Prospective Trial

Efficacy of Medical Ozone for Treatment of Chronic Musculoskeletal Pain with Abnormal Mitochondrial Redox State: Prospective Randomized Clinical Trial

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Background: Chronic primary musculoskeletal pain is multifaceted and 20% of the adult population lives with severe chronic pain and experience symptoms such as intense pain, depression, weakness, sleep problems, decreased quality of life and decreased emotional well-being.

Objectives: This paper studies the efficacy of trigger point injections with ozone compared to standard steroid injection or combination therapy for the treatment of chronic musculoskeletal pain in patients with abnormal mitochondrial redox state.

Study Design: This is a prospective randomized clinical study conducted with 51 patients experiencing chronic musculoskeletal pain.

Setting: Medical Research Institute Hospital, Alexandria University.

Methods: By computer-generated random numbers the 51 patients were divided into 3 groups. Group A (17 patients) received ozone injection, group B (17 patients) received betamethasone injection and group C (17 patients) received combined ozone and betamethasone injections. The groups were compared based on the intensity of pain and correction of mitochondrial redox state of the patients.

Results: Three days after intervention, the visual analog scale (VAS) scores reported by patients were lower in group A compared to group B (with a mean difference 1.27, 95% confidence interval (CI) of 0.15-2.39 ($P < 0.02$). One and 3 weeks after intervention, VAS scores of patients were lower in groups A and C compared to group B. At one week the mean difference between A and B was 1.2, with a 95% CI of 0.15-2.25 ($P < 0.02$) and the mean difference between C and B was 1.73 with a 95% CI of 0.69-2.78 ($P < 0.001$). At 3 weeks the mean difference between A and B was 1.5 with a 95% CI of 0.2-2.87 ($P < 0.01$) and the mean difference between C and B was 2.27 with a 95% CI of 0.93-3.60 ($P < 0.0001$). The reduced/oxidized glutathione ratio after intervention was higher in groups A and C compared to group B ($P > 0.008$). The mitochondrial copy number was higher in group A compared to group B ($P < 0.002$).

Limitation: This study didn't allow for the comparison of the experimental groups with a placebo or control group for musculoskeletal pain conditions in orderto establish the role of an abnormal mitochondrial redox state on the pathogenesis of patients from an ethical view.

Conclusions: Ozone therapy or combined ozone and betamethasone treatment are effective techniques for management of pain since it produced a significant reduction of muscle pain and increase of the pain free interval experienced by patients. Ozone therapy causes pain improvement which increases with time and it improves muscle oxygenation and mitochondrial function.

Trial Registration: This study was approved by the Ethics Committee of Medical Research Institute (IORH: IOR 00088812) and was registered at the Pan African Clinical Trial Registry (www.pactr.org) under the identification number PACTR201908620943471. The registration this

experiment started on 07/08/2019. This study's protocol followed the CONSORT guidelines and was performed under the relevant guidelines.

Key words: Ozone, steroid, fibromyalgia, trigger point, redox state, antioxidant, chronic pain

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Chronic primary musculoskeletal pain is a pain that lasts for 3 to 6 months or beyond the time of normal healing. Musculoskeletal disorders are the most common source of chronic musculoskeletal pain. Myofascial trigger points (MTrPs) are loci of hypersensitivity within a tender, taut, palpable band of muscle (1). Paresthesia, muscle weakness without primary atrophy, restricted mobility, proprioceptive disorders with impaired coordination, and autonomic reactions can all be caused by MTrPs (2). Due to the increasing prevalence of MTrPs, there is a need for non-surgical and effective solutions such as physical therapy, pharmacologic treatment, and injection-based therapy. Currently, MTrPs can be treated noninvasively by the spray and stretch technique, transcutaneous electrical stimulation, physical therapy, and massage. Invasive treatments for MTrPs include injections with local anesthetics with or without corticosteroids, botulinum toxin and dry needling. The mechanism of action of the trigger point injection (TPI) is thought to the disruption of trigger points by the mechanical effect of the needle or the chemical effect of the agents injected, which results in relaxation and lengthening of the muscle fiber. The effect of the injectate may include local vasodilation, dilution, and removal of nociceptive substrates. Injection therapy can be used when the pain or functional impairments caused by MTrPs persist despite oral medication or exercise. Corticosteroid injections are the most often used treatment for musculoskeletal diseases, however, they only give temporary symptom relief while raising the risk of tissue atrophy. As a result, physicians have developed an interest in alternative injectants, such as ozone injections (3).

Ozone (O_3) gas was discovered during the mid-1800s. It is a molecule composed of 3 oxygen atoms in a dynamically unstable arrangement. Ozone therapy is an alternative medical treatment that introduces ozone or ozonide's into the body. Ozone therapy isn't considered a viable treatment option by certain government-regulating agencies, including the U.S. Food and Drug Administration (FDA), which prohibits the application of ozone therapy (4). Currently, there are only 14 countries where ozone therapy has been regularized, either throughout the country or in some parts of it. More research is needed to better understand the uses and benefits, as well as potential risks of this therapy.

The FDA prohibits the use of O_3 "in any medical condition for which there is no proof of safety and effectiveness", stating that "ozone is a toxic gas with no known useful medical application in specific, adjunctive, or preventive therapy. In order for ozone to be effective as a germicide, it must be present in a concentration far greater than that which can be safely tolerated by man and animals"(5).

Ozone is considered therapeutic because of its anti-inflammatory, antioxidant, and analgesic effect. Ozone activates cellular metabolism, reduces prostaglandin synthesis, and reduces oxidative stress by inducing the synthesis of antioxidant enzymes (superoxide dismutase, glutathione peroxidase, and catalase). Additionally, O_3 leads to the amelioration of the tissue oxygen supply through hemorheological action, vasodilatation, and angiogenesis stimulation (5,6). There are no reported allergic side effects or destructive adverse effects on tendons or cartilage from the use of O_3 (O_2-O_3). Thus, O_3 is an effective therapy that can be used to treat diabetes mellitus, hypertension and gastritis (7). On the other hand, steroids have an anti-inflammatory action by limiting capillary dilatation and permeability, which restricts polymorphs and macrophages from accumulating and inhibits release of vasoactive kinins (8).

Mitochondrial content determines the aerobic capacity of a muscle and patients experiencing chronic musculoskeletal pain have abnormal mitochondrial content. This can cause them to have low levels of adenosine triphosphate (ATP), which propagates muscle contracture. Low ATP levels result in compressed capillary circulation which can produce a hypoxic environment (9). The majority of patients with confirmed mitochondrial oxidative defects present with raised blood lactate levels which is associated with an elevated lactate/pyruvate (L/P) ratio, which indicates a change in the cellular redox state of the patient's body (10). Lactate facilitates the response of acid-sensing ion channels (ASIC-3) to low pH. Lactate exposure also leads to reactive oxygen species (ROS) generation, which directly interact with the nociceptive system (11,12). This study aims to de-

termine the efficacy of TPI with medical O_3 compared to standard steroid injections or combination therapy for the treatment of chronic musculoskeletal pain in patients with abnormal mitochondrial redox states. The primary outcome of this study is effect of the TPI with O_3 versus steroids, versus both O_3 and steroids on pain measured by the visual analog scale (VAS) and the secondary outcome is to study the efficacy of medical O_3 by observing the reduced/oxidized glutathione ratio (as a marker of plasma redox status) and mitochondrial copy number (as a marker of mitochondrial biogenesis).

Methods

Ethics and Registration

Informed and written consent was obtained from all patients prior to their inclusion in the study, according to the ethical code (IORH: IOR00088812). This study was registered at the Pan African Clinical Trial Registry (www.pactr.org) on July 8th, 2019, with the identification number PACTR201908620943471. This study was performed and this manuscript was prepared in accordance with the Consolidated Standards of Reporting Trials (CONSORT) 2010 statement guidelines (13).

Study Design

This prospective, clinical, randomized study was carried out at the Anesthesia and Pain Management Department, Alexandria Medical Research Institute, from January 2019 to January 2021. In this study, 51 patients underwent ozone treatment or non-O_3 treatments (with 17 patients per group) in a pain clinic and the resulting average difference in their VAS score was compared.

Patient Inclusion and Exclusion Criteria

The study included patients of both genders between 20-60 years, who had been experiencing chronic musculoskeletal pain for more than 6 months. This study enrolled patients who experienced pain with 4–8 trigger points, reported a VAS higher than 4, and had a serum lactate/pyruvate ratio higher than 10/1 (14). Patients with bleeding tendency, glucose-6-phosphate deficiency, a skin infection at the site of injection, systemic infection, hypersensitivity to the used medication, diabetes, or recent history of steroid therapy in the last three months were excluded from the study.

Randomization and Blinding

All patients were randomly divided into 3 groups using computer-generated random numbers and all patients were blind to the regrade injection substances they received:

Group A: (n = 17) received a trigger point injection of 5 mL of 12 µg/mL O_3 from the EXT50 O_3 generator (Longevity resources) for each point.

Group B: Control group (n = 17) received a trigger point injection of 0.5 mg betamethasone injectable suspension diluted in 2 mL sterile water for each point.

Group C: (n = 17) received a trigger point injection of 5 mL of 12 µg/mL O_3 from the EXT50 O_3 generator and 0.5 mg of betamethasone injectable suspension diluted in 2 mL sterile water for each point.

Procedure

All the injections were performed by the main researcher as follows:

An intravenous line was inserted into the patient and then they were positioned either in a sitting position with their head on the table (for intervention at the patient's neck) or in the prone position (for intervention at the patient's back). The site/s of injection was then demarcated.

After scrubbing with alcohol, the trigger point was stabilized by pinching between the thumb and index finger to prevent the trigger point from rolling away from the advancing needle. Under sterile condition, a needle (22G,1-2 inch) was inserted 1–2 cm away from the trigger point at an angle of 30° to the skin. When the needle contacted the trigger point muscular twitch was felt. After aspiration to ensure that the needle is not in a blood vessel, the solution was injected directly into the trigger point in a fanning approach (Fig. 1) (15).

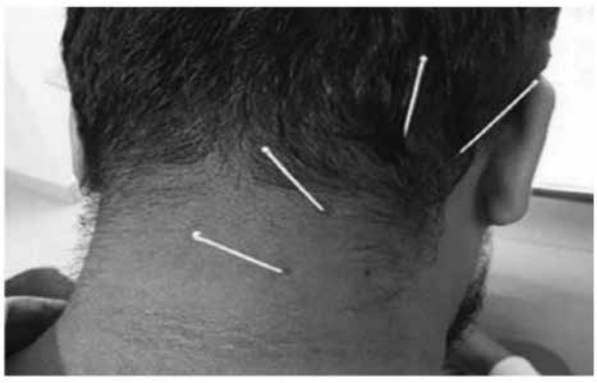

Fig. 1. *Solution injected into the trigger point.*

The O_3 used was freshly obtained from an O_3 generator (EXT50 O_3 generator, Longevity resources) (Fig. 2). The generator is composed of a high voltage tube through which medical oxygen (O_2) passes, dividing into molecules that generate ozone. The dial on the generator was pointed at 12 mic/mL during O_3 generation. A 5 mL syringe was connected to the exit of the generator to collect the gas. The syringe hub was carefully covered with a needle and was held in upright to avoid leakage.

Betamethasone was prepared by diluting 1 mL of the ampule that contains 5 mg of betamethasone as betamethasone dipropionate and 2 mg of betamethasone as betamethasone sodium phosphate in 7 mL sterile water so each mL of the prepared solution contains 1 mg of betamethasone. In a 5 mL syringe, we diluted 0.5 mL containing 0.5 mg betamethasone in 2 mL sterile water to be injected in one trigger point. For regrade combined injection, the same needle was used for the steroid injection after the O_3 injection.

After injection, the area was palpated to ensure that no other tender points existed. If additional tender points were palpable, they were isolated, needled and injected and this was done for up to 8 additional points. Pressure was then applied to the injected area for 2 minutes to promote hemostasis before it was covered with a simple adhesive bandage.

Fig. 2. *EXT50 ozone generator (Longevity resources, Canada*

Additionally, all patients were allowed analgesia in the form of acetaminophen 500 mg up to 3 times a day and were instructed to stop taking acetaminophen 48 hours before pain assessment. All patients were also taught how to use the VAS to rate their experienced pain between 0 and 10 (0 being no pain;10 being worst imaginable pain) (16). Lastly, all patients were instructed to avoid vigorous muscular activity 3 days before laboratory tests.

Outcome Measures

1- The age, gender, weight, height, body mass index (BMI), and total number of the injected trigger points were documented.
2- The patients' pain assessment by the VAS was recorded before intervention and also 3 days, one week and 3 weeks after intervention.
3- Laboratory investigations: Two blood samples were obtained; the first before intervention and the second 3 days after intervention. The blood samples were used for the following tests:
   - The isolation of peripheral mononuclear cells for the analysis of mitochondrial DNA (mtDNA) copy number (as a marker of mitochondrial biogenesis) using quantitative real-time polymerase chain reaction (qPCR).
   - The lactate present in the serum was determined using the colorimetric L lactate Assay Kit (cat.no. ab65331; Abcam) and pyruvate was assayed using colorimetric Pyruvate Assay Kit (cat.no. ab65342; Abcam) according to the manufac¬turer's protocols.
   - The plasma redox status was measured as reduced/oxidized glutathione (GSH/GSSG) ratio using the enzymatic method described by Griffith (17).

Sample Size Calculation: -

A minimal total sample size of 45 patients (15 per group) is needed to detect an average difference of VAS among the treated condition (alternative hypothesis) compared to the nontreated condition (null hypothesis) with a common estimated group SD of one and with 95% confidence level and 80% power using the one-way ANOVA test (18). We enrolled 51 eligible participants to account for dropouts and lost follow-ups.

Statistical Analysis

Data was collected and entered to the computer using the SPSS (Statistical Package for Social Science) program for statistical analysis (version 26). The data was then described by calculating the minimum, maximum, median and inter-quartile range (IQR). Additionally, the data was categorically described using frequency and percentage. The Kolmogorov-Smirnov test was used to normalize the data and box and whiskers plots were used to visualize it (19). Comparisons were carried out between 2 independent, normally distributed parameters using the one-way ANOVA test. Comparisons were carried out between two independent parameters which were not normally distributed using the Kruskal-Wallis test (20). Post-hoc pair-wise comparisons were made using the Bonferroni test to determine the nature of the differences between groups (21). Comparisons were carried out between 2 related parameters which were not normally distributed using Wilcoxon Signed Rank tests (22). An alpha level was set to 5% with a significance level of 95%, and a beta error accepted up to 20% with a power of study of 80%.

Results

Fifty-one patients in the pain clinic were selected to participate in this trial. They were then randomized and allocated to one of 3 groups. Seventeen patients received O_3 injection (group A), 17 patients received betamethasone injection (group B) and 17 patients received both O_3 and betamethasone injections (group C). Three patients, one from each group, refused the intervention, and 3 patients, one from each group, have incomplete follow-ups. The remaining 45 patients fulfilled all criteria for analysis (Fig. 3). The groups were compared based on the baseline characteristics of age, weight, height, BMI, gender, and number of injection sites and it was determined that there was no statistically significant difference among the 3 studied groups (Table 1). Additionally, there was no statistically significant difference in the VAS scores reported by the 3 studied groups before intervention ($P = 0.856$).

Three Days after Intervention

We conducted a one-way ANOVA test to compare the VAS scores reported by the different groups 3 days after the intervention. Group A reported a mean VAS score of 5.07 ±1.28, Group B reported a mean VAS score of 6.33 + 1.04, and Group C reported a mean VAS score of 5.67 + 1.34 (Table 2, Fig. 4). There was a statistically significant difference among the VAS scores reported by the 3 groups ($P = 0.02$). The Bonferroni test was used to determine the nature of the differences between the groups. This analysis revealed that the VAS score reported by group A was lower than that reported by group B with a mean difference of 1.27, and a 95% confidence interval (CI) of 0.15-2.39 ($P > 0.02$).

One Week after Intervention

We conducted a one-way ANOVA test to compare the VAS scores reported by the different groups one week after intervention. Group A reported a mean VAS score of 3.27 ± 0.88, Group B reported a mean VAS score of 4.47 + 0.99, and Group C reported a mean VAS score of 2.73 + 1.48 (Table 3, Fig. 5). There was a statistically significant difference among the VAS scores reported by the 3 groups ($P = 0.01$). The Bonferroni test revealed that the VAS scores reported by group A and C were lower than that reported by group B. Between groups A and B there was a mean difference of 1.2, and a 95% CI of 0.15-2.25 ($P > 0.02$) and between groups C and B there was a mean difference of 1.73, and a 95% CI of 0.69-2.78 ($P < 0.001$).

Three Weeks after Intervention

We conducted also a one-way ANOVA test to compare the VAS scores reported by the different groups 3 weeks after intervention. Group A reported a mean VAS score of 2.73 ± 1.43, Group B reported a mean VAS score of 4.27 + 0.96, and Group C reported a mean VAS score of 2.00 + 1.85 (Table 4, Fig. 6). There was a statistically significant difference among the VAS scores reported by the 3 groups ($P = 0.001$). The Bonferroni test revealed that the VAS scores reported by group A and C were lower than that reported by group B. Between groups A and B there was a mean difference of 1.53, and a 95% CI of 0.15-2.25 ($P < 0.01$) and between groups C and B there was a mean difference of 1.73, and a 95% CI of 0.69-2.78 ($P < 0.001$).

Mitochondrial DNA (mtDNA) Copy Number

mtDNA levels after intervention was statistically significantly higher when compared to that before intervention in group A ($P = 0.001$), and C ($P = 0.001$), but not in group B ($P = 0.118$).

Comparing the 3 groups, there was no statistically significant difference before intervention ($P = 0.924$) but was statistically significantly different after intervention ($P = 0.002$). After intervention, mtDNA levels were statistically significantly higher in group A compared to B ($P = 0.001$). However, comparing mtDNA levels after

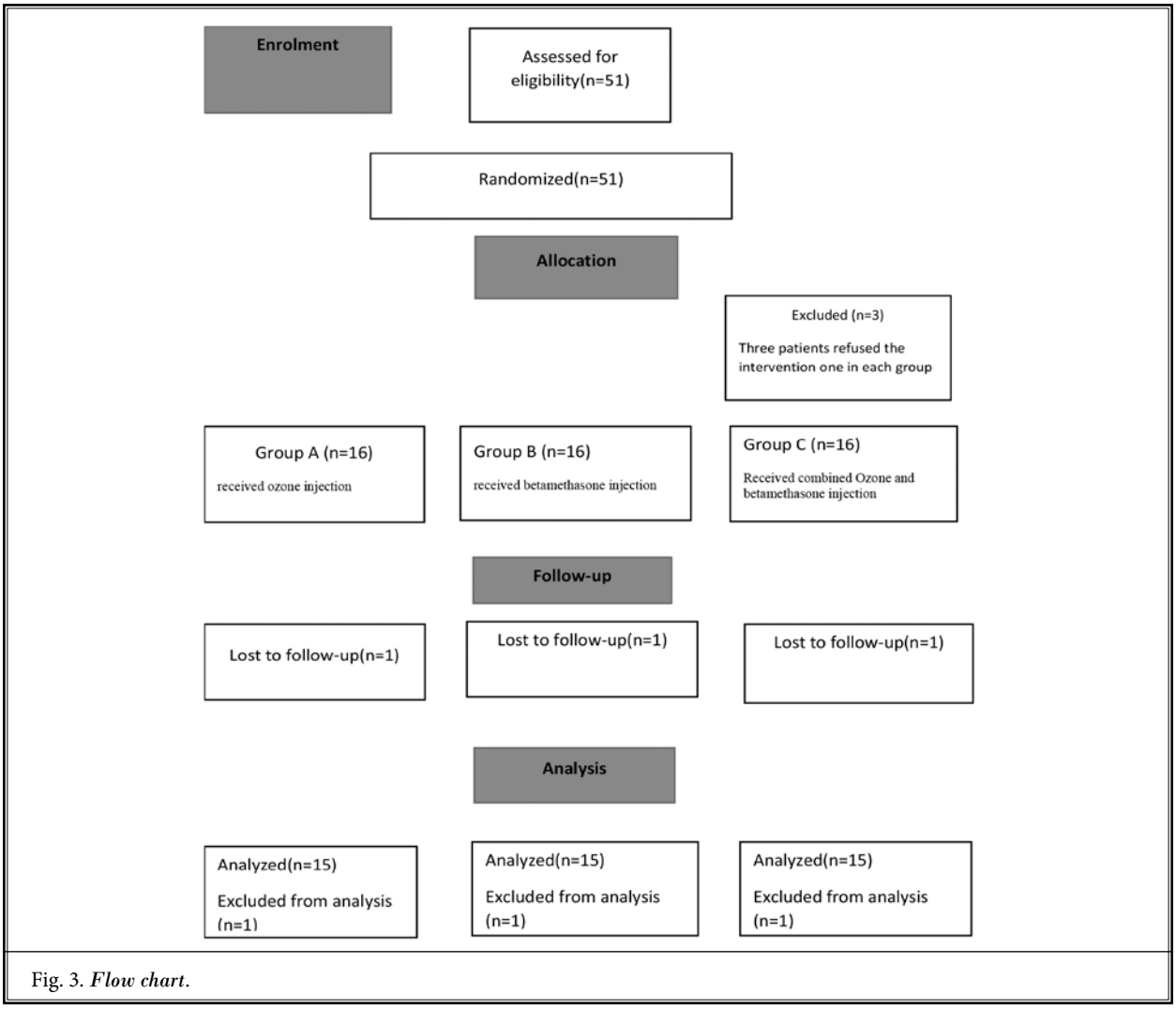

Fig. 3. *Flow chart*.

Table 1. *Baseline characteristics*.

Baseline characteristic	Group A n = 15			Group B n = 15			Group C n = 15		
	Min-max	Median	IQR	Min-max	Median	IQR	Min-max	Median	IQR
Age in years	27-60	47	37-55	30-55	42	37-48	22-60	50	38-54
Weight	60-93	79	68-84	58-87	77	75-82	56-93	77	68-82
Hight	155-182	168	162-173	158-188	173	166-178	158-188	170	160-177
BMI	21-32.90	27.60	24.80-30.70	20.80-30.48	25.39	23.66-27.94	21.90-34.60	25.39	23.66-27.94
Number of injected points	4-8	6	6-8	4-8	6	6-8	4-8	6	6-8
Gender Men (n) Women (n)		7 (46.67%) 8 (53.33%)			8 (53.33%) 7 (46.67%)			7 (46.67%) 8 (53.33%)	

n: Number of patients
Min-max: Minimum-maximum
IQR: interquartile range

Table 2. *Three days after intervention.*

Mean (SD)				
	Group A (n = 15)	Group B (n = 15)	Group C (n = 15)	*P* Value
VAS after 3 days	5.07(1.28)	6.33(1.04)	5.67(1.34)	0.02*

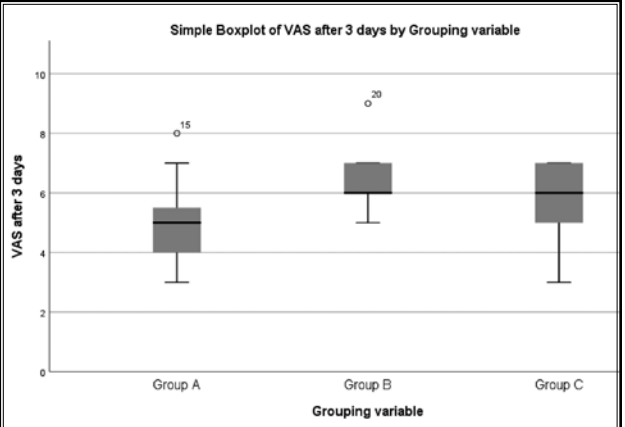

Fig. 4. *VAS after 3 days.*

intervention between group A with C, and group B with C shows no statistically significant difference (*P* = 0.336 and *P* = 0.148 respectively) (Table 5, Fig. 7).

Reduced/Oxidized Glutathione (GSH/GSSG) Ratio

The GSH/GSSG ratio 3 days after intervention was statistically significantly higher when compared to that before the intervention in groups A, B and C (*P* = 0.001, 0.002 and 0.001 respectively).

Comparing the 3 groups, there was no statistically significant difference in the GSH/GSSSG ratio between the groups before intervention (*P* = 0.863) but there was a statistically significant difference between the groups 3 days after intervention (*P* = 0.008). Three days after intervention, the GSH/GSSG ratio was statistically significantly higher in group A and C compared to group B (*P* = 0.028, 0.016 respectively). Meanwhile, there was no statistically significant different in the GSH/GSSG ratio between group A and group C three days after intervention (*P* = 1.000) (Table 6, Fig. 8).

Discussion

The rising musculoskeletal pain prevalence has prompted the search for non-surgical treatments such as physical therapy, pharmacological treatment, and injection-based treatment. Injection therapies are usually recommended when pain or functional limitations persist despite oral medication or exercise.

Table 3. *One week after intervention.*

Mean (SD)				
	Group A (n = 15)	Group B (n = 15)	Group C (n = 15)	*P* Value
VAS after one week	3.27 (0.88)	4.47 (0.99)	2.73 (1.48)	0.001*

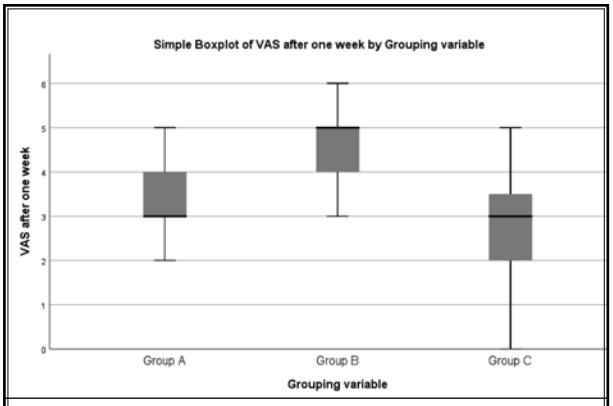

Fig. 5. *VAS after one week.*

Table 4. *Three weeks after intervention.*

Mean (SD)				
	Group A (n = 15)	Group B (n = 15)	Group C (n = 15)	*P* Value
VAS after 3 weeks	2.73 (1.43)	4.27 (0.96)	2.00 (1.85)	0.0001*

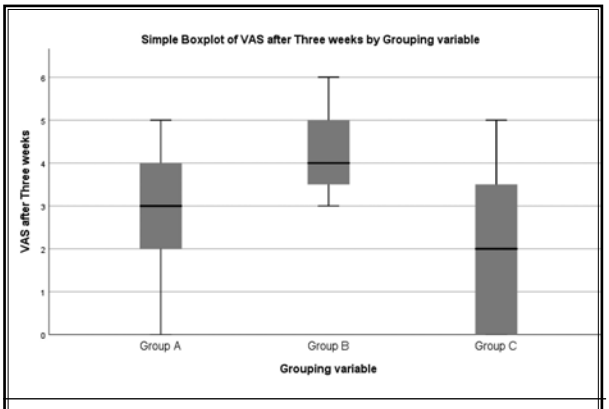

Fig. 6. *VAS after 3 weeks.*

The most frequent treatment for musculoskeletal problems is corticosteroid injections due to their anti-inflammatory effect. Additionally, a combination of oxygen and ozone gases has been used in medicine

Table 5. *mtDNA copy number before and after intervention in the three studied groups.*

	Group			Kruskal Wallis Test
	A n = 15	B n = 15	C n = 15	
mtDNA copy number (Before) - Min-max - Median IQR	 44-56 51.00 47-54	 44-56 50 47-53	 43-56 50 46-54	*P* = 0.924
mtDNA copy number (After) - Min-max - Median IQR	 51-62 57 54-60	 46-57 51 48-54	 48-60 54 51-57	*P* = 0.002*
Wilcoxon Signed Ranks Test	*P* = 0. 001*	*P* = 0.118 NS	*P* = 0.001*	
Pairwise comparison using Bonferroni test				
P1	*P* = 0.001 *			
P2	*P* = 0.336			
P3	*P* = 0.148			

n: Number of patients; Min-max: Minimum – maximum; *: Statistically significant ($P < 0.05$); NS: Statistically not significant ($P > 0.05$); IQR: interquartile range; P1: Group (A) against group (B); P1: Group (A) against group (C); P1: Group (B) against group (C)

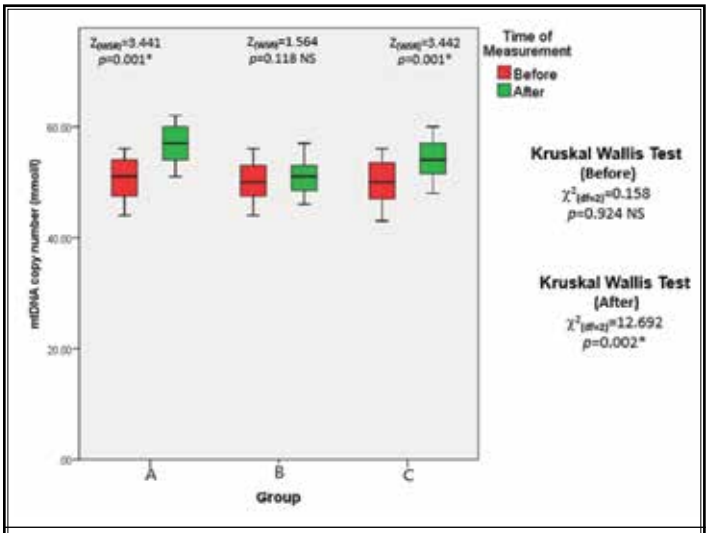

Fig. 7. *mtDNA copy number before and after intervention in the 3 studied groups.*

since the 1930s, and they have recently been utilized to alleviate pain. While ozone therapy is not FDA approved as a medical treatment, more research is needed to better understand the uses and benefits, as well as potential risks to this therapy.

Recently, researchers have discovered that the brief, calculated oxidative stress caused by ozone therapy may correct permanent imbalances induced by persistent or severe oxidative injury. It has become evident that modest, repeated ozone treatments boost the activity of enzymes like superoxide dismutase, catalase, and glutathione peroxidase, producing a state of oxidative stress adaptation with major benefits. When injected into trigger points, O_3-O_2 expands and disrupts the tissue and fascia, perhaps correcting the trigger point pathology.

In the present study, before intervention, there was no statistically significant difference in VAS score among the 3 studied groups (P = 0.856). 3 days after intervention, the VAS scores reported by patients injected with O_3 were statistically significantly lower than those reported by patients injected with betamethasone. On the other hand, there was no statistically significant difference in the VAS scores of patients injected with O_3 or betamethasone compared to patients injected with both ozone and betamethasone.

However, one week and 3 weeks after intervention, patients injected with O_3 and patients injected with both O_3 and betamethasone reported statistically significantly lower VAS scores when compared to patients injected only with betamethasone. Moreover, there was no statistically significant difference in the VAS scores reported by patients injected with only O_3 and patients injected with both O_3 and betamethasone. These results might be due to the delayed action of steroids, like betamethasone, since their anti-inflammatory effect takes longer to be elicited as it involves the active moiety of the steroid entering cells and combining with receptor protein annexin-1 to alter messenger RNA production (23). Since

Table 6. *GSH/GSSG ratio before and after intervention in the 3 studied groups.*

	Group			Kruskal Wallis Test
	A n = 15	B n = 15	C n = 15	
GSH GSSG Ratio (Before) - Min-max - Median IQR	 20.21-23.37 21.32 20.47-22	 18.41-24.29 21.74 19.69-22.99	 18.09-24.71 21.35 20.32-23.60	*P* = 0.863 NS
GSH GSSG Ratio (After) - Min-max - Median IQR	 22.67-26.86 24.32 23.26-25.67	 19.39-25.86 23.20 21.47-23.70	 21.43-26.90 24.42 23.60-25.43	*P* = 0.008 *
Wilcoxon Signed Ranks Test	*P* = 0.001*	*P* = 0.002*	*P* = 0.001*	
Pairwise comparison using Bonferroni test				
P1	*P* = 0.028 *			
P2	*P* = 1.000			
P3	*P* = 0.016 *			

n: Number of patients; Min-max: Minimum-maximum; WSR: Wilcoxon Signed Ranks Test; IQR: interquartile range

there was no statistical significance to the difference between VAS scores reported by patients who received only the ozone injection compared to patients who received both the O_3 and the steroid injection, it can be assumed that the action of these therapies is not synergistic in nature.

The literature is scarce in comparing the effects of O_3 with steroids treatments of the myofascial pain syndrome, but several studies have compared them in different clinical situations (7,24).

In a 2019 retrospective cohort study of Ulusoy et al, treated patients with chronic lateral epicondylitis by injecting them with either O_3 or corticosteroid (24). They found that before the injection procedure, there was no difference in the pain scores reported by patients in both groups. Assessment of pain scores just after the injections demonstrated that patients in the 2 groups reported similar pain scores. Interestingly, analysis of pain on the 3, 6, and 9 months following the injections demonstrated that patients who received O_3 injections reported significantly lower pain scores. This study used a corticosteroid dosage of 1 mL of betamethasone dipropionate (6.43 mg) and 1 mL betamethasone sodium phosphate (2.63 mg) and they used an O_3 dosage of 30 µg/mL. Thus, the corticosteroid dosage, O_3 dosage and pathology in this study from Ulusoy et al are different from those of the current study.

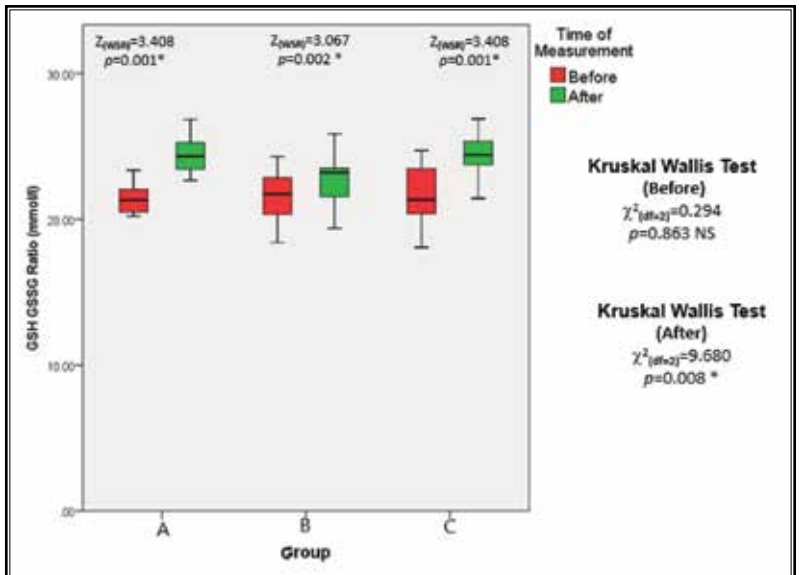

Fig. 8. *GSH/GSSG ratio before and after intervention in the 3 studied groups.*

Contrary to the results of this study, O_3 treatment did not have any benefits compared to classical treatment with steroids and local anesthetic in another study by Babaei-Ghazani et al in 2019 (8). In the study, patients were injected with either a mixture of triamcinolone 40 mg/mL with 2 mL of 1% lidocaine or they were injected with 8 mL of O_3 of 12 µg/mL and 2 mL of 1% lidocaine. After patients were injected in the sub acromial bursa in shoulder impingement under ultrasound guidance, the study recorded the patients' reported VAS score and dis-

ability scores related to the condition. They observed that the patients who received the steroid injection reported greater pain improvement (the mean improvement was 4.47 points on the VAS in comparison with the 2.87 point improvement of the O_3 group). Although the results might seem to discourage ozone treatment, the short-term pain relief and the potential side effects of steroids are yet to be considered. Additionally, this study was designed to assess the efficacy of a single O_3 injection instead of multiple injections like this study.

In the current study, patients who received local injection of trigger points with 0.5 mg of betamethasone reported a significant reduction in their VAS scores after 3 weeks of the intervention, with a median percent reduction of 50%. This is due to the anti-inflammatory action of the corticosteroids predominately affecting the cytokines, through which it inhibits the cellular mediated immunity and decreases the inflammatory cell accumulation and vascular response (25). There are different theories regarding the mechanism of action of corticosteroids as an adjuvant to local anesthetics in treatment of chronic pain syndromes. Steroids induce a degree of vasoconstriction, so one theory is that the drug acts by reducing local anesthetic absorption. A more attractive theory holds that corticosteroids increase the activity of inhibitory potassium channels on nociceptive C-fibers (via glucocorticoid receptors), thus decreasing their activity (26).

In the present study, patients who received both the ozone and betamethasone injection demonstrated a significant median percent decrease of 75% in the VAS scores they reported 3 weeks after intervention. This decrease is probably due to the synergistic action of both ozone and steroids.

Changes in mitochondrial physiology can occur following stress or corticosteroid treatment (27). Time and dosage-dependent effects on mitochondrial oxidation have been reported, with an acute or low to moderate dosage increasing mitochondrial oxidation and a long-term or high-dose decreasing mitochondrial oxidation. In vivo studies found that corticosteroid treatment decreases mitochondrial DNA copy number (28).

Interestingly when ozone and steroid treatment were combined mtDNA levels of patients increased, which might denote that the antioxidant effect of ozone could have surpassed the deleterious effects of the steroids on the mitochondria. However, this increase did not reach the degree of significance when compared to the patients who received only the steroid injection, which might be explained by the low dose and one-time injection of O_3 in this study.

Oxidative stress is commonly described as an imbalance of pro-oxidants and antioxidants, which is described as the plasma redox status in humans. Comparing the 3 groups, there was no statistically significant difference in the GSH/GSSSG ratio of patients before intervention. However, 3 days after intervention, patients in all 3 groups has higher GSH/GSSG ratios. Furthermore, 3 days after intervention, the GSH/GSSG ratio was statistically significantly higher in patients who received the O_3 and both the O_3 and betamethasone compared to patients who only received betamethasone injection.

A study published by Safwat et al also supports these results. In this 2014 study, O_3 was effective in increasing the hepatic and renal GSH content as well as in normalizing hepatic glutathione peroxidase (GPx) activity in elderly rats (29). Similarly, another study showed that prophylactic O_3 therapy corrected decreased GSH content, adenosine triphosphate/adenosine diphosphate ratio, mitochondrial superoxide dismutase, and complex IV (cytochrome-c oxidase) activity in aged rats (30).

No conclusive data is available on the direct effect of steroids on the GSH/GSSG ratio, however there is evidence suggesting the opposite effect in different systems (31). A study on the effect of the steroid dexamethasone on the redox status of patients with ataxia telangiectasia found that dexamethasone enhances total GSH while having no effect on GSSG (32). Another study found that steroids increased the activity of glutathione redox-cycle enzymes, encouraging resynthesis of reduced glutathione and stability of intracellular redox state in preterm infants given antenatal betamethasone (33).

On the other hand, a study which investigated the mechanism of glucocorticoid-induced decrease of GPx enzyme activity found that in an in vitro model of E18 fetal rat hippocampal cultures, corticosteroid administration decreased levels of GSH and GPx (34).

The effects of O_3 and steroids on the GSH/GSSG in the present study might be due to the noticeable beneficial effect of O_3 on the redox state over the potential opposite effect of the low dose steroid used. Additionally, the type of steroid used as well as time of measurement may have affect these results.

Ozone has an antioxidant effect by increasing the transcription of antioxidant enzymes. Thus, ozone treatment enhances mitochondrial function and bio-

genesis. Thus, it can be concluded that medical ozone therapy is an effective technique for the management of pain as it had significant effect by reducing the muscle pain and increasing pain free intervals experienced by patients. Pain improvement increases with time, so ozone treatment can reduce overall analgesic demand. Corticosteroids can produce short-term symptomatic improvement and they had variable effects on mitochondrial respiratory function and biogenesis in patients. Furthermore, there were no known adverse effects from this intervention. Recommendations and future planning for further study should include controlled trials for specific musculoskeletal pain conditions to establish the role of abnormal mitochondrial redox state in patients' pathogenesis and also, a larger sample size to increase the generalizability of the findings.

Availability of the Data and Materials

The datasets generated and analyzed during the current study are not publicly available due to institutional restrictions but are available from the corresponding author on reasonable request.

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