Randomized Controlled Trial

Clinical Effects and Safety of the Use of Methylene Blue for the Treatment of Lumbar Facet Joint Syndrome

Xiuqin Yu, MD, Jinyuan Zhang, PhD, Mingxia Wang, MD, Yu Yang, MD, Wei Zhang, MD, Dan Su, MD, Lijun Liao, PhD, Hao Yang, PhD, Hongwei Fang, MD, Xiangrui Wang, PhD

From: Department of Pain, Shanghai East Hospital, Tongji University School of Medicine, Shanghai, China

Address Correspondence: Hongwei Fang, MD and Xiangrui Wang, PhD Department of Pain, Shanghai East Hospital, Tongji University School of Medicine, No.150 Jimo Road, Shanghai 200120, China. E-mail: hongwei_fang163@163.com

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Background: Lumbar facet joint syndrome (LFJS) has been suggested to be a main source of low back pain. Methylene blue (MB), an inhibitor of nitric oxide synthesis with potential analgesic and anti-inflammatory properties, has been widely applied for a variety of pain-related diseases. However, no studies have been conducted on the treatment of LFJS patients using MB.

Objectives: The purpose of this study was to evaluate the therapeutic effects of intra-articular injection of MB on LFJS patients.

Study Design: A prospective, randomized, controlled clinical trial.

Setting: Department of pain, Shanghai East Hospital.

Methods: A total of 120 eligible patients with LFJS were randomly divided into an MB group and a control group. Numeric Rating Scale (NRS), Oswestry Disability Index (ODI), Pittsburgh Sleep Quality Index (PSQI), Patient Health Questionnaire-9 (PHQ-9) were used to evaluate the pre-operation and post-operation states of the patients, and adverse events were recorded. The patients participating in this study were followed up for a period of 6 months.

Results: A total of 104 patients were followed up for the entire 6 months period. The control group included 51 patients, and the MB group included 53 patients. In both groups, the NRS scores, ODI scores, PHQ-9 scores, and PSQI scores decreased at different time points after treatment, compared to baseline. Moreover, the NRS scores were significantly lower than that of the control group at 3 months and 6 months after operation ($P < 0.05$). The ODI, PSQI, and PHQ-9 scores of the MB group were also respective significantly lower than that of the control group at 3 months and 6 months after operation ($P < 0.05$). As for the clinical efficacy, the total effective treatment rate of the MB group was significantly higher than that of the control group at 6 months after the procedure ($P < 0.05$). On the first day after operation, the incidence of hyperglycemia in patients with diabetes in the MB group was significantly lower than that of the control group ($P < 0.05$).

Limitations: Firstly, the patients enrolled were recruited from a single center, and the sample size was small. Secondly, the patients were only followed-up for a period of 6 months after treatment. Thirdly, double blinding was not used in the design of this research study.

Conclusion: Ultrasound-guided intra-articular MB injection is a safe and effective therapy for patients with LFJS. Intra-articular injection with MB can significantly reduce pain intensity, improve patient lumbar function, pain-related depression and sleep quality, increase total effective rate with no severe adverse side effects.

Key words: Facet joint syndrome, methylene blue, intra-articular injection, ultrasound guidance

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Low back pain is a widely prevalent health condition that is responsible for considerable suffering worldwide (1). Recent research has shown that low back pain results in a higher number of years of living with a disability than any other health condition (2). Many people with low back pain have ongoing and recurrent complaints and bear a high disease burden (3). At a societal level, low back pain is also responsible for substantial costs by way of health care expenditure, disability insurance, and work absenteeism (4). The term lumbar facet joint syndrome (LFJS) has been used to define low back pain originating from facet joints (5,6), which is a main source of chronic low back pain in approximately 15%-52% of cases (6). Trauma, degenerative arthritis, chondromalacia, and segmental instability are the common causes of facet joint pain (7), of which degenerative osteoarthritis is the most common cause (8).

As true synovial joints, facet joints are rather identical to other peripheral joints and consist of a synovial capsule, synovial membrane, hyaline cartilage, and subchondral bone (9). The joint space has a capacity of 1-2 mL (6). Similar to many other peripheral synovial joints, LFJS is induced through many mechanisms, such as capsular stretch, entrapment of synovial villi between the articular surfaces, nerve impingement by osteophytes, and the release of inflammatory substances (5,10). At present, the focus is on osteoarthritic changes that lead to LFJS. Reports have suggested that facet joint osteoarthritis is osteoarthritis with narrowing, joint space narrowing, osteophytosis, joint hypertrophy, subchondral sclerosis, and bony deformity, and is similar to traditional peripheral osteoarthritis (11-13). Numerous studies have found that multiple inflammatory cytokines, such as tumor necrosis factor-α (TNFα), interleukin-1β (IL-1β), and interleukin-6 (IL-6), as well as inflammatory mediators, such as prostaglandins, are enriched in the facet joint tissues in degenerative lumbar facet joints (14,15).

At present, the main treatments of LFJS include conservative treatment, minimally invasive interventional therapy, and surgical treatment. Among them, intra-articular injection is a minimally invasive technique, which is easy to administer and widely used in clinical practice (16). As for image-guided methods, ultrasound has been increasingly used and allows for real-time identification of anatomical structures and improves procedural success. Ultrasound-guided intra-articular injection can effectively mitigate facet joint pain with a lower incidence of undesirable complications, such as extradural hematoma, pulmonary complications, and nerve injury (17,18). In a majority of studies, the injected mixture contained a long-acting corticosteroid (either soluble or nonsoluble) and a local anesthetic (19,20). However, the quantitative analysis of the histology, pathology, and ultrastructure of articular chondrocytes, has found that corticosteroids can cause wear and tear on the surface of articular cartilage and decrease the hardness of articular cartilage, inducing chondrocyte degeneration and inhibiting its function (21). Therefore, it is important to identify safer and more effective clinical drugs for LFJS treatment.

Methylene blue (MB), an inhibitor of nitric oxide synthase and guanylate cyclase, has been widely applied for a variety of pain-related diseases due to its characteristic abilities, such as the blocking of pain transmission, antioxidant, and anti-inflammatory effects (22,23). Studies have shown that, based on its anti-oxidative and anti-inflammatory properties, MB can be used for the treatment of methemoglobinemia, onychomycosis (toenail), recurrent genital herpes simplex, esophageal cancer, septic shock, acute hepatic failure, neurodegenerative diseases, and ischemic brain injury (24-27). It has been reported that intradiscal injection of methylene blue can significantly reduce pain intensity and improve disc degeneration without obvious adverse side effects (28,29). The above-mentioned research has indicated that MB exerts good anti-inflammatory and analgesic effects and could be a potential method of treatment for LFJS. At present, no studies have been conducted on the treatment of LFJS patients using MB.

Therefore, we conducted a prospective randomized clinical trial to evaluate the efficacy and safety of ultrasound-guided intra-articular injection of MB for LFJS treatment.

Methods

General Information

This prospective randomized, single-blind study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Review Committee of Shanghai East Hospital ([2020] Pre-study No. 070). Our study was registered with the Chinese Clinical Trial Registry (registration No. ChiCTR2000034132). The patients included were recruited from among patients who received treatment at the Pain Department of Shanghai East Hospital from June 2020 to November 2020. At recruitment, written informed consent was

obtained from each patient. The pre-enrollment evaluation was performed by an experienced pain physician and included a diagnosis of LFJS pain based on physical examination and magnetic resonance imaging (MRI).

Inclusion criteria: 1) over 18 years of age; 2) persistent chronic low back pain for at least 3 months, and MRI-proven LFJ osteoarthritis and hypertrophy in the lumbar segments (30); 3) low back pain with or without radiation pain in the buttocks indicating articular process syndrome (aggravation of pain in extension or bending of the spine toward the affected side; aggravation of pain in sitting for a long time, going up the steps and maintaining a posture for a long time); 4) failure of conservative treatment (physical therapy, drug therapy, etc.); 5) informed consent to participate voluntarily provided by the patient or their family members.

Exclusion criteria:1) under 18 years of age; 2) a history of lumbar trauma or lumbar surgery; 3) abnormal lumbar imaging findings but no symptoms of low back pain; 4) low back pain caused by other diseases, such as intervertebral disc disorder, lumbar tuberculosis or tumor, radiculopathy, intraspinal diseases, radiating pain from other areas, cardiovascular diseases, and rheumatic immune system diseases; 5) systemic steroid therapy received within 1 month or intracapsular injection of steroids within 6 months; 6) pregnant or lactating women, patients with mental illness or other major diseases; 7) poor compliance or loss during follow-up.

Test Group

A computer-generated random assignment sequence was used to divide 120 patients with LFJS into 2 groups, the control group and the MB group. To ensure the secrecy of the groups, a random numeric sequence number for each order was sealed in an opaque envelope. A nurse who did not participate in the study opened the envelopes and determined the group assignment. Patients were hidden about their therapeutic schedule. Each group contained 60 patients at the beginning of the clinical trial.

Therapeutic Schedule

After the patients were admitted to hospital, routine examinations, including blood tests for coagulation, blood glucose, liver function, and kidney function were performed. All treatment procedures were performed in the operating room. Standard monitors, including electrocardiogram, noninvasive blood pressure, pulse oximetry, and heart rate, were applied. The treatment segments were marked before operation. Then, patients were placed in the prone position, and a thin pillow was put under the anterior lower abdomen. Following standard skin asepsis, a sterile surgical towel was placed on the body (Fig. 1A). A low resolution (12-16 MHz) was selected on the linear array transducer probe (S Nerve, Sonosite, Bothell, WA) and a coupling agent was applied and covered with a sterile film. The marked areas were scanned at the sagittal and coronal planes, and the ultrasound probe was fixed when the facet joints were detected. Prior to needle insertion, the skin was infiltrated with 3 mL of 1% lidocaine (production batch number C20A038, Shandong Hualu Pharmaceutical Co. Ltd.). A 22-gauge Tuohy needle (Tuoren, Xinxiang, Henan, China) was inserted into the articular cavity after being guided by ultrasound. When the puncture needle broke through the skin, the progress of the needle was observed in real time and clearly on the screen. Then, the mixture was injected into the target position (Fig. 1B).

As for mixture, for the MB group, 2 mL of 1% methylene blue (production batch number 2002123, Jichuan Pharmaceutical Group Co. Ltd.) and 5 mL of 2% lidocaine (production batch number C20A038, Shandong Hualu Pharmaceutical Co. Ltd.) was diluted with 20 mL of normal saline. Patients received approximately 1 mL of the medication cocktail. For the control group, 1 mL of diprospan (containing 5 mg of betamethasone dipropionate and 2 mg of betamethasone sodium phosphate) (production batch number 0001162870, Shanghai Schering-Plough Pharmaceutical Co. Ltd.) and 5 mL of 2% lidocaine diluted to 20 mL using normal saline. The injection dose was the same as for the MB group.

Assessment Criteria

Primary Indicators

The numeric rating scale (NRS) is an 11-point numerical scale with one extreme labelled as no pain (0) and the other extreme as worst pain imaginable (10). It is a valid and reliable scale. The patient was asked to indicate the level of pain immediately before the session and 1 week, 1 month, 3 months, and 6 months after the intervention.

Secondary Indicators

1. Lumbar function The Oswestry Disability Index (ODI) is a self-administered questionnaire containing 10 questions concerning the intensity of pain,

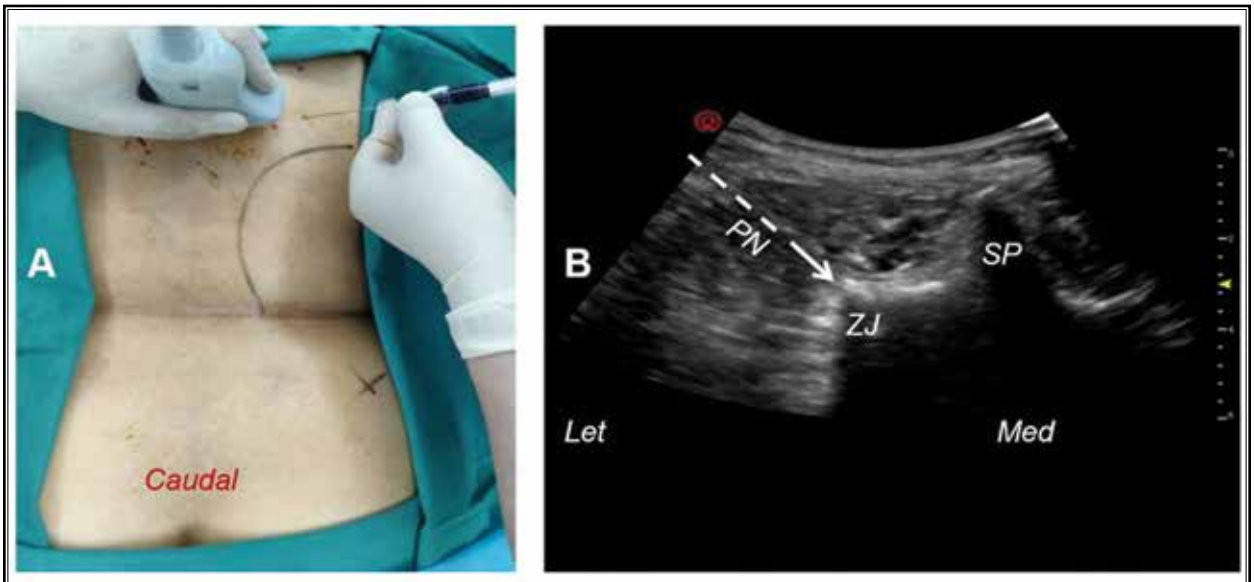

Fig. 1. *Schematic diagram of lumbar facet joint injection therapy, A) Patient was prone position, the anterior lower abdominal pad with thin pillow, then the skin was sterilized by povidone iodine and the sterile surgical towel was placed on the patient; Ultrasonic positioning on the right side of lumbar facet joint (ZJ), dotted line was puncture needle route. B) Puncture needle was injected into the lumbar facet joint, then doctor proceed to inject MB or steroids treatment.*
Abbreviations: SP, spinous process; ZJ, zygapophysial joints; PN, puncture needle.

lifting, ability to care for oneself, ability to walk, ability to sit, sexual function, ability to stand, social life, sleep quality, and ability to travel. Each item on the ODI has 6 options that each represent a score from 0 to 5. A percentage score indicates the final result: total score of the patient/total raw score possible × 100%. If the patient has failed to complete all items, the total raw score will be calculated after removing the score of the uncompleted items. ODI was assessed before operation, 1 month, 3 months, and 6 months after the intervention.

2. Sleep quality The Pittsburgh Sleep Quality Index (PSQI) is a 19-item questionnaire that assesses several aspects of sleep quality (sleep duration, disturbances, quality, efficiency, sleep onset latency, medication and daytime dysfunction). A global score of sleep quality is the sum of the various components of the questionnaire. The higher the score the worse the sleep quality. PSQI was assessed at baseline, 1 month, 3 months, and 6 months after the operation.
3. Depression assessment The Patient Health Questionnaire (PHQ-9) is a reliable and valid measure of depression severity and is scored as follows: a score of 5 to 10 points is considered to indicate mild depression; a score of 10 to 15 points, moderate depression; and a score of 15 to 20 points, moderately severe depression; a score of > 20 points indicates severe depression. Depression assessment was assessed at the same time points as the ODI and PHQ-9.
4. Postoperative analgesic usage All patients with mild and moderate pain (4 ≤ NRS scores ≤ 6) were administered celecoxib (0.2 g, qd) for pain relief, and patients who had severe pain (NRS scores > 7) were administered Tramadol (100 mg, q12h) during the first 3 days after surgery. During the follow-up period, patients who experienced serious drug-related side effects (gastrointestinal ulcers, cardiovascular problems, etc.) with oral celecoxib were replaced with tramadol. Patients were administered tramadol orally, starting with 100 mg per night and gradually increasing the dosage to avoid drug-related side effects (such as dizziness, nausea, and vomiting). After discharge, medication was administered based on the above mentioned principles of remedial drug administration. Celecoxib was not used continuously for more than 3 months. The number of patients in the 2 groups who were administered the remedial drugs were recorded at 1 week, 1 month, 3 months, and 6 months after surgery.

5. Treatment effects The effect of treatment was evaluated using the NRS score, pain symptoms, and local physical symptoms. The treatment effect was divided into 4 grades: "completely cured," "significant positive effect," "effective," and "invalid." The "completely cured" grade indicated that disease symptoms and physical signs of the disease had disappeared and good quality of life had been restored. A "significant positive effect" grade indicated that disease symptoms were significantly alleviated and the quality of life was improved, but there was still intermittent tolerable pain. An "effective" grade indicated that disease symptoms were alleviated, but the effect was not permanent and did not last for a long time. An "invalid" grade indicated that disease symptoms were not improved, and the quality of life was the same as before. The "effective rate" was estimated by the number of [(cured + significant effect + effective)/ total number] × 100%. Treatment effect was only assessed at 6 months after surgery.
6. Adverse Complications The incidence of adverse reactions (hyperglycemia, hypertension, nausea, and vomiting) in both groups was observed and recorded on the first day after operation. Liver and kidney function were monitored preoperatively and during the first 6 months after operation. Each adverse event was recorded in detail and reported in time.

Sample Size

Since there was no reference for the effectiveness of intra-articular injection with MB in LFJS patients, a preliminary trial was conducted before the formal research study began, as suggested and approved by the Institutional Review Board of Shanghai East Hospital. The preliminary trial indicated that the effective rate of treatment was 60% (6/10) in the control group and 80% (8/10) in the MB group at 1 week after operation. Therefore, the sample size calculation was based on a 60% effective rate in the control group and an 80% effective rate in the MB group. Assuming a 2-sided α = 0.05 and a statistical power of 0.8, and estimating a 10% loss to follow-up, the sample size was calculated as n = 56 for each group.

Statistical Analysis

Numerical variables are presented as the mean ± standard deviation (SD), and categorical variables are reported as numbers or percentages. The Kolmogorov-Smirnov test was used to assess the normality of measurement data. Normally distributed data were analyzed using the independent t-test and repeated measures analysis of variance (ANOVA), while non-normally distributed data were compared using the Mann-Whitney U test. Differences in count data were analyzed using the chi-square test or Fisher's exact test. A P value of < 0.05 was considered to indicate statistical significance. Statistical analysis was performed using version 22.0 SPSS software (SPSS Inc., Chicago, IL).

Results

A total of 104 patients were followed-up for a period of 6 months. The control group included 51 patients (5 patients were lost during follow-up and 4 patients exited the experiment), and the MB group included 53 patients (7 patients were lost during the follow-up period) (Fig. 2).

Preoperative Patient Characteristics

The demographic characteristics of the patients, including age, gender, body mass index (BMI), disease duration, NRS, PSQI, and PHQ-9 scores were recorded before surgery, and no significant differences were found between the 2 groups. (P > 0.05) (Table 1).

NRS Scores

There was no significant difference in the NRS scores between the 2 groups before operation. Compared with the preoperative baseline scores, the NRS scores were significantly decreased at different time points in both groups. At 1 week and 1 month after operation, the NRS scores of the MB group was slightly lower than that of the control group (P > 0.05), while at 3 months and 6 months, it had decreased significantly (P < 0.05) (Fig. 3, Table 2).

ODI Scores, PSQI Scores, and PHQ-9 Scores

There was no significant difference in the baseline ODI scores, PSQI scores, and PHQ-9 scores between the 2 groups. The ODI scores, PSQI scores, and PHQ-9 scores of the patients in both groups respective significantly decreased at each postoperative follow-up time point compared with the preoperative baseline, while the scores of the MB group were significantly lower than those of the control group at 3 months and 6 months (P < 0.05) (Fig. 4-6, Table 3).

Postoperative Analgesic Usage

Fewer patients in the MB group accepted Celecox-

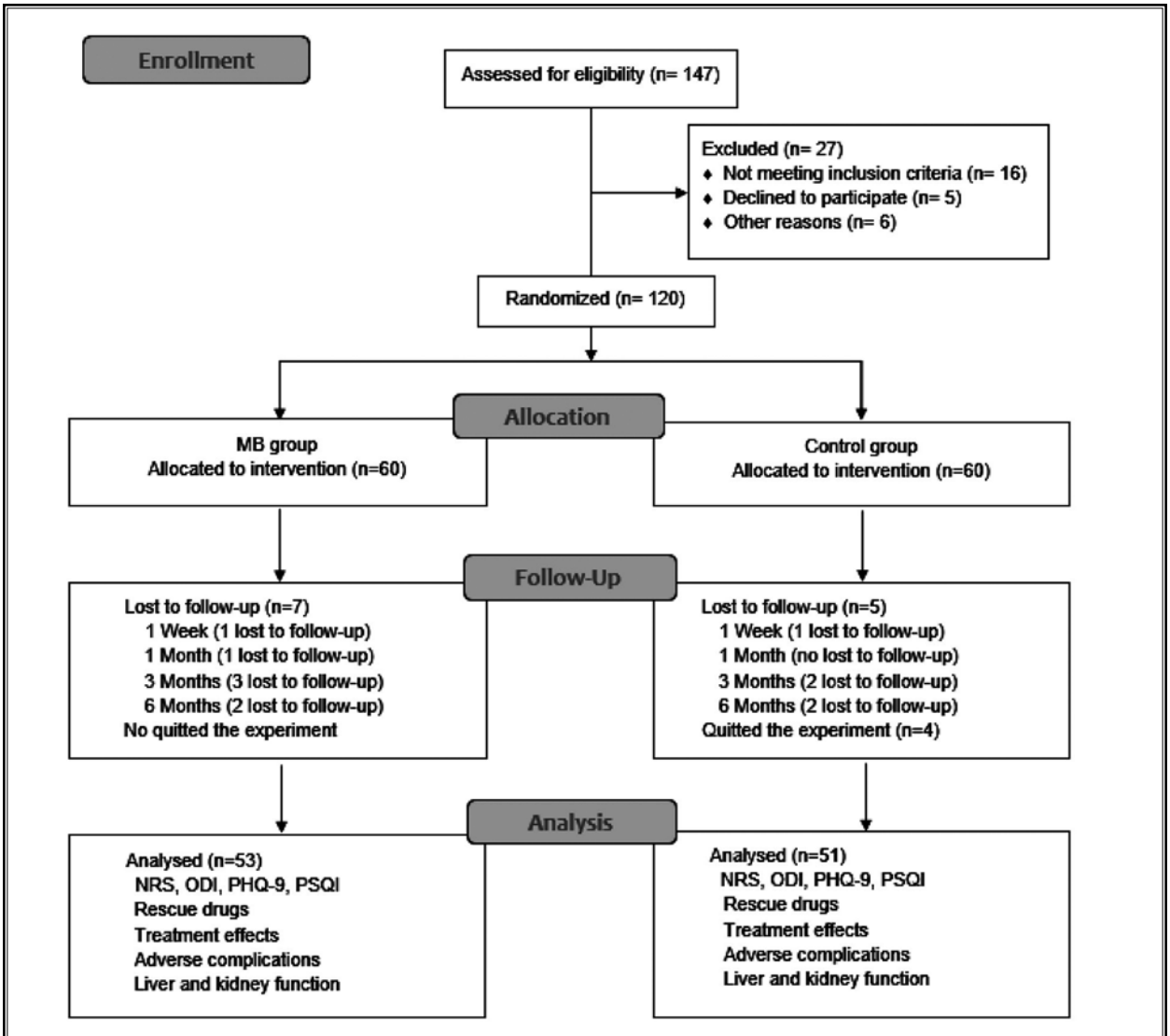

Fig. 2. *Consort flow diagram.*

ib and Tramadol for analgesia at 1 week, 1 month, 3, and 6 months after surgery, compared with the control group, but there was no statistical difference in the results. ($P > 0.05$) (Table 4).

Treatment Effects

The total effective rate was 78.4% in the control group and 94.3% in the MB group at 6 months after surgery. There was a statistical difference between the 2 groups ($P < 0.05$) (Table 5).

Adverse Complications

On the first day after operation, the incidence of hyperglycemia in patients with diabetes in the MB group was significantly lower than that of the control group ($P < 0.05$). There were no significant differences in the incidence of other adverse reactions (hypertension, nausea, and vomiting) between the 2 groups ($P > 0.05$). At 6 months after operation, no abnormal liver or kidney function was reported in either of the 2 groups (Table 6).

Discussion

LFJS is one of the most common causes of chronic low back pain and is highly prevalent. LFJS affects patients from all walks of life and affects the elderly in

Table 1. *General preoperative characteristics of enrolled patients (mean ± SD).*

	MB group (n = 53)	Control group (n = 51)	*P* value
Age (years)	62.4 ± 12.4	64.2 ± 11.4	0.673
BMI (cm2/kg)	24.3 ± 3.4	23.2 ± 2.4	0.778
Gender (M/F)	20/33	22/29	0.213
Duration of pain (months)	10.6 ± 5.5	11.1 ± 5.1	0.157
Preoperative symptoms			
NRS score	7.8 ± 2.4	8.1 ± 2.6	0.824
ODI score	63.5 ± 15.8	66.4 ± 12.7	0.966
PHQ-9 score	17.1 ± 3.1	18.5 ± 3.0	0.076
PSQI score	16.0 ± 4.4	15.4 ± 5.4	0.657
Liver function (abnormal/normal)	0/53	0/51	
Renal function (abnormal/normal)	0/53	0/51	

BMI, body mass index; NRS, Numeric Rating Scale; ODI, Oswestry Disability Index;
PHQ-9, Patient Health Questionnaire 9; PSQI, Pittsburgh Sleep Quality Index.

Table 2. *Comparison of NRS scores between the 2 groups (mean ± SD).*

	MB group (n = 53)	Control group (n = 51)	*P* value
Pre-operation	7.8 ± 2.4	8.1 ± 2.6	0.824
Post-operation			
1 Week	2.2 ± 0.8	2.7 ± 1.9	0.089
1 Month	1.9 ± 1.2	2.4 ± 1.9	0.456
3 Months	2.7 ± 0.7 [a]	3.4 ± 1.6	0.003
6 Months	2.6 ± 1.0 [b]	3.8 ± 1.7	< 0.001

[a] $P < 0.05$ versus the control group, [b] $P < 0.001$ versus the control group

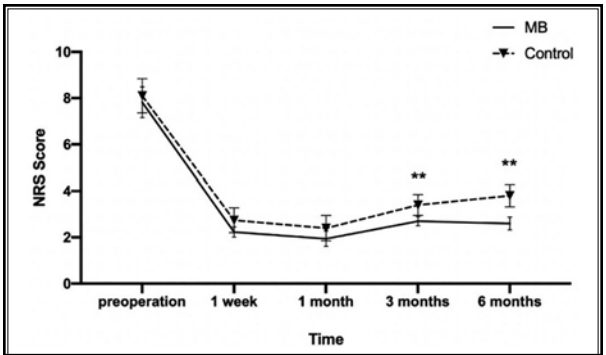

Fig. 3. *NRS scores at different times of the 2 groups. (** P < 0.001 versus the control group)*

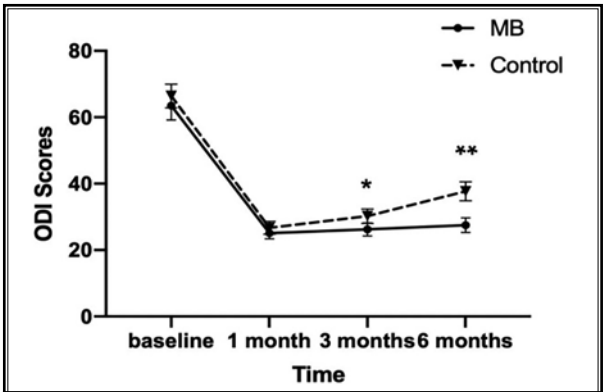

Fig. 4. *ODI scores at different times of the 2 groups. (** P < 0.001 versus the control group, * P < 0.05 versus the control group)*

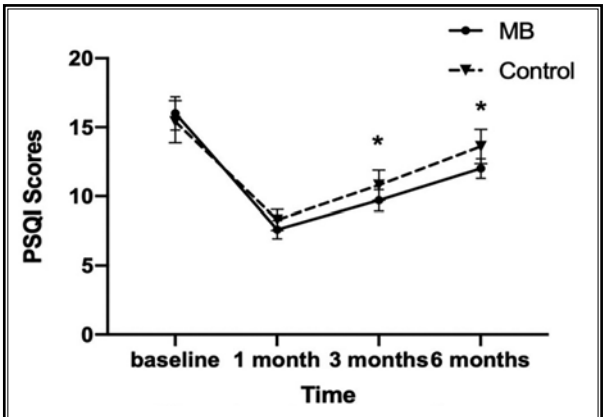

Fig. 5. *PSQI scores at different times of the 2 groups. (* P < 0.05 versus the control group)*

particular (31,32). Along with escalating health-care costs, LFJS frequently results in a significant physical and psychological impairment and a decline in the performance of social responsibilities, including work and involvement with family (31).

Lumbar facet joints are movable synovial joints formed by adjacent superior and inferior articular processes of the vertebral arch. The medial branch of the posterior ramus of the spinal nerve runs caudally around the base of the superior articular process of the vertebral body subsequent to the facet joint and continues in the groove between the superior articular process and the transverse process, finally branching off to innervate the adjacent upper and lower facet joints, and is widely distributed in the facet joint capsule and synovium in the form of nerve endings. Therefore, the facet joints of the lumbar spine are innervated by at

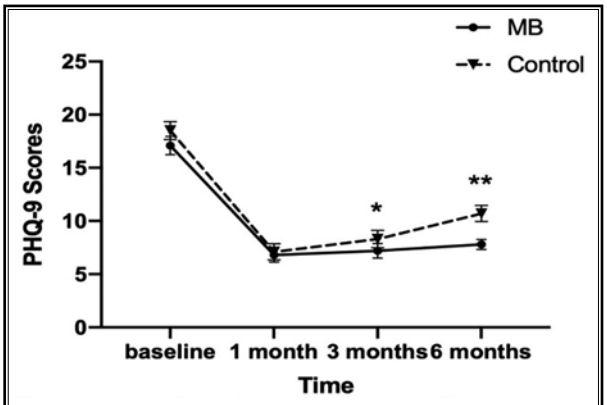

Fig. 6. *PHQ-9 scores at different times of the 2 groups. (** $P < 0.001$ versus the control group, * $P < 0.05$ versus the control group)*

Table 3. *ODI, PHQ-9, and PSQI scores of the 2 groups (mean ± SD).*

	MB group (n = 53)	Control group (n = 51)	*P* value
ODI			
Pre-operation	63.5 ± 15.8	66.4 ± 12.7	0.966
Post-operation 1 month	25.1 ± 6.3	26.7 ± 6.8	0.432
Post-operation 3 months	26.2 ± 7.1 [a]	30.2 ± 7.6	0.038
Post-operation 6 months	27.5 ± 7.9 [b]	37.7 ± 10.1	< 0.001
PHQ-9			
Pre-operation	17.1 ± 3.1	18.5 ± 3.0	0.076
Post-operation 1 month	6.8 ± 2.4	7.1 ± 2.7	0.182
Post-operation 3 months	7.2 ± 2.5 [a]	8.3 ± 2.9	0.040
Post-operation 6 months	7.8 ± 1.7 [b]	10.7 ± 2.7	< 0.001
PSQI			
Pre-operation	16.0 ± 4.4	15.4 ± 5.4	0.657
Post-operation 1 month	7.6 ± 2.4	8.3 ± 2.7	0.127
Post-operation 3 months	9.7 ± 2.8 [a]	10.8 ± 3.9	0.049
Post-operation 6 months	12.0 ± 2.6 [a]	13.6 ± 4.4	0.015

[a] $P < 0.05$ versus the control group, [b] $P < 0.001$ versus the control group

Table 4. *Remedial drugs usage assessment in patients.*

	MB group (n = 53)	Control group (n = 51)	*P* value
Numbers of patients taking remedial drugs (n)			
Celecoxib			
1 Week	3	3	0.961
1 Month	4	6	0.466
3 Months	5	7	0.493
6 Months	5	8	0.335
Tramadol			
1 Week	0	0	
1 Month	0	0	
3 Months	1	3	0.289
6 Months	1	3	0.289

Table 5. *Efficacy assessment in patients.*

	MB group (n = 53)	Control group (n= 51)	*P* value
Efficacy (n)			0.024*
Heal	15	9	
Excellent	23	18	
Effectively	13	13	
Ineffectively	3	11	

*$P < 0.05$ versus the control group

Table 6. *Comparison of adverse complications between the 2 groups.*

	MB group (n = 53)	Control group (n = 51)	*P* value
One day Post-operation			
Adverse reactions (n)			
Hyperglycemia	1/14	13/13	< 0.001*
Hypertension	2	1	0.581
Dizziness	3	5	0.428
Nausea	1	2	0.535
During 6 months Post-operation			
Liver function (abnormal/ normal)	0/53	0/51	
Renal function (abnormal/ normal)	0/53	0/51	

*$P < 0.001$ versus the control group

least 2 medial branches of the posterior rami of spinal nerves (33), with an abundance of segmental anastomosis and variation among the nerve branches of the facet joints, which form the complex pathogenesis of lumbar and leg pain (34). Usually, pain increases along with the intensity of stress, exercise, extension of the spine, and rotational motions (35). Unfortunately, this

region is highly sensitive to pain, and the level of pain is intensified due to dense innervation (33,36). Although various methods of treatment are available for LFJS, they are not fully effective in relieving LFJS. Researchers believe that intra-articular injections are more suitable as nerve blocks for LFJS.

MB exerts antioxidant, anti-inflammatory, neuroprotective, and mitochondria protective effects and has been widely used for the treatment of methemoglobinemia, malaria, cyanide poisoning, septicopyemia, Alzheimer's disease, and other diseases (26,27,37-39). Previously, MB has been used for the treatment of various other pain conditions through different routes of administration. When administered intradiscally, MB attenuated chronic discogenic low back pain (40). Pretreatment with intravenous MB diminished the effective propofol dose required during anesthesia and decreased the level of pain on injection with propofol (41). Perianal injection of MB administrated intradermally was useful in reducing postoperative pain after open hemorrhoidectomy and postoperative pain after sphincterotomy (23). Oral rinse of MB is an effective and safe treatment for refractory pain resulting from oral mucositis related to cancer treatment (42). Our latest findings suggest that continuous thoracic paravertebral infusion with MB induces significant effects on postherpetic neuralgia (PHN)(43). The evidence mentioned above indicates that MB is a safe and effective analgesic for the treatment of various painful conditions. In this study, we comprehensively assessed the analgesic effects of intra-articular injection of MB in patients with LFJS. We found that the patients' NRS scores in both groups were significantly lower during the whole follow-up period than that of the baseline. Moreover, NRS scores in the MB group were significantly lower than that of the control group at 3 months to 6 months after surgery. We also found that intra-articular injection of MB could effectively improve lumbar function (ODI scores, Table 3, Fig. 4) and decrease the number of patients requiring oral rescue drugs (Celecoxib and Tramadol). And as for its clinical efficacy, the effective rate in the MB group was significantly higher than that of the control group (94.3% vs 78.4%). (Table 5)

Several mechanisms may be related to the neuropathic pain and neuroinflammation relieving effects in LFJS induced by MB. First, the mechanisms of neuropathic pain may involve the activation of NO- and cGMP-dependent signaling pathways in the spinal cord (44,45). There is evidence that both the central and peripheral nervous systems are involved in nociceptive processing. The release of NO is apparently required for the maintenance of hyperexcitability, as high doses of NO-donors can cause hyperalgesia (46). However, MB exerts direct inhibitory effects on NO synthase (NOS), which are both constitutive and inducible, and blocks the accumulation of cyclic guanosine monophosphate (cGMP) by inhibiting the enzyme guanylate cyclase (42,47). In addition, various studies have demonstrated that 5-hydroxytryptamine (5-HT) is present in central and peripheral serotonergic neurons and that it is released from platelets and mast cells after tissue injury, exerting analgesic effects depending on the site of action and the receptor subtype. MB is a potent reversible inhibitor of monoamine oxidase A (MAO-A) and thus influences 5-HT expression levels. Based on these properties, MB was found to effectively block these pathways and exert anti-nociceptive effects (48,49). In addition, activation of glial cells, such as microglia and astrocytes, leads to the production of neuroinflammation, the latter of which plays a crucial role in the induction and maintenance of pathological neuralgia. Activated glial cells release proinflammatory cytokines, such as TNF-α, IL-1β, and chemokines, to stimulate and sensitize spinal cord nociceptive neurons, while MB has been reported to exert anti-inflammatory effects on a series of disease models (50). Zhao et al (51) found that a single thoracic paravertebral injection of MB could notably inhibit the generation of plasma IL-6, TNF-α, and cortisol in PHN patients, indicating that the anti-inflammatory properties of MB may be an explanation for its analgesic effect. Furthermore, MB treatment could mitigate the genesis of neuropathic pain by attenuating the activation of canonical inflammasomes (52). It was reported that MB attenuated the activation of canonical inflammasomes, such as NLRP3, NLRC4, and AIM2, as well as the activation of non-canonical inflammasomes (53). The anti-inflammasome properties of MB were further confirmed using mice models (52). MB inhibited upstream signals, such as inflammasome assembly, phagocytosis, and the gene expression of inflammasome components via the inhibition of NF-κB signaling (52). Recently, studies have confirmed that mitochondrial dysfunction plays a central role in the formation of neuropathic pain, neuroinflammation, and oxidative stress (54); ample evidence has suggested that mitochondria are a promising target for the development of neuroprotection (55). Importantly, MB could compete with molecular oxygen for the transfer of electrons by xanthine oxidase, and inhibit the formation of free oxygen radicals and superoxides on mitochondrial

intima (56,57), thus achieving anti-inflammatory and analgesic effects. All of these antioxidant and anti-inflammatory properties may be responsible for the effectiveness of MB for LFJS.

Patients with chronic pain are more likely to develop insomnia, anxiety, and depression and chronic pain may also have a negative impact on a patients' quality of life. Some studies have shown that LFJS patients often perform abnormal activity in brain regions associated with anxiety and depression, such as the limbic system and frontal lobe (58,59). Therefore, we explored the effect of MB on sleep quality and depression status of LFJS patients. Our results manifested that LFJS patients at 6 months after operation in both groups showed significantly lower PSQI and PHQ-9 scores than those at baseline. In addition, the scores of patients in the MB group were significantly lower than that in the control group.

In this study, we also found that steroids can cause changes in blood glucose levels. On the first day post-operation, the number of diabetic patients with hyperglycemia was significantly higher in the control group than in the MB group, suggesting that methylene blue may be a better choice for LFJS patients.

Limitations

There are also several limitations in our study. Firstly, the patients enrolled were recruited from a single center, and the sample size was small. Secondly, the patients were only followed-up for a period of 6 months after treatment. Thirdly, double blinding was not used in the design of this research study.

Conclusions

In conclusion, ultrasound-guided intra-articular MB injection is a novel, safe, and effective method of therapy for LFJS. Intra-articular injection of MB can significantly reduce pain intensity, improve patient lumbar function, pain-related depression and sleep quality, and increase the total effective rate with no severe adverse side effects.

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Authorship Contributions

Xiuqin Yu, Jinyuan Zhang contribute to study design, analysis, and manuscript drafting. Mingxia Wang, Yu Yang contribute to data analysis and manuscript editing. Wei Zhang, Dan Su contribute to patient follow-up, data collection. Lijun Liao, Hao Yao contribute to study concept, design. Hongwei Fang, Xiangrui Wang contribute to study concept, design, analysis, and manuscript editing.

Authorship

All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Compliance with Ethics Guidelines

This study was approved by the Institutional Review Board and Ethics Committee of Shanghai East Hospital ([2020] Pre-study No. 070). The present clinical research was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients before inclusion.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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