Efficacy of Medical Ozone for Treatment of Chronic Musculoskeletal Pain with Abnormal Mitochondrial Redox State

Taha Tairy Dardeer Alsawy Alsawy
Aswan University

Laila Saber Abdel Aziz Sabry Sabry
University of Alexandria

Ahmed Fawzy Elmulla Elmulla
University of Alexandria

Maher Abdul-Nabi Kamel Kamel
University of Alexandria

Ayman Mohamady Eldemrdash Eldemrdash (aymaneldemrdash@yahoo.com)
Aswan University

Engi Yousry Hashem Hashem
University of Alexandria

Research Article

Keywords: Medical ozone, Steroid, Musculoskeletal, Trigger point, Redox state

Posted Date: January 11th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2436605/v1

License: ☑️ ☐️ This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Background

Chronic musculoskeletal pain is multifaceted and 20% of the adult population lives with severe chronic pain and have negative consequences, intense pain, depression, weakness, sleep problems, loss of enjoyment of life and decreased emotional well-being. This work done to study efficacy of trigger point injections with ozone for treatment of chronic musculoskeletal pain in patients with abnormal mitochondrial redox state compared to standard steroid injection or combination therapy.

Methods

This is a prospective randomized clinical study conducted on 51 patients with chronic musculoskeletal pain at Medical Research Institute Hospital, Alexandria University from January 2019 to January 2021. Patients randomly using computer-generated random numbers into 3 groups, 17 received ozone injection, 17 betamethasone injection and 17 combined Ozone and betamethasone injection. Groups were compared regarding intensity of pain, correction of mitochondrial redox state and normalizing Lactate/Pyruvate ratio.

Results

There were differences between 3 groups as regard VAS; three days after intervention (p < 0.021) as it was lower in group A compared to group and at one and three weeks after intervention (p < 0.001) where it was lower in groups A, C when compared with group B. There were differences in lactate/pyruvate ratio (percentage change) between the 3 groups (p < 0.004) as it was lower in groups A and C when compared with group B. There were differences between 3 groups as regard mitochondrial copy number (p < 0.002) as it was higher in group A when compared with group B. There were differences between the 3 groups as regard reduced/oxidized glutathione (p > 0.008) as it was higher in groups A and C when compared with group B.

Conclusions

Trigger point injections with ozone can relief musculoskeletal pain as it had significant effect in reduction of muscle pain and increasing pain free interval. Pain improvement increases with time. Ozone improves muscle oxygenation, mitochondrial function.

Introduction

Chronic musculoskeletal pain is the pain that lasts for three to six months or beyond the time of normal healing. Musculoskeletal disorders are the most often faced source of chronic musculoskeletal pain, Myofascial trigger points (MTrPs) are loci of hypersensitivity within a tender, taut, palpable band of muscle. MTrPs are characterized by referred pain on palpation and elicitation of a local twitch response with application of mechanical pressure[1]. Paresthesia, muscle weakness without primary atrophy, restricted mobility, proprioceptive disorders with impaired coordination, and autonomic reactions can also be caused by MTrPs.
and due to increasing the prevalence of this has led to a need to non-surgical solutions and effective such as physical therapy, pharmacologic treatment, and injection-based therapy. This MTrPs can have treated non-invasively by spray and stretch, transcutaneous electrical stimulation, physical therapy, and massage. Invasive treatments include injections with local anesthetics with or without corticosteroids, or botulinum toxin, or dry needling. The mechanism of action of trigger point injection (TPI) is thought to be disruption of the trigger points by the mechanical effect of the needle or the chemical effect of the agents injected, resulting in relaxation and lengthening of the muscle fiber. The effect of the injectate may include local vasodilation, dilution, and removal of the nociceptive substrates. Injection therapy can be used when pain or functional impairments persist despite oral medication or exercise. Corticosteroid injections are the most often used treatment for musculoskeletal diseases; they 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 injection [3].

Ozone(O3) gas was found during the mid-1800s. It is a molecule composed of three oxygen atoms in a dynamically unstable arrangement. Ozone was known for therapeutic effects because of the anti-inflammatory, antioxidant and analgesic effects by activating the cellular metabolism, reducing prostaglandin synthesis, making the redox system function properly by reducing oxidative stress through induction of the synthesis of antioxidant enzymes (superoxide dismutase, glutathione peroxidase, and catalase) and, in addition, amelioration of the tissue oxygen supply through hemorheological action, vasodilatation, and angiogenesis stimulation[4][5]. There are no reported allergic side effects or destructive adverse effect on tendons or cartilage with use of ozone(O3). It can be used for diabetes mellitus, hypertension, gastritis. So, on which increase the effectiveness of O therapy [6]. While Steroids have anti-inflammatory action by limiting capillary dilatation and permeability that restrict polymorphs and macrophages accumulation and inhibit release of vasoactive kinins [7].

Mitochondrial content determines the aerobic capacity of a muscle and is impaired in chronic musculoskeletal pain leading to decrease of adenosine triphosphate (ATP) that in turn propagates contracture and the resulting compressed capillary circulation can cause a hypoxic environment[8]. The majority of confirmed mitochondrial oxidative defects present with a raised blood lactate and this is often associated with elevated lactate/pyruvate(L/P) ratio, which means a change in the cellular redox state[9]. Lactate appears to facilitate the response of acid-sensing ion channels (ASIC-3) to low PH. Lactate exposure leads to reactive oxygen species (ROS) generation[10]. The increased lactate induces ROS, which directly interacts with the nociceptive system or, in turn, activates the algetic[11].

This work aims to study efficacy of trigger point injection with medical ozone for treatment of chronic musculoskeletal pain in patients with abnormal mitochondrial Redox state compared to standard steroid injections or combination therapy. The primary outcome is effect of the trigger point injection with ozone versus steroids versus both ozone and steroids on pain measured by visual analogue scale and the secondary outcome is to study efficacy of medical ozone in normalizing Lactate/Pyruvate ratio through correction of mitochondrial redox state.

Study Design And Methods

The current prospective clinical randomized study carried out at the Medical Research Institute Hospital, Anesthesia and Pain Management Department, Alexandria University. The study conducted on 51 patients, presented to the pain clinic (17 per group) according to the department of Biostatistics of the Medical Research
Institute to detect an average difference of visual analogue scale (VAS) among the ozone treated condition compared to the non-ozone treated conditions.

**Trial registration**

This study was approved by the Ethics Committee of Medical Research Institute, Alexandria University. (IORH: IOR 00088812) and registered at the Pan African Clinical Trial Registry (www.pactr.org), identification number for the registry was (PACTR201908620943471). The registration time of this experiment 07/08/2019. The study protocol followed the CONSORT guidelines. The study protocol was performed in the relevant guidelines.

**Inclusion criteria:**

1. Patients with chronic musculoskeletal pain for more than 6 months.
2. Patients with 4–8 trigger pointes.
3. VAS more than 4.
4. Serum lactate/pyruvate ratio more than 10/1[12].
5. Both sexes between 20-60 years.

**Exclusion Criteria:**

1. Bleeding tendency.
2. Glucose 6 phosphate deficiency.
3. Skin infection at the site of injection.
4. Systemic infection.
5. Hypersensitivity to the used medication.
6. Diabetic patients.
7. Recent history of steroid therapy in the last three months.

An informed written consent was taken from all the patients prior to their inclusion in the study, according to the ethical guidelines of the Medical Research Institute, Alexandria University. (IORH: IOR 00088812). A statement to confirm that all methods were carried out in accordance with relevant guidelines and regulations. A statement to confirm that all experimental protocol were approved by a name institutional and/or licensing committee

All patients randomly divided using computer-generated random numbers in the three groups and concealed:

**Group A:** (n. 17) received a trigger point injection of 5ml of 12µg/ml ozone using EXT50 ozone generator (Longevity resources, Canada) for each point.

**Group B:** Controlled group (n. 17) received a trigger point injection 0.5mg betamethasone injectable suspension diluted in 2ml sterile water for each point.

**Group C:** (n. 17) received a trigger point injection of 5ml of 12µg/ml ozone using EXT50 ozone generator and 0.5mg betamethasone injectable suspension
diluted in 2ml sterile water for each point.

- All patients were allowed to use analgesia in the form of paracetamol 500mg three times daily and instructed to stop it 48 hours before pain assessment.

- All patients were taught how to use the visual analogue scale (VAS) between 0 and 10 (0 = no pain; 10 = worst imaginable pain) [13].

- All patients were instructed to avoid vigorous muscular activity 3 days before laboratory tests.

**Procedure**

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

Intravenous line was inserted then the patient positioned either in sitting position with the head on the table for intervention at the patient neck or in prone position for intervention at the patient back. The site/s of injection was demarcated.

After scrubbing with alcohol, the trigger point was stabilized with a pinch between the thumb and index finger to prevent the trigger point from rolling away from the advancing needle. The needle (25G, 1-2 inch) was inserted 1–2 cm away from the trigger point at an acute angle of 30° to the skin, when the needle contacted the trigger point muscular twitch was felt. After the aspiration to ensure the needle not in blood vessel, the solution injected directly into the trigger point and in a fanning approach [14].

Ozone was freshly obtained from an ozone generator (EXT50 ozone generator (Longevity resources, Canada). It is composed of a high voltage tube through which medical oxygen (O2) passes, dividing into molecules that generate ozone, dial was pointed at 12 mic /ml. A 5ml syringe was connected to the exit of the generator to collect the gas. The syringe hub was carefully covered with a needle and will be held in the upright position to avoid leakage.

Betamethasone prepared by diluting one ml of the ampule that contains 5mg of betamethasone as betamethasone dipropionate and 2mg of betamethasone as betamethasone sodium phosphate in 7ml sterile water so each ml of the prepared solution contains one mg of betamethasone. In a 5ml syringe, we diluted 0.5ml containing 0.5mg betamethasone in 2ml sterile water to be injected in one trigger point.

After injection, the area was palpated to ensure that no other tender points exist. If additional tender points are palpable, they were isolated, needled and injected to a total of 4-8 points. Pressure was then applied to the injected area for two minutes to promote hemostasis and it was covered with a simple adhesive bandage.

**Data collection and Measurements:**

1. The age, sex, weight, height, body mass index and total number of the injected trigger points was documented.

2. The pain assessment by the visual analogue scale (VAS) between 0 and 10 (0 = no pain; 10 = worst imaginable pain) [13].
Timing of measurements:

1. The pain assessment by VAS was done before intervention then three days, one week and three weeks after intervention.

2- Laboratory Investigations: Two blood samples were obtained; the first before intervention and the second three days after intervention.

Laboratory investigations:

1. The blood samples were obtained and used for the isolation of peripheral mononuclear cells for the analysis of mitochondrial DNA copy number (mtDNA) (as a marker of mitochondrial biogenesis) using quantitative real-time polymerase chain reaction (qPCR).

2. Serum lactate was determined using colorimetric L-lactate Assay kit (cat.no. ab65331; Abcam) and pyruvate were assayed using colorimetric Pyruvate Assay Kit (cat. no. ab65342; Abcam) according to the manufacturer's protocols.

3. Plasma Redox status as reduced/oxidized Glutathione (GSH/GSSG) ratio was assayed using the enzymatic method described by Griffith [15].

Outcomes: -

The primary outcome is effect of the trigger point injection with ozone versus steroids versus both ozone and steroids on pain measured by visual analogue scale and the secondary outcome is to study efficacy of medical ozone in normalizing Lactate/Pyruvate ratio through correction of mitochondrial redox state.

Sample size calculation: -

A minimal total sample size of 45 patients (15 per group) is needed to detect an average difference of Visual Analogue Scale among the treated condition (Alternative Hypothesis) compared to the non-treated condition (Null Hypothesis) with common estimated group standard deviation of 1 and with 95% confidence level and 80% power using One-Way ANOVA test. (PASS program version 20). References:(Elvis AM, Ekta JS. Ozone therapy: A clinical review. Journal of Natural Science Biology, and Medicine.2011;2(1):66-70. We enrolled 51 eligible participants to avoid drop out and lost follow up.

Statistical Analysis

- Data were collected and entered to the computer using SPSS (Statistical Package for Social Science) program for statistical analysis (version 26)
- Data were entered as numerical or categorical, as appropriate.
- Kolmogorov-Smirnov test of normality revealed significance in the distribution of some variables, so the non-parametric statistics was adopted [16].
- Data were described using minimum, maximum, median and inter-quartile range (IQR).
- Categorical variables were described using frequency and percentage.
Comparisons were carried out between two studied related not-normally distributed subgroups using Wilcoxon Signed Ranks test [17].

Comparisons were carried out between more than two studied independent not-normally distributed subgroups using Kruskal-Wallis test [18].

Post-hoc pair-wise comparisons when Kruskal-Wallis test was significant was carried out using Dunn-Sidak test for multiple comparison or Bonferroni test [19]

Box and Whiskers plots were used accordingly.

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%.

Result

Fifty-one patients in the pain clinic were identified as possible participants, randomized and were allocated to three equal groups, 17 patients received ozone injection, 17 patients received betamethasone injection and 17 patients received combined Ozone and betamethasone injection of these, three patients were not eligible due to; refused the intervention, only left 48 eligible participants. The remaining 45 patients fulfilled all criteria for analysis, three patients lost follow-up in the study (Fig.1).

The groups were compared baseline characteristics as regarding age, weight, height, BMI, sex and number of injection sites there was no statistically significant difference among the three studied groups (Tab.1)

Table (1) baseline characteristics: -
<table>
<thead>
<tr>
<th>Baseline characteristic</th>
<th>Group A</th>
<th></th>
<th>Group B</th>
<th></th>
<th>Group C</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=15</td>
<td>n=15</td>
<td>n=15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Min-Max</td>
<td>Median</td>
<td>IQR</td>
<td>Min-Max</td>
<td>Median</td>
<td>IQR</td>
</tr>
<tr>
<td>Age in years</td>
<td>27-60</td>
<td>47</td>
<td>37-55</td>
<td>30-55</td>
<td>42</td>
<td>37-48</td>
</tr>
<tr>
<td>Weight</td>
<td>60-93</td>
<td>79</td>
<td>68-84</td>
<td>58-87</td>
<td>77</td>
<td>75-82</td>
</tr>
<tr>
<td>Height</td>
<td>155-182</td>
<td>168</td>
<td>162-173</td>
<td>158-188</td>
<td>173</td>
<td>166-178</td>
</tr>
<tr>
<td>Number of injected points</td>
<td>4-8</td>
<td>6</td>
<td>6-8</td>
<td>4-8</td>
<td>6</td>
<td>6-8</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (n&amp;%)</td>
<td>7 (46.67)</td>
<td>8 (53.33)</td>
<td>7(46.67)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female (n&amp;%)</td>
<td>8 (53.33)</td>
<td>7(46.67)</td>
<td>8 (53.33)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n: Number of patients

Min-Max: Minimum-Maximum

IQR: interquartile range

**Visual Analogue Scale (VAS): (Table 2 and Figure 2,3,4 and 5)**

Comparing the three groups; **Before intervention**, there was no statistically significant difference in VAS among the three studied groups \((P=0.856)\).

**Three days after intervention**, there was statistically significant difference in VAS \((P=0.021)\) where group **A** showed statistically significantly more reduction when compared with group **B** \((P=0.022)\) but there was no statistically significant difference when group **A** and group **B** compared with group **C** \((P=0.567), (P=0.436)\) respectively.

**One week after intervention**, there was statistically significant difference in VAS \((P=0.001)\) where group **A** and **C** showed statistically significantly more reduction when compared with group **B** \((P=0.020)\) and \((P=0.001)\) respectively but not when group **A** compared with group **C** \((P=0.634)\).

**Three weeks after intervention**, there was statistically significant difference in VAS \((P=0.001)\) where group **A** and **C** showed statistically significantly more reduction when compared with group **B** \((P=0.006)\) and \((P=0.001)\) respectively but not when group **A** compared with group **C** \((P=0.457)\). *(Tab.2) & (Fig 4,5 and 6)*.
Table (2): VAS before, after intervention in the three studied groups:

<table>
<thead>
<tr>
<th>VAS</th>
<th>Group</th>
<th>Kruskal Wallis Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>VAS (Before)</td>
<td>n=15</td>
<td>n=15</td>
</tr>
<tr>
<td>Min-Max</td>
<td>6 – 9</td>
<td>6 – 9</td>
</tr>
<tr>
<td>Median</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>IQR</td>
<td>7-9</td>
<td>7-9</td>
</tr>
<tr>
<td>P=0.856</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAS (After 3 Days)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min-Max</td>
<td>3 – 8</td>
<td>5 – 9</td>
</tr>
<tr>
<td>Median</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>IQR</td>
<td>4-6</td>
<td>6-7</td>
</tr>
<tr>
<td>P=0.021*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAS (After 1 Week)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min-Max</td>
<td>2 – 5</td>
<td>3 – 6</td>
</tr>
<tr>
<td>Median</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>IQR</td>
<td>3-4</td>
<td>4-5</td>
</tr>
<tr>
<td>P=0.001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAS (After 3 Weeks)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min-Max</td>
<td>0 – 5</td>
<td>3 – 6</td>
</tr>
<tr>
<td>Median</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>IQR</td>
<td>2-4</td>
<td>3-5</td>
</tr>
<tr>
<td>P=0.001*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

VAS: Visual analogue scale

n: Number of patients

Min-Max: Minimum – Maximum

IQR: Interquartile range

*: Statistically significant (p<0.05)

Serum lactate: (Table 3)
Serum lactate level measured 3 days after intervention was statistically significantly lower when compared with before intervention in the three studied groups; Group A \((P=0.01)\), group B \((P=0.001)\) and group C \((P=0.001)\).

Comparing the three groups; There was no statistically significant difference in serum lactate among the three studied groups neither before nor 3 days after intervention \((P=0.742)\) and \((P=0.353)\) respectively. (Tab.3)

**Table (3): Serum lactate before and after intervention in the three studied groups**

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th>Kruskal Wallis Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>n=15</td>
<td>n=15</td>
</tr>
<tr>
<td><strong>Lactate (Before) (mmol/l)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Min-Max</td>
<td>1.62-2.45</td>
<td>1.67-2.35</td>
</tr>
<tr>
<td>- Median</td>
<td>1.96</td>
<td>1.87</td>
</tr>
<tr>
<td>- IQR</td>
<td>1.84-2.17</td>
<td>1.74-2.14</td>
</tr>
<tr>
<td><strong>Lactate (After) (mmol/l)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Min-Max</td>
<td>1.47-2.19</td>
<td>1.51-2.24</td>
</tr>
<tr>
<td>- Median</td>
<td>1.78</td>
<td>1.76</td>
</tr>
<tr>
<td>- IQR</td>
<td>1.64-1.86</td>
<td>1.57-1.98</td>
</tr>
<tr>
<td><strong>Wilcoxon Signed Ranks Test</strong></td>
<td>p=0.001*</td>
<td>p=0.001*</td>
</tr>
</tbody>
</table>

n: Number of patients

Min-Max: Minimum - Maximum

IQR: interquartile range

**Serum pyruvate**: (Table 4)

Serum pyruvate level measured 3 days after intervention was not statistically significantly different when compared with before intervention in all studied groups; in group A \((P=0.055)\), in group B \((P=0.512)\) and group C \((P=0.232)\).

Comparing the three groups; There was no statistically significant difference in serum pyruvate among the three studied groups neither before nor after intervention \((P=0.495)\), \((P=0.086)\) respectively. (Tab.4)

**Table (4): Serum pyruvate(mmol/l) before and after intervention in the three studied groups**
<table>
<thead>
<tr>
<th></th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Pyruvate (Before) (mmol/l)</td>
<td>n=15</td>
</tr>
<tr>
<td>- Min-Max</td>
<td>0.12-0.17</td>
</tr>
<tr>
<td>- Median</td>
<td>0.15</td>
</tr>
<tr>
<td>- IQR</td>
<td>0.14-0.16</td>
</tr>
<tr>
<td>Pyruvate (After) (mmol/l)</td>
<td></td>
</tr>
<tr>
<td>- Min-Max</td>
<td>0.13-0.17</td>
</tr>
<tr>
<td>- Median</td>
<td>0.15</td>
</tr>
<tr>
<td>- IQR</td>
<td>0.14-0.16</td>
</tr>
<tr>
<td>Wilcoxon Signed Ranks Test</td>
<td>P=0.055</td>
</tr>
</tbody>
</table>

n: Number of patients

Min-Max: Minimum - Maximum

WSR: Wilcoxon Signed Ranks Test

IQR: interquartile range

**lactate/pyruvate(L/P) ratio (percentage changes) (%) : (Tab.5 and Fig.6)**

L/P ratio after intervention was statistically significantly lower when compared with before intervention in the three studied groups; A(P=0.001), B(P=0.002) and C(P=0.001).

Comparing the three groups; There was no statistically significant difference in L/P ratio among the three studied groups before and after intervention(P=0.869 NS),(P=0.418 NS) while there was statistically significant difference in percentage change of L/P ratio among the three studied groups(P=0.004) where the percentage change of L/P ratio in group A and C showed statistically significantly more reduction when compared with group B(P=0.017) respectively and showed no statistically significant reduction when group A compared with group C(P=1.000).

**Table (5): lactate/pyruvate ratio (percentage change) (%) in the three studied groups**
<table>
<thead>
<tr>
<th>Group</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>Kruskal Wallis Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=15</td>
<td>n=15</td>
<td>n=15</td>
<td></td>
</tr>
<tr>
<td><strong>Lactate Pyruvate ratio (percentage change) (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td>p=0.004*</td>
</tr>
<tr>
<td>- Min-Max</td>
<td>-19.37-</td>
<td>-16.00-</td>
<td>-26.82-2.47</td>
<td></td>
</tr>
<tr>
<td>- Median</td>
<td>-5.00</td>
<td>-3.95</td>
<td>-12.59</td>
<td></td>
</tr>
<tr>
<td>- IQR</td>
<td>-13.20</td>
<td>-6.62</td>
<td>-20.19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-17.48</td>
<td>-10.38</td>
<td>-8.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-9.66</td>
<td>-4.33</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Pairwise comparison using Games-Howell test

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th>P=0.010 *</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td></td>
<td></td>
<td>P=0.017 *</td>
</tr>
<tr>
<td>P2</td>
<td></td>
<td></td>
<td>P=1.000</td>
</tr>
<tr>
<td>P3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n: Number of patients

Min-Max: Minimum-Maximum

IQR: interquartile range

WSR: Wilcoxon Signed Ranks Test

P1: Group(A) against group(B)

P1: Group(A) against group(C)

P1: Group(B) against group(C)

**Mitochondrial DNA (mtDNA) copy number:** (Table 6)

mtDNA after intervention was statistically significantly higher when compared with before intervention in the group A(P=0.001); and C(P=0.001) but not in group B(P=0.118).

Comparing the three groups, there was no statistically significant difference before intervention (P=0.924) but was statistically significantly different after intervention(P=0.002) where mtDNA after intervention was statistically significantly high when group A compared with B(P=0.001) while in comparison of group A with C and B with C there was no statistically significantly difference (P=0.336), (P=0.148) respectively. (Tab 6)

**Table (6): mtDNA copy number before and after intervention in the three studied groups**
<table>
<thead>
<tr>
<th></th>
<th>A n=15</th>
<th>B n=15</th>
<th>C n=15</th>
<th>Kruskal Wallis Test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>mtDNA copy number (Before)</strong></td>
<td></td>
<td></td>
<td></td>
<td>P=0.924</td>
</tr>
<tr>
<td>Min-Max</td>
<td>44-56</td>
<td>44-56</td>
<td>43-56</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>51.00</td>
<td>50</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>IQR</td>
<td>47-54</td>
<td>47-53</td>
<td>46-54</td>
<td></td>
</tr>
<tr>
<td><strong>mtDNA copy number (After)</strong></td>
<td></td>
<td></td>
<td></td>
<td>P=0.002*</td>
</tr>
<tr>
<td>Min-Max</td>
<td>51-62</td>
<td>46-57</td>
<td>48-60</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>57</td>
<td>51</td>
<td>54</td>
<td>P=0.001*</td>
</tr>
<tr>
<td>IQR</td>
<td>54-60</td>
<td>48-54</td>
<td>51-57</td>
<td></td>
</tr>
</tbody>
</table>

**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)

**Plasma redox state, Reduced/Oxidized glutathione (GSH/GSSG) ratio**: (Table 7)

GSH/GSSG ratio 3 days after intervention was statistically significantly higher when compared with before the intervention in the three studied groups; group **A** (p=0.001), group **B** (p=0.002) and group **C** (p=0.001).
Comparing the three groups, there was no statistically significant difference in GSH/GSSSG ratio before intervention ($P = 0.863$) but was statistically significant difference 3 days after intervention ($p = 0.008$) where GSH/GSSG ratio after intervention was statistically significantly high when group A and C compared with group B ($P = 0.028$), ($P = 0.016$) respectively while not when group A compared with group C ($P = 1.000$). (Tab. 7)

Table (7): GSH/GSSG ratio before and after intervention in the three studied groups

<table>
<thead>
<tr>
<th>Group</th>
<th>A (n=15)</th>
<th>B (n=15)</th>
<th>C (n=15)</th>
<th>Kruskal Wallis Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH GSSG Ratio (Before)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Min-Max</td>
<td>20.21-23.37</td>
<td>18.41-24.29</td>
<td>18.09-24.71</td>
<td></td>
</tr>
<tr>
<td>- Median</td>
<td>21.32</td>
<td>21.74</td>
<td>21.35</td>
<td>P=0.863 NS</td>
</tr>
<tr>
<td>GSH GSSG Ratio (After)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Min-Max</td>
<td>22.67-26.86</td>
<td>19.39-25.86</td>
<td>21.43-26.90</td>
<td>P=0.008 *</td>
</tr>
<tr>
<td>- Median</td>
<td>24.32</td>
<td>23.20</td>
<td>24.42</td>
<td></td>
</tr>
<tr>
<td>- IQR</td>
<td>23.26-25.67</td>
<td>21.47-23.70</td>
<td>23.60-25.43</td>
<td></td>
</tr>
<tr>
<td>Wilcoxon Signed Ranks Test</td>
<td>p=0.001*</td>
<td>p=0.002*</td>
<td>p=0.001*</td>
<td></td>
</tr>
<tr>
<td>Pairwise comparison using Bonferroni test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n: Number of patients

Min-Max: Minimum-Maximum

WSR: Wilcoxon Signed Ranks Test

IQR: interquartile range

**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 can be
introduced when pain or functional limitations are significant despite oral medication or exercise.

The most frequent treatment for musculoskeletal problems is corticosteroid injections. They’re anti-inflammatory, which is why they’re so widely utilized in pain treatment. Since the 1930s, a combination of oxygen and ozone gases has been used in medicine, recently utilized to alleviate pain. Surprisingly, researchers have discovered that the brief, calculated oxidative stress obtained by ozone therapy may correct permanent imbalances induced by persistent or severe oxidative injury, and it is becoming evident that modest, repeated ozone treatment boosts the activity of superoxide dismutase, catalase, and glutathione peroxidase, producing a state of oxidative stress adaptation with major benefits. When injected into trigger points, ozone-oxygen expands and disrupts the tissue and fascia, perhaps correcting the trigger point pathology.

In the current study, **VAS** after TPI with ozone(5ml of 12mic/ml) was statistically significantly lower when compared with before intervention in group A at all the studied times, with a median percent decrease of -66% after 3 weeks. This might be caused by the transient and acute oxidative state which leads to multiple beneficial biological responses including activation of antioxidant systems, improvement of the blood flow and subsequently oxygen transfer, which might dilute the pain metabolites and nociceptive substances[20].

Furthermore, Ozone therapy also down regulates TNF and TNFR2 which are inflammatory mediators and it induces an analgesic effect through phosphodiesterase A2 blockage [21].

This is in accordance with Raeissadat et al., (2018) [22] who compared ozone injection versus lidocaine and dry needling of MTrP in patients with MPS, they reported a significant decrease in VAS (MD=−3.6 ± 1.4, p-value = 0.001) in patients who received 8cc of oxygen/ozone with a concentration of 15µg/ ml. Despite the three interventions led to a significant decrease in the VAS but they favored ozone therapy and lidocaine injection to dry needling due to the significant decrease in the VAS after the 4 weeks follow Up.

Ozone therapy is presently making great progress in the treatment of musculoskeletal diseases including shoulder joints both in shoulder adhesive capsulitis sub acromial bursitis, hip bursitis, rheumatoid arthritis, herniated disc, lumbar facet joint syndrome, carpal tunnel syndrome, osteoarthritis, and others [23].

Similarly, a study used intramuscular ozone injection in individuals suffering from LBP, showed that the mean pre-treatment VAS score was 5.6, while after treatment, a reduction of 2.3 point of the VAS scale (mean value was 3.3) obtained[24]. And also, the study of Özcan et al., (2019)[25] who compared the pain scores of patients before and after paravertebral ozone/oxygen(O/O) injections for low back pain, they found that there was a significant improvement in the statistical comparison of VAS score between the pre injection and first month (P < 0.000) and there was no significant difference in the statistical comparison of VAS score between the first and third months (P < 0.05).

In the current study, local injection of the trigger points with 0.5mg betamethasone showed significant reduction in the VAS after 3 weeks with a median percent reduction of (-50%), this is due to the anti-inflammatory action of the corticosteroids predominately affecting the cytokines, it inhibits the cellular mediated immunity, so it decreased the inflammatory cell accumulation and vascular response[26].

In the present study the VAS after 3 weeks showed a significant median percent decrease of (-75%) in the combined therapy group of both ozone and betamethasone, probably due to the synergistic action of both
ozone and steroids.

The current study is especially different because the steroid injection was done without combining it with local anesthetics, more over to the best of our knowledge, there has not been enough evidence about ozone utilization in MPS patients in the form of a well-designed randomized controlled trials.

In the present study, before intervention, there was no statistically significant difference in VAS among the three studied groups (p = 0.856). Three days after intervention, there was statistically significant difference in VAS where group A showed statistically significant more reduction when compared with group B but there was no statistically significant difference when group A and group B were compared with group C. One week and three weeks after intervention, there was statistically significant difference in VAS where group A and C showed statistically significantly more reduction when compared with group B but not when group A was compared with group C. These results might be because of delayed action of the steroids as their anti-inflammatory takes longer to be elicited as it involves its active moiety entering cells and combining with receptors protein annexin-1 to alter messenger RNA production[27].

Although there was a decrease in the VAS in the combined therapy group compared to the ozone only group but this reduction lacked statistical significance proposing that the action is not synergistic in nature though this postulation needs more studies to prove it.

The literature is scarce in comparing the ozone with steroids in the myofascial pain syndrome, but several studies compared them in different clinical situations [28][6]

In a retrospective cohort study of Ulusoy et al., (2019) [28] that was performed on 2 groups of patients with chronic lateral epicondylitis, ozone group and corticosteroid group and found that before the injection procedure, there was no difference between groups of corticosteroids and ozone with respect to pain scores. Assessment of pain scores just after the injections yielded that the two groups had similar results with respect to the pain score. Interestingly, analysis of pain on the 3rd, 6th and the 9th months following injections demonstrated that ozone group had significantly better scores. This study is different from the current study in several aspects as they used different dosage of corticosteroid and ozone, in their study corticosteroid dosage was (1ml of betamethasone dipropionate(6.43mg) and betamethasone sodium phosphate(2.63mg) and ozone dosage were(30µg/ml), in addition to the different pathology.

On the contrary to the current results, Ozone did not prove any superiority to the classical treatment with steroids and local anesthetic in other study, Babaei-Ghazani et al., (2019) [6] injected either a mixture of (triamcinolone 40mg/ml with two ml of lidocaine 1%) or (8ml of ozone of 12µg/ml and 2ml of lidocaine 1%) in the sub acromial bursa in shoulder impingement under ultrasound guidance, they studied the VAS in addition to other disability scores related to the condition. When they evaluated the VAS, they reported that the patients who provided the steroid injection reported greater pain improvement (the mean improvement was 4.47 point in comparison with 2.87 point in ozone group). Although the results might seem discouraging for the ozone treatment but the short time pain relief and the potential side effects of the steroids are yet to be considered especially that the study was design to assess the efficacy of a single ozone injection not multiple ones.
In the current study, L/P ratio following intervention was statistically significantly lower when compared with before intervention in the three studied groups which might be due the correction of the local hypoxia induced by the deactivation of the trigger points. Though the pathogenesis of the MPS is not deeply clarified. The local hypoxia generated by the sustained contractile activity in the sarcomeres of the trigger points was postulated to be responsible for the increase in the lactate levels in the myofascial pain syndrome. As in the exercising muscles there is also reliance on ‘anaerobic’ metabolism also known as” oxygen deficit” state which directly proportional to exercise intensity and is reflected in the extent of muscle creatine phosphate degradation and the accumulation of lactate [29].

Pyruvate was measured together with lactate which helps distinguish hypoxic from non-hypoxic lactate sources; in anaerobic circumstances, pyruvate is converted to lactate, increasing the L/P ratio[30]. In accordance with Redant et al.,(2019)[31],the current study was conducted on patients with an initial L/P ratio above 10 which confirms the hypoxic source of hyperlactatemia (Type1 hyperlactatemia),A condition that was not corrected with either ozone or steroids but it was partially corrected in the combined treatment(group C) with ozone and steroids as the range of L/P ratio was 8.30–14.70.

Comparing the three groups; There was no statistically significant difference in L/P ratio among the three studied groups pre and post intervention, while there was statistically significant difference in percentage change of L/P ratio among the three studied groups(\(P= 0.004\)) where the percentage change of L/P ratio in group A and C showed statistically significantly more reduction when compared with group B(\(P= 0.010\),\(P= 0.017\))respectively and exhibited no statistically significant reduction when group A compared with group C. These results might be due to better wash of the lactate, probably the ozone treatment was more effective in the improvement of the oxygenation of the muscles and correction of the oxygen deficit state responsible for the anaerobic metabolism, though more research is needed to prove this observation.

In accordance with the current study, Clavo et al., (2003) [32] detected that there were significant changes in muscle PO before and after ozone therapy and concluded that ozone therapy can modify the level of oxygenation in resting muscles, particularly of those that are most hypoxic. Also, Soloveva, (2016) [33] that studied processes of energy metabolism of blood under the influence of different ozone concentrations during long use in the experiment and detected that the lactate level in erythrocytes was significantly decreased when using an ozone concentration of 3000mcg/l by 35%, also LDH activity increased.

There is a scarcity of research on the effect of corticosteroids on lactate levels, and the results are contradictory[34]. In contrary to present study, Boysen,(2009)[35]Prospective, controlled experimental study about effect of oral prednisone for two weeks in two different doses 1mg(low dose) and 4mg(high dose)on serum lactate and found that low and high groups had significantly higher blood lactate concentrations at the fourth day and 14th day. This difference can be explained by that steroid induced type B hyperlactatemia due to the hyperglycemic effect of glucocorticosteroids which does not affect the L/P ratio. Also, these results were different from the current study probably because the patients in the current study received a single injection of relatively small dose of 0.5mg betamethasone per trigger point (median of 6trigger points).

mtDNA encodes proteins that are essential for cellular ATP production, mtDNA after intervention was statistically significantly higher when compared with before intervention in the group A and C but not in group B. Comparing the three groups, mtDNA was not statistically significantly different before intervention but was
statistically significantly different after intervention where mtDNA after intervention was statistically
significantly higher only when group A was compared with B.

It could be speculated as exposure to low \( \text{O} \) concentrations for therapeutic purposes was found to act on
nuclear factor erythroid (Nrf2). The serum Ozone was able to activate Nrf2 in a manner dependent dose. The
activation of nuclear respiratory factor-1 by Nrf2 has been demonstrated to drive mitochondrial biogenesis.
Through a positive feedback loop, Nrf2 increases the mitochondrial transcription factor A, a master regulator of
mitochondrial biogenesis [36].

In accordance with the current results, Hori et al., (2009) concluded in their study that the exposure of yeast
Saccharomyces cerevisiae to ROS due to the incubation in hydrogen peroxide, lead to an increase in the mtDNA
due to the excision-repair enzyme which introduces a double stranded break at the mtDNA replication origin
ori5 consequently initiating a rolling-circle mtDNA replication by DNA pairing protein (Mitochondrial
Homologous Recombination) and consequently increasing the mtDNA.

Following stress or corticosteroid treatment, changes in mitochondrial physiology have been reported
[37]. Time- and dosage-dependent effects on mitochondrial oxidation were 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, they found that corticosteroid decrease mitochondrial DNA copy number [38].

Interestingly when ozone and steroid treatment were combined mtDNA was increased which might denote that
the antioxidant effect of ozone could have surpassed the deleterious effects of the steroids on the
mitochondria but the increase did not reach the degree of significance when compared to group B, this might
be explained by low dose and a onetime injection of Ozone in this study.

Oxidative stress is commonly described as an imbalance of pro-oxidants and antioxidants, which may be
determined in humans as plasma redox status. GSH/GSSG., GSH/GSSG ratio 3 days after intervention was
statistically significantly higher when compared with before intervention in all of the studied groups.

Comparing the three groups, there was no statistically significantly different in GSH/GSSSG ratio before
intervention but was statistically significantly different 3 days after intervention where GSH/GSSG ratio after
intervention was statistically significantly high when group A and C were compared with group B.

This agreed by Safwat et al., (2014) [39] found that \( \text{O} \) has an anti-aging impact via lowering liver and kidney
damage by its antioxidant property. \( \text{O} \) was effective in increasing hepatic and renal GSH content as well as
normalizing hepatic Glutathione peroxidase (GPx) activity in elderly rats. Similarly, prophylactic \( \text{O} \) therapy
corrected decreased GSH content, adenosine triphosphate/adenosine diphosphate ratio, mitochondrial
Superoxide dismutase, and complex IV (cytochrome-c oxidase) activity in aged rats. Ozone enhances
glutathione redox index, complex I [40].

No conclusive data are available on a direct effect of steroids on GSH/GSSG ratio, there is evidence suggesting
opposite effects in different systems [41]. A study on the effect of dexamethasone on redox status in ataxia
telangiectasia found that dexamethasone enhances total GSH while having no effect on GSSG [42]. Another
study found that steroid 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[43].

On the other hand, a study investigated the mechanism behind glucocorticoid-induced decrease of GPx enzyme activity: In an in vitro model of E18 fetal rat hippocampal cultures, they found corticosteroid administration decreased levels of GSH and GPx [44].

The effects of Ozone and steroid on GSH/GSSG in the present study might be due to the noticeable beneficial effect of ozone on the redox state over the potential opposite effect of the low dose steroid used, also type of steroid as well as time of measurement may have a role.

From the current study, it was concluded that:

- Medical ozone therapy is an effective technique for management of pain as it had significant effect in reduction of muscle pain and increasing pain free interval in patients. Pain improvement increases with time. So, it will reduce analgesic demand.
- Ozone treatment improves muscle oxygenation. It also enhances the mitochondrial function and biogenesis.
- Ozone has anti-oxidant effect through improve the transcription of antioxidant enzymes.
- Corticosteroids have short term symptomatic improvement, they had variable effect on mitochondrial respiratory function and biogenesis.

Recommendation

- More controlled clinical trials are required to evaluate the role of ozone therapy in management of specific musculoskeletal painful conditions.
- More controlled studies comparing effect of medical ozone versus dry needling and local anesthetics for management of musculoskeletal pain conditions.
- Future controlled trials for specific musculoskeletal pain conditions are needed to establish the role of abnormal mitochondrial redox state in their pathogenesis.

Declarations

Ethics approval and consent to participate

The study was approved by the Medical Ethics Committee at the Medical Research Institute, Alexandria University. (IORH: IOR 00088812). An informed written consent was taken from all the patients prior to their inclusion in the study, according to the ethical guidelines of the All procedures in the study involving human participants were performed in accordance with the ethics standards of the institutional and national research committee

Consent for publication

Declared consent for publication Not Applicable
Competing interests

The authors declare no conflicts of interest.

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.

Funding

No special funding.

Authors’ contributions

The authors confirm contribution to the paper as follows:

- **study conception and design**: Ahmed Fawzy Elmulla and Laila Saber Abdel Aziz Sabry.
- **Data collection**: Taha Tairy Dardeer Alsawy, Engi Yousry Hashem and Maher Abdul-Nabi Kamel.
- **Analysis and interpretation of results**: Ahmed Fawzy Elmulla.
- **Draft manuscript preparation**: *Ayman Mohamady Eldemrdash, Engi Yousry Hashem and Taha Tairy Dardeer Alsawy*:

All authors reviewed the results and approved the final version of the manuscript.

Acknowledgements

The authors gratefully thank the Departments of Anesthesiology and Intensive Care of the Medical Research Institute, Alexandria University, and Residency Study Department of the center for allowing and supporting this study.

Author details

- **Laila Saber Abdel Aziz Sabry, Anesthesia and Pain Management Medical Research Institute University of Alexandria** lillysabry@hotmail.com
- **Ahmed Fawzy Elmulla, Anesthesia and Pain Management Medical Research Institute University of Alexandria** aelmulla@yahoo.com.uk
- **Engi Yousry Hashem, Anesthesia and Pain Management Medical Research Institute University of Alexandria**, engi_yousry@yahoo.com
- **Maher Abdul-Nabi Kamel, Biochemistry, Medical Research Institute University of Alexandria** maherrashwan@hotmail.com
- **Taha Tairy Dardeer Alsawy, Anesthesia, intensive care and Pain Management, Faculty of Medicine, Aswan University**, tahatairy@gmail.com
- ***Ayman Mohamady Eldemrdash, Anesthesia, intensive care and Pain Management, Faculty of Medicine, Aswan University*, aymaneldemrdash@yahoo.com
References


Figures
**Figure 1**

Flow chart
Independent-Samples Kruskal-Wallis Test

Figure 2

VAS before injection

Grouping variable
Figure 3

VAS after 3 days
Figure 4

VAS after 1 week

Independent-Samples Kruskal-Wallis Test
Figure 5

VAS after 3 weeks
Figure 6

lactate/pyruvate ratio percentage change in the three studied groups