobserved outcomes within each randomized group did not change significantly over time.[33,49] The means ± standard errors of the mean (SEM) of variables were calculated, except for age and number of offspring, for which SD was used. A Kolmogorov-Smirnov normality test was applied: At a level of 0.05, the data were considered to be from a normally distributed population. Normally distributed data were compared by using one-way analysis of variance (ANOVA) and Fisher exact test, and pre- and posttreatment values were compared by using paired *t* test. A paired *t* test was also used to compare Δ values. At the 0.05 level, results not considered to be from a normally distributed population were compared using the paired-sample Wilcoxon signed rank test. Nonparametric variables were compared by using chi-square test. Spearman's correlation coefficient was used to identify linear correlations of VAS scores with serum and salivary melatonin levels. Statistical significance was set at $P \leq .05$.

Results

A flowchart of the study design, including patient recruitment and dropouts, is shown in Fig 1. During a 12-month period, 32 patients with a previous diagnosis of BMS were screened for participation. Twenty patients (16 females and 4 males; mean ± SD age: 64.4 ± 11.5 years) were enrolled in the study. The patients' sociodemographic data, clinical history, and current drug use are shown in Table 2.

During the first phase of the intervention, 8 of the 20 (40%) patients dropped out because of side effects (n = 4; self-reported heavy tremor, sexual disturbances, blurred vision, and severe heavy-headedness), lack of efficacy (n = 2), improvement in pain (n = 1), or loss to follow-up (n = 1). The dropouts were equally distributed between the placebo (n = 4) and melatonin (n = 4) groups, which allowed an LOCF approach for ITT analysis without reducing the statistical power. The patients who completed the study adhered to the therapy for more than 80% of the total therapy according to self-reports, although some did not return the compresses to be checked.

Primary Outcomes: Pain Evaluation

Patient Impression of Pain Change. Most patients did not report significant changes in pain intensity during placebo or melatonin treatment (Figs 2a and 2b): half of the patients (n = 10, 50%) perceived no change in pain after placebo treatment, and 60% (n = 12) perceived no change after melatonin treatment. Interestingly, a moderate improvement in pain was reported after melatonin treatment in 20% of cases (n = 4), slightly higher than that after placebo treatment (n = 3, 15%), although this difference was not statistically significant. Worsening of pain was recorded by 5% of patients (n = 1) after melatonin treatment and by 10% after placebo treatment (n = 2).

Pain Intensity. The primary outcome was improvement in BMS symptoms as measured by a VAS. At all time points, VAS scores were > 3 (moderate to severe pain). In the placebo group, the baseline VAS score was 7.8 ± 0.3 and decreased

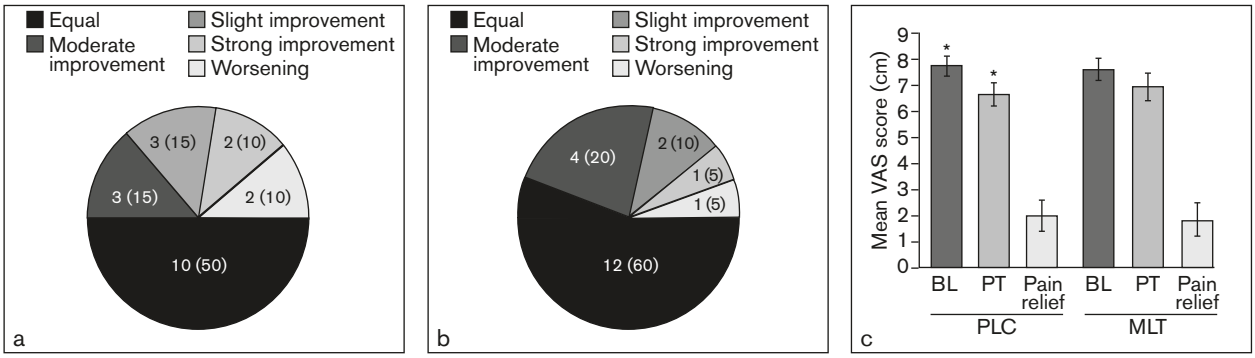

Fig 2 Number (%) of patients reporting changes in pain for **(a)** placebo and **(b)** melatonin treatments. No differences were statistically significant (chi-square test). **(c)** Mean visual analog scale (VAS) scores for pain intensity and pain relief (pain relief was evaluated only posttreatment). Error bars indicate standard errors of the mean (SEM). MLT = melatonin; PLC = placebo; BL = baseline; PT = posttreatment. *$P \leq .05$ (paired *t* test).

Table 3 Comparing Clinical Outcomes as Difference Between Baseline and Posttreatment Values

	Δ VAS pain intensity (mean ± SEM)	Δ % oral sites (mean % ± SEM)	Δ MOS-SLP9 (mean ± SEM)	Δ HAM-A (mean ± SEM)	Δ ESS (mean ± SEM)
Placebo	1.2 ± 0.4	5 ± 4.2	2.2 ± 3.1	0.7 ± 0.8	−0.3 ± 0.5
Melatonin	0.6 ± 0.5	1 ± 3.3	3.1 ± 2.1	2.1 ± 1	−0.2 ± 0.4

VAS = visual analog scale; MOS = Medical Outcomes Survey Sleep Scale; HAM-A = Hamilton Rating Scale for Anxiety; ESS = Epworth Sleepiness Scale; SEM = standard error of the mean.

Table 4 Medical Outcomes Survey (MOS) Sleep Scores (N = 20)

Treatment and time point	SLPQRAW (h)	SLPD4	SLPSNR1	SLPSOB1	SLPA2	SLPS3	SLP9
PLC BL	5.6 ± 0.2	38.7 ± 5.8	19 ± 6	12 ± 5.8	50 ± 8.7	19.6 ± 3.4	33.8 ± 3.8
PLC PT	5.5 ± 0.3	34.7 ± 6.1	15 ± 6.1	21 ± 7.3	57.5 ± 8.8	20 ± 3.7	31.6 ± 4.5
MLT BL	5.3 ± 0.3	37 ± 5.8	12 ± 4.4	20 ± 8	56 ± 8.3	20 ± 4.1*	32.2 ± 4.6
MLT PT	5.7 ± 0.4	32.4 ± 5.3	13 ± 4.6	22 ± 7.5	59.5 ± 9.1	26 ± 4.7*	29.3 ± 4.7

Data are reported as mean ± standard error of the mean (SEM).
PLC = placebo; MLT = melatonin; BL = baseline; PT = posttreatment; SLPQRAW = sleep quantity (refers to hours of sleep per night in the last week, lower scores indicate worse sleep problems); SLPD4 = sleep disturbance (trouble falling asleep, how long to fall asleep, unquiet sleep, awakening during sleep time, and trouble falling asleep again); SLPSNR1 = snoring; SLPSOB1 = awakening with shortness of breath or headache; SLPA2 = sleep adequacy (enough sleep to feel rested upon waking in the morning); SLPS3 = daytime somnolence (feeling drowsy during the day, having trouble staying awake during the day, taking naps); SLP9 = sleep problems index II (sleep adequacy, respiratory impairment, somnolence, time to fall asleep, quiet sleep, and drowsy during the day).
*$P \leq .05$ (paired-sample *t* test for normally distributed populations; paired-sample Wilcoxon signed rank test for not normally distributed populations).

to 6.7 ± 0.4 posttreatment. In the melatonin group, the VAS score at baseline was 7.6 ± 0.4 and after treatment was 7.0 ± 0.5. VAS scores did not differ significantly among the four time points, but there was a significant difference in the placebo group between baseline and posttreatment ($P \leq .05$). The mean ΔVAS was 1.2 ± 0.4 for placebo and 0.6 ± 0.5 for melatonin; these values did not differ significantly (Table 3). Patients consistently reported low pain relief scores: 2.0 ± 0.6 after placebo treatment and 1.9 ± 0.6 after melatonin treatment.

Number of Oral Sites Involved. Overall, no change in the number of oral sites affected by pain was recorded. At baseline, the mean percentage of involved oral sites was 42% ± 6% in the placebo group and 35% ± 5% in the melatonin group; at the end of treatment, the number of sites was unchanged after melatonin treatment (35% ± 6%) and decreased slightly after placebo treatment (37% ± 7%), albeit not significantly. Δ% values were similar: 5% ± 4.2% in the placebo group and 1% ± 3.3% in the melatonin group (Table 3).

Secondary Outcomes: Sleep Disturbances and Anxiety

Sleep Quality. The MOS subscale scores are shown in Table 4. At the time of enrollment (first baseline), optimal sleep quantity (SLPQRAW), defined as 7 to 8 hours, was recorded in five patients (25%); the remaining 75% (n = 15) reported a reduced sleeping

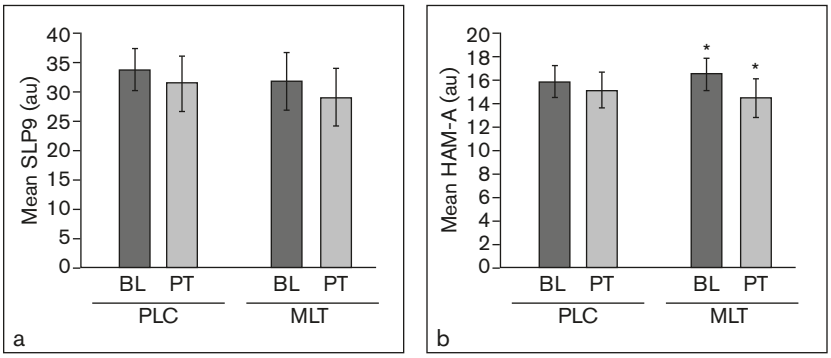

Fig 3 (a) Sleep quality before and after treatments as evaluated with the Medical Outcomes Survey (MOS) sleep problems index II (SLP9). **(b)** Anxiety before and after treatments as evaluated with the Hamilton Rating Scale for Anxiety (HAM-A) score. Error bars indicate standard errors of the mean (SEM). MLT = melatonin; PLC = placebo; BL = baseline; PT = posttreatment; au = arbitrary unit. *$P \leq .05$ (paired *t* test).

time, with an average of 5.3 ± 1.5 hours of sleep. Melatonin treatment slightly increased the number of hours slept during the night, but placebo treatment did not (Table 4). In two patients, this increase corresponded to optimal sleep quantity. The amount of sleep experienced by the first of these patients increased from 6 to 8 hours per night after melatonin treatment but remained at 6 hours after placebo treatment, which corresponded to a slight improvement in VAS score (ΔVAS: 0.2). The amount of sleep experienced by the second patient increased from 5 to 8 hours per night after melatonin treatment but decreased from 5 to 4 hours per night after placebo treatment, and this patient showed a marked improvement in VAS score (ΔVAS: 4.6). Melatonin treatment was also correlated with a slight improvement in sleep disturbances (SLPD4) (Table 4). At baseline, the SLP9 score was 33.8 ± 3.8 in the placebo group and 32.2 ± 4.6 in the melatonin group. The SLP9 score decreased in both groups posttreatment, albeit not significantly (Fig 3a; Table 3).

Anxiety. At the time of enrollment, the HAM-A score in BMS patients ranged from mild (n = 13, 65%) to moderate (n = 3, 15%) to severe (n = 4, 20%). After melatonin treatment, a statistically significant decrease in comparison with baseline was recorded ($P \leq .05$; Fig 3b). Interestingly, a patient in the melatonin group showed a reduction in HAM-A score from 22 to 8. ΔHAM-A values were consistently higher in the melatonin group than in the placebo group (Table 3).

Oral Bioavailability. Melatonin bioavailability was determined only in patients who completed the study (n = 12) for whom all serum and saliva samples were available. At baseline, serum melatonin levels were less than 5 pg/mL in all patients; after treatment, the melatonin concentration peaked at 1,520 ± 646 pg/mL but remained at physiologic levels after placebo treatment (26 ± 16 pg/mL) ($P \leq .05$; Fig 4a). No such difference was detected in saliva (Fig 4b). The correlation between serum and salivary melatonin levels was positive after melatonin treatment ($\rho = 0.1$), but negative after placebo treatment ($\rho = -0.5$; $P < .05$). Serum and salivary melatonin levels were correlated with the VAS score in each patient (Fig 4c). Notably, after melatonin treatment, serum melatonin concentrations increased markedly in half of the patients (n = 6) but remained at physiologic levels in the rest (Fig 4d). No strong correlation between VAS score and melatonin concentration was found for either treatment, although values were negatively associated; ie, a higher melatonin level corresponded to a lower VAS score ($\rho = -0.2$ for serum and −0.3 for saliva).

Side Effects

Table 5 summarizes the side effects recorded during the trial. The most frequent side effect was self-reported sleep impairment, which was slightly more common during melatonin than placebo treatment (n = 10 and n = 6, respectively). The ESS index was also used to evaluate diurnal somnolence. Overall, ESS values corresponded to normal daytime sleepiness, except in one patient in whom the values revealed moderately excessive daytime sleepiness at baseline and posttreatment (ESS score = 13). ESS scores increased after both treatments, supporting mild daytime sleepiness, with no significant difference between the melatonin and placebo groups (Fig 5; Table 3). Headache, dizziness, nausea and vomiting, and impaired concentration were reported slightly more frequently during placebo treatment, while appetite alteration was more marked during melatonin treatment; however, these differences were not significant (Table 5). Placebo also induced heart palpitations and severe tremor in two female patients, while melatonin produced sexual disturbances and blurred vision in a male patient and severe heavy-headedness in a female patient; these side effects caused the patients to drop out of the trial.

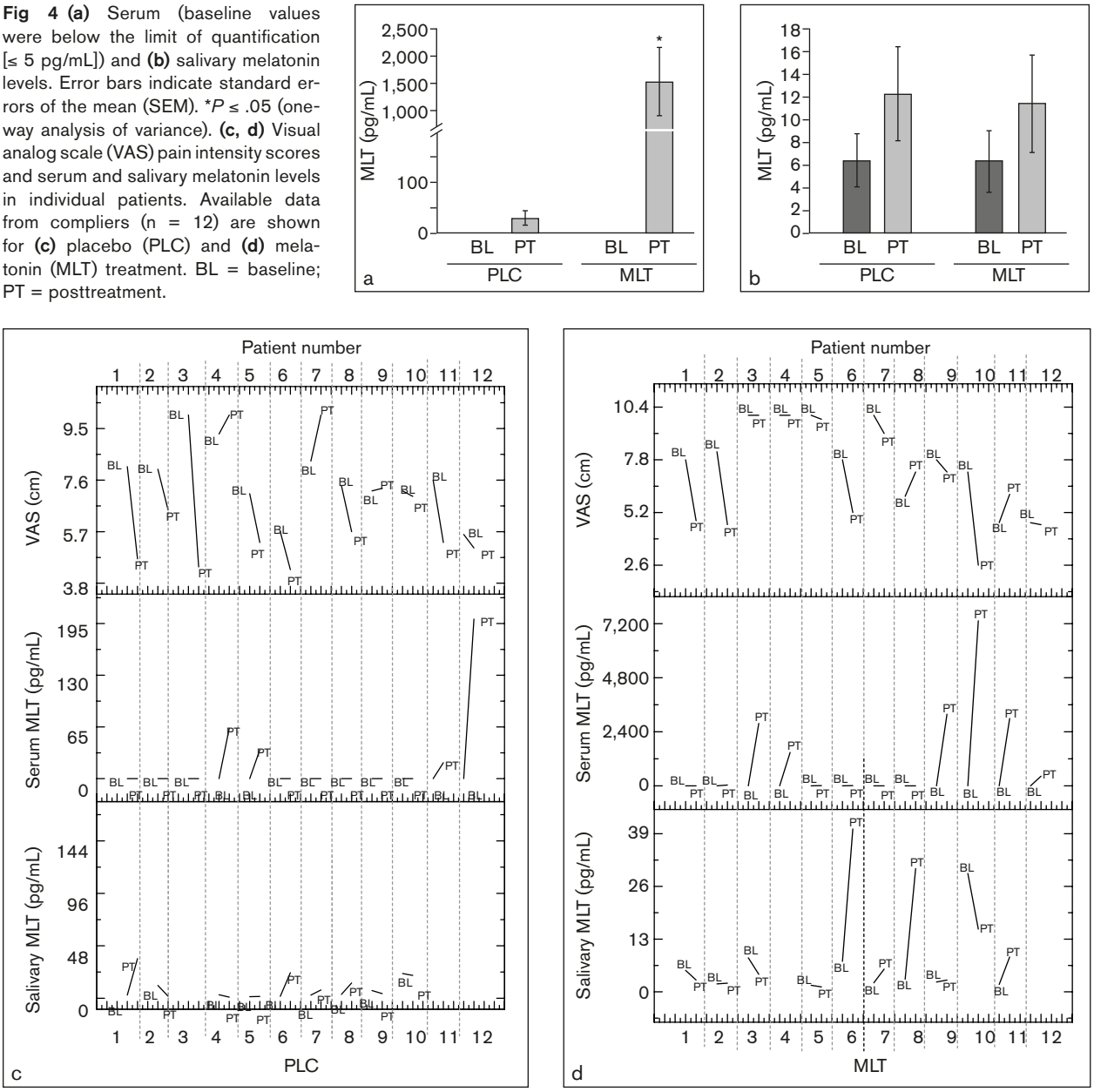

Fig 4 (a) Serum (baseline values were below the limit of quantification [≤ 5 pg/mL]) and **(b)** salivary melatonin levels. Error bars indicate standard errors of the mean (SEM). *$P \leq .05$ (one-way analysis of variance). **(c, d)** Visual analog scale (VAS) pain intensity scores and serum and salivary melatonin levels in individual patients. Available data from compliers (n = 12) are shown for **(c)** placebo (PLC) and **(d)** melatonin (MLT) treatment. BL = baseline; PT = posttreatment.

Discussion

To the best of the authors' knowledge, the present study is the first randomized, triple-blinded, placebo-controlled trial of 8-week melatonin treatment in BMS patients with pain intensity used as the primary outcome. The rationale of the study lies in the role of melatonin in BMS treatment and its mechanisms of action. Besides systemically regulating mood, the immune system, and circadian rhythms,[22] melatonin may be useful for treatment of oral diseases—including BMS—due to its neuroprotective, antioxidant, and anti-inflammatory activities.[28,30,50–52]

The present study has shown that melatonin and placebo have comparable efficacy in reducing pain caused by BMS, as patient-reported pain was largely unchanged in both treatments, showing only slight improvements in ΔVAS posttreatment. This lack of difference can be attributed to the effect of placebo on BMS patients, as stated in a recent systematic review: "On average, treatment with placebos produced a response that was 72% as large as the response to active drugs."[53] The interpretation of this result must also consider incomplete compliance with the melatonin therapy, as suggested by the physiologic serum melatonin concentrations,[54] despite self-reported satisfactory adherence to therapy.

Table 5 Side Effects Recorded by Patients Who Completed the Study (n = 12)

	Sleep disturbances, n	Headache, n	Dizziness, n	Nausea and vomiting, n	Impaired concentration, n	Appetite alteration, n	Other
Placebo							
Slight	1	0	0	1	0	1	
Moderate	2	2	3	0	3	0	
Severe	3	1	1	1	2	0	
Total	6	3	4	2	5	1	2[a]
Melatonin							
Slight	3	1	0	0	1	1	
Moderate	4	1	2	0	1	1	
Severe	3	0	0	0	0	0	
Total	10	2	2	0	2	2	2[b]

[a]Palpitations, severe tremor.
[b]Sexual disturbances, blurred vision, severe heavy-headedness.

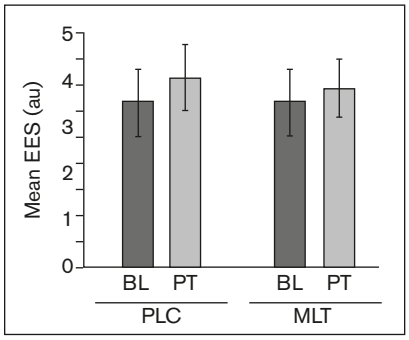

Fig 5 Diurnal somnolence before and after treatments as evaluated with the Epworth Sleepiness Scale (ESS). There were no significant differences. MLT = melatonin; PLC = placebo; BL = baseline; PT = posttreatment; au = arbitrary unit.

This study did not detect an association between melatonin and improved sleep quality, which is consistent with a recent meta-analysis of melatonin for sleep disorders in patients with neurodegenerative diseases.[29] This is important, as sleep disturbances are associated with BMS.[11–14] Anxiety and depression, which are independently associated with both sleep disturbances and BMS,[11,15] may have confounded the results. Thus, the results of this study should be interpreted with caution because sleep assessment was based on self-reports, not polysomnographic analysis. Sleep deprivation was found in 75% of BMS patients who slept less than 7 hours per night, and melatonin treatment improved sleep quantity slightly in these patients. Interestingly, a significant increase in sleep quantity was observed in two patients treated with melatonin who reached the optimal number of hours slept, although only one of them showed a marked improvement in VAS score. Sleep quality was not significantly affected by either treatment. In the US general population, the Medical Outcomes Study reported a mean SLP9 score of 25.8.[55] In the present study, the mean values (around 30) were higher than these cut-offs, suggesting poor sleep quality in BMS patients. These findings are consistent with a previous report of an SLP9 score of about 42 in BMS patients.[13]

The change in anxiety levels was also evaluated, as this is important for managing BMS in terms of coping with pain and improving quality of life. Overall, a moderate to severe anxiety level was found in 35% of cases, consistent with the 8% to 50% in previous reports.[9] Interestingly, melatonin resulted in a significantly greater reduction in anxiety than placebo,[5] but this is likely clinically irrelevant. However, this finding is consistent with evidence supporting use of melatonin as a premedication (orally or sublingually) to reduce preoperative anxiety in adults.[56]

Despite its lack of efficacy, melatonin exhibited a generally safe pharmacologic profile, as reported previously.[23,38,56] There were no differences in side effects or dropout rate between the melatonin and placebo groups. The main side effect with both treatments was self-reported sleep impairment, suggesting that patients perceived that melatonin (independently of the presence of the active ingredient) influenced their sleepiness. Indeed, no significant changes in more objective measures of sleep (SLP9 and ESS) were observed. Other minor side effects (eg, dizziness, headache, and nausea) were similar in the melatonin and placebo groups. This suggests a nocebo effect (ie, negative, adverse reactions subsequent to intake of a placebo[57]), as has been reported during treatment of BMS patients.[58] The nocebo effect results in some patients experiencing side effects despite taking a placebo and can result in the patients dropping out of trials, as occurred in two cases in this study.[59]

The limitations of this study must be considered when interpreting the findings. These include the small sample size, the high rate of dropouts, the lack of body mass index measurements (which may have influenced pharmacokinetics), and questionable patient compliance. Despite the pragmatic approach to patient recruitment and the good safety profile of melatonin, these limitations hamper the generalizability

of the findings. In addition, the hospital setting and consequent involvement of oral medicine specialists in BMS further hinder direct translation of the findings to general practice.

Given the correlation between BMS and sleep quality, future studies should investigate sleep quality improvement as a treatment to alleviate BMS pain, as well as the ability of melatonin to ameliorate sleep quality and/or quantity in BMS patients who are responsive to it.

Conclusions

Although recent evidence suggests an association between sleep disorders and BMS, melatonin was not superior to placebo in reducing BMS-related pain under this study's experimental conditions. Sleep quality did not change, and while anxiety improved slightly after melatonin treatment, this was probably not to a clinically relevant degree. High-dose melatonin showed a generally good safety profile, and the side effects were comparable to those in the placebo group.

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