Online First
The European Research Journal 2026 Research Article
DOI: https://doi.org/10.18621/eurj.1264 *Family Medicine*

Evaluation of Ozone Therapy Effectiveness by the Red Cell Distribution Width/Albumin Ratio

Meryem Betos Koçak[1], Mehmet Akif Nas[2], İdeal Beraa Yılmaz Kartal[2]

[1]Department of Family Medicine, University of Health Sciences, Hamidiye Faculty of Medicine Balıkesir Atatürk City Hospital, Balıkesir, Türkiye; [2]Department of Family Medicine, Atatürk University, Faculty of Medicine, Erzurum, Türkiye

Abstract:

Objective: Ozone is an inorganic gas utilized for medical purposes. Ozone therapy has gained increasing popularity in recent years and is employed in the treatment of chronic diseases associated with inflammation. The red cell distribution width/albumin ratio (RAR) has recently emerged as an inflammatory marker. The aim of this study was to evaluate the efficacy of ozone therapy in patients with spinal pain and to investigate the effect of ozone therapy on inflammation through changes in the RAR value.

Methods: This prospective pilot study was conducted in a pre- and post-treatment design on patients with spinal pain. In addition to demographic data, visual analog scale (VAS) scores and RAR values were recorded before and after ozone therapy. All patients underwent major ozone autohemotherapy. Data were analyzed using the SPSS software.

Results: During the study period, 93 patients presented to the ozone therapy unit. After applying the exclusion criteria, 66 patients were included in the analysis. The mean pre-treatment VAS score was 6.74±1.53, while the mean post-treatment VAS score was 4.08±1.54 ($P<0.05$). The mean pre-treatment RAR value was 3.37±0.64, which decreased to 3.14±0.50 following ozone therapy ($P<0.05$).

Conclusion: Our findings demonstrate that major ozone autohemotherapy is effective in alleviating spinal pain. Furthermore, the reduction in RAR values before and after treatment indicates that major ozone autohemotherapy exerts an anti-inflammatory effect.

Keywords: Ozone Therapy, Red Cell Distribution Width (RDW), RDW/Albumin Ratio, Spinal Pain

Ozone (O_3), or trioxygen, is an inorganic gas that is an allotrope of oxygen, with lower stability compared to diatomic dioxygen (O_2) [1]. Ozone has been used for medical purposes for more than a century [2]. Medically, ozone is employed as a therapeutic agent in pathologies such as chronic hypoxia, inflammation, and redox imbalance [3]. Ozone therapy is an innovative method that has attracted increasing interest in medical science. With its versatile therapeutic potential, ozone therapy has been utilized across various medical fields. The fundamental principle of ozone therapy is to enhance tissue oxygenation, thereby improving oxygen delivery to hypoxic tissues, which creates optimal conditions for metabolic and reparative processes [4].

Ozone therapy has been shown to be effective in a range of diseases associated with chronic oxidative stress and inflammation, including chronic pain

Submitted: March 17, 2026 **Accepted:** April 28, 2026 **Published Online:** May 5, 2026

How to cite this article: Betos Koçak M, Nas MA, Yılmaz Kartal İB. Evaluation of Ozone Therapy Effectiveness by the Red Cell Distribution Width/Albumin Ratio. Eur Res J. 2026. doi: 10.18621/eurj.1264

Corresponding author: Meryem Betos Koçak, MD., Assist. Prof., Phone: +90 226 460 40 00, E-mail: meryembetoskocak@gmail.com

Available Online at https://www.eurj.org.tr

syndromes. Administration of ozone initiates a controlled and safe process of "micro-oxidation," which modulates multiple cellular antioxidant and inflammatory systems [3]. In addition to improving tissue hyperoxygenation, ozone therapy has notable anti-inflammatory and analgesic properties, making it an effective treatment option for painful syndromes affecting muscles, tendons, and joints [5]. The mechanisms underlying the analgesic effects of ozone therapy in musculoskeletal disorders have been previously described. Among them, the most prominent is its ability to reduce inflammation via modulation of arachidonic acid metabolism. Ozone decreases the breakdown of arachidonic acid into pro-inflammatory prostaglandins, thereby reducing inflammatory mediators and alleviating pain. Other proposed mechanisms include: (i) enhancement of hyperoxygenation, improving oxygen delivery in conditions such as vasospasm and disc herniation where blood flow is compromised; (ii) reduction of water retention in the nucleus pulposus by degrading glycosaminoglycan chains, thereby decreasing disc volume and contributing to pain relief; and (iii) stimulation of fibroblastic activity, promoting collagen deposition and tissue repair, which further supports pain reduction [6–9].

The red cell distribution width (RDW) /albumin ratio (RAR), defined as the ratio of RDW to serum albumin, is a novel inflammatory marker. An elevated RAR, reflecting increased RDW and decreased albumin levels, may predict severe inflammation [10]. RAR has been reported to be elevated in numerous inflammation-associated diseases and serves as a prognostic indicator in conditions such as sepsis, cancer, diabetic ketoacidosis, and acute respiratory distress syndrome [11].

The aim of our study was to investigate the efficacy of ozone therapy in reducing spinal pain. In addition, we sought to evaluate the therapeutic effect of ozone therapy using simple, inexpensive, and readily accessible biochemical and hematological parameters. Our objective was not only to demonstrate the clinical effectiveness of ozone therapy, but also to support it with laboratory-based evidence. While various markers have previously been studied to assess the efficacy of ozone therapy, in our study we aimed to evaluate its effect using RAR, a simple, cost-effective, easily obtainable, objective, and sensitive indicator.

METHODS

Study Design and Participants

This prospective, pre–post pilot study included patients presenting with spinal pain who applied to the ozone therapy unit of Balıkesir Atatürk City Hospital. Patients admitted for ozone therapy between October 1, 2024, and July 31, 2025, were enrolled.

Participation in the study was voluntary, and written informed consent was obtained from all patients. For each participant, a dedicated study form was completed by the same physician, who both created the forms and conducted face-to-face interviews with the patients. The study form included demographic data (age, sex, marital status, residence [classified as urban or rural], educational level [high school or below, undergraduate and above], employment status, smoking status, body mass index), pain scores (measured using the Visual Analog Scale [VAS] immediately before initiation of ozone therapy and on the third day after completion of treatment) [12], The VAS scale used was a 10-point horizontal line ranging from 0 (no pain) to 10 (the most severe pain imaginable), the number and dosage of ozone therapy sessions (mg), side effects, and laboratory data obtained on the day ozone therapy was initiated and three days after its completion (RDW-CV [%], albumin [g/dL], and the RDW/albumin ratio [RAR]).

All patients were instructed on the use of the VAS to grade their pain from 0 (no pain) to 10 (worst imaginable pain) [13]. Data were obtained by recording all variables in patient-specific study forms. Patients were advised to avoid intense physical activity three days before laboratory testing. During the study period, patients did not receive physiotherapy, and no new medications were prescribed, although continuation of their existing treatments was allowed.

The minimum sample size was calculated using G*Power 3.1. To detect a mean difference in VAS scores in a paired design, with a 95% confidence interval, 90% power, and Cohen's d = 0.5, at least 49 patients were required. In our clinic, only major ozone autohemotherapy (MAH) is performed; other techniques (e.g., bagging, trigger point injection, intradiscal injection) are not applied.

Inclusion criteria [14]:

•Discogenic pain [15]

•Patients with spinal stenosis not considered candidates for surgery
•Patients with radicular compression
•Pain lasting more than 6 weeks
•VAS score > 4

Exclusion criteria:

•Patients refusing participation
•Patients unable to tolerate or complete the procedure
•Patients discontinuing ozone therapy or failing to complete the treatment protocol
•Incomplete or incorrect data records
•Pregnancy or breastfeeding
•Known coagulation disorders or current use of oral anticoagulants
•Glucose-6-phosphate dehydrogenase deficiency
•Local skin infection at the injection site
•Active systemic infection
•History of steroid therapy within the past 3 months
•Cancer
•Recent surgery
•Physiotherapy within the past 3 months prior to ozone therapy
•Chronic inflammatory diseases (e.g., rheumatoid arthritis, SLE, Sjögren's syndrome)
•Iron deficiency anemia
•Chronic liver disease
•Renal failure
•Bone marrow disorders

Procedure

Data were obtained from the completed patient study forms. Ozone therapy was performed by a family physician specialist certified by the Turkish Ministry of Health for ozone therapy application. All procedures were conducted by the same physician using an identical protocol. Patients were fully informed about the ozone therapy procedure before initiation. VAS scores were recorded before the first session and three days after the final session.

Ozone therapy was performed according to protocols previously described in the literature [16–19]. All patients underwent MAH, with ozone generated using a SALUTEM (Turkey) medical device. Disposable ozone-resistant medical materials were used, including a glass ozone bottle, 19/21 G stainless steel transfusion needles, ozone-resistant blood transfusion sets, ozone-resistant tubing, butterfly needles, bacterial filters, and 50 mL siliconized syringes (Medipac Medical®, Germany; Bexen Medical®, Spain).

Each patient received 10 MAH sessions, three times per week, in accordance with the Italian Scientific Society of Oxygen–Ozone Therapy (SIOOT) protocols. The procedure began with both the ozone and blood transfer sets closed. After venous access was established, 100 mL of blood was withdrawn into the glass bottle. Ozone was then introduced at a concentration of 30 µg/mL using 50 mL syringes, repeated twice to ensure equal volumes of blood and the oxygen–ozone mixture. The oxygen (95–100%) and ozone (1–5%) mixture was delivered at a flow rate of 0.43 L/min, with final gas pressure maintained at atmospheric levels.

The collected blood was mixed with ozone for 5 minutes to achieve homogeneous distribution and maximize therapeutic efficacy. Sodium citrate (3.13%) was used as an anticoagulant, in line with the recommendations of the World Federation of Ozone Therapy. The ozonated blood was reinfused into the patient's circulation within 10–15 minutes.

Ethical approval was obtained from the local ethics committee, and the study was conducted in accordance with the Declaration of Helsinki (Balıkesir Atatürk City Hospital Ethics Committee, Date: 19.09.2024, Decision No: 2024/09/49).

Statistical Analysis

Data were analyzed using SPSS version 23.0. Results were expressed as mean, standard deviation, median, minimum, maximum, percentage, and frequency. The Shapiro–Wilk and Kolmogorov–Smirnov tests were used to evaluate the normality of continuous variables. Normally distributed continuous variables were compared between independent groups using the independent-samples t-test, while non-normally distributed variables were compared using the Mann–Whitney U test. Categorical variables were analyzed using the chi-square or Fisher's exact tests. For within-group comparisons, the paired-samples t-test was applied for normally distributed variables, and the Wilcoxon signed-rank test was used for non-normally distributed variables. A P-value of <0.05 was considered statistically significant.

RESULTS

During the study period, 93 patients who met the inclusion criteria applied to the ozone therapy unit of our hospital. When the exclusion criteria were applied, 27 patients were excluded from the study. Ultimately, the study was completed with 66 patients. The flow chart is presented in Figure 1.

The age of the included patients ranged from 26 to 64 years, with a mean age of 43.05±8.84 years. Of the participants, 42 (63.6%) were female. A total of 49 (74.2%) patients were married, and 61 (92.4%) patients resided in urban or district centers. Twenty-five (37.9%) patients had an education level of high school or below. Fifty-one (77.3%) patients were employed, and 37 (56.1%) patients were smokers.

Patient height ranged from 155 to 181 cm, with a mean of 168.32±6.96 cm, while body weight ranged from 53 to 98 kg, with a mean of 71.55±10.99 kg. The mean body mass index (BMI) was 25.15±2.70, with a minimum of 20.1 and a maximum of 31.3. Only one patient experienced transient palpitations following the procedure, which resolved spontaneously within a short time and did not recur; this patient successfully completed all sessions without further complications. No other adverse effects were reported in any patient.

Pre- and post-treatment VAS scores, RDW values, albumin levels, and RAR values of the study population are summarized in Table 1.

DISCUSSION

Interest in ozone as an adjunctive treatment in medicine has increased steadily over the past two decades. While approximately 120 scientific articles were published in 2001, this number rose to 309 by 2021. According to Google Trends data, global interest in the medical use of ozone has expanded to include more countries since 2004. It is evident that the clinical use of ozone represents a significant opportunity for clinicians. As a contemporary subject, many researchers are attempting to elucidate the role of ozone in medicine [20]. In our study, we investigated the use of ozone therapy, and its benefits were demonstrated through objective biochemical and hematological inflammatory markers. Notably, our findings revealed a reduction in the commonly used inflammatory marker RAR following ozone therapy.

Ozone therapy has been known for more than 150 years, and its beneficial effects have been reported in various studies. Due to its potent, low-cost, and non-pharmacological effects, ozone has been widely utilized in the management of more than 50 pathological conditions, including degenerative disorders, neurological, orthopedic, and genitourinary diseases. It has been suggested that ozone contributes to the treatment of these disorders by modulating oxidative–antioxidative mechanisms [18].

Ozone therapy can generally be administered

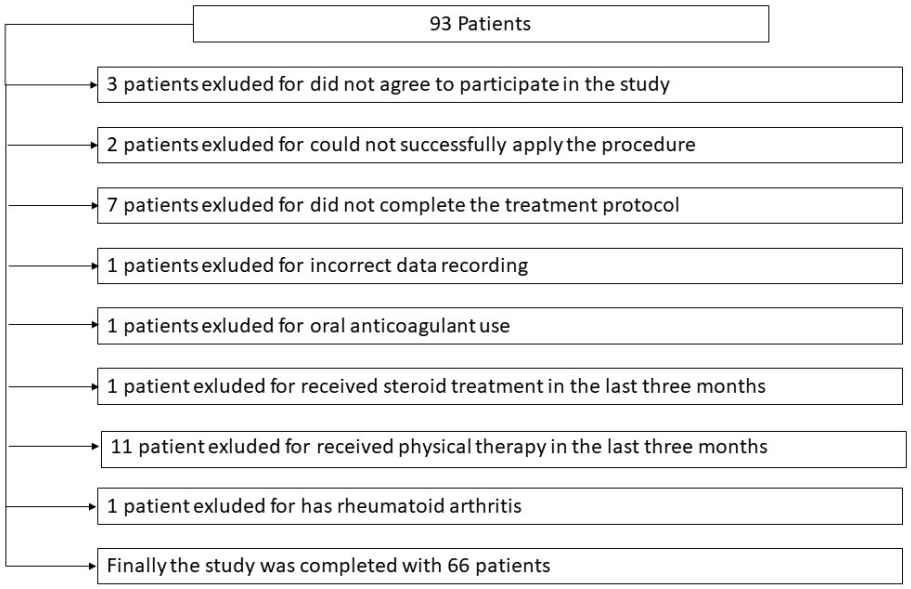

FIGURE 1. Flowchart of Study.

TABLE 1. Pre- and Post-Treatment VAS Scores, RDW, Albumin, and RAR Values

Variable	Mean	Minimum	Maximum	P-value*
VAS[1]	6.74±1.53	5	10	**<0.001**
VAS[2]	4.08±1.54	1	7	**<0.001**
RDW[1] (%)	14.06±1.56	12.2	18.9	**<0.001**
RDW[2] (%)	13.37±1.15	11.9	17.1	**<0.001**
Albumin[1] (g/dL)	4.20±0.36	3	4.9	**<0.001**
Albumin[2] (g/dL)	4.29±0.36	3.1	5.14	**<0.001**
RAR[1] (%/g/dL)	3.37±0.64	2.54	5.9	**<0.001**
RAR[2] (%/g/dL)	3.14±0.5	2.45	5.16	**<0.001**

Data are shown as mean±standard deviation or minimum-maximum.
VAS, visual analog scale; RDW, red cell distribution width; RAR, red cell distribution width/albumin ratio.
[1]before ozone therapy, [2]after ozone therapy, *the independent-samples t-test
Statistically significant P-values are shown in bold.

either locally or systemically. Locally, it may be applied to spinal muscles or tendons, or directly to open wounds via sealed bags preventing air leakage. Systemically, it can be performed using an ozone-generating device, where blood withdrawn from the patient is ozonated and subsequently reinfused into the patient. This systemic method is also known as major ozone therapy, and it should only be performed by highly qualified experts and professionals. Major ozone therapy has been employed in the treatment of inflammatory musculoskeletal disorders, infected postoperative or traumatic wounds, and numerous other inflammatory pathologies [20]. In our study, we utilized major ozone therapy. While much of the literature focuses on local ozone applications, systemic use of major ozone therapy has been relatively rare in clinical studies, making our work an important contribution to the field.

In a study by Eldemrdash *et al*. [12] investigating the efficacy of medical ozone for the treatment of chronic musculoskeletal pain, three groups were formed: Group A received only medical ozone, Group B received dexamethasone, and Group C received both medical ozone and dexamethasone. Based on VAS scores one week after intervention, Groups A and C reported lower VAS scores compared to Group B [12]. Similarly, Chirumbolo *et al*. [21] observed improvement in musculoskeletal pain in patients with myalgic encephalomyelitis/chronic fatigue syndrome following major ozone therapy. Their results indicated that major ozone therapy was beneficial for musculoskeletal pain [21]. Consistent with these findings, our study also demonstrated a reduction in pain with major ozone therapy.

RDW reflects anisocytosis among red blood cells, which is often a consequence of inflammation. Elevated RDW indicates increased erythrocyte destruction, ineffective erythropoiesis, and/or shortened erythrocyte lifespan [10]. Albumin, a negative acute-phase protein, possesses physiological properties including anti-inflammatory, antioxidant, anticoagulant, and antiplatelet aggregation activities, in addition to its colloid osmotic effects [11, 22]. Under conditions of oxidative stress and chronic inflammation, albumin undergoes oxidation, leading to hypoalbuminemia; this explains why decreased albumin levels are frequently observed in inflammatory diseases [23].

Both RDW and albumin concentration have been proposed as integrative biomarkers of the multidimensional dysfunctional physiological state associated with inflammation, oxidative stress, and nutrition. Since these markers represent different aspects of pathological processes, their integration is considered more valuable [22]. To obtain more sensitive data in our study, we used the RDW-to-albumin ratio (RAR).

RAR is a novel, reliable, inexpensive, and easily obtainable inflammatory marker derived from routine laboratory tests. A strong inflammatory response can

lead to a significant increase in RAR [10, 24]. In a cohort study, Hao *et al*. [22] demonstrated that elevated RAR values were strongly and independently associated with an increased risk of all-cause mortality, including deaths due to malignancies, cardiovascular disease, cerebrovascular disease, respiratory illness, diabetes, and other causes. Although the underlying biological mechanisms remain unclear, this association is most likely related to chronic inflammation. Therefore, RAR, as a marker of chronic inflammation, is thought to be directly linked to mortality [22].

In a study of 3,381 cancer patients, Lu *et al*. [24] investigated all-cause mortality and identified a cut-off value for RAR of 5.51. Patients with RAR values above this threshold had higher mortality rates, which the authors attributed to the role of systemic inflammation in cancer progression [24]. Similarly, Qiu *et al*. [23], in their study of 1,174 patients with chronic obstructive pulmonary disease, reported a cut-off value of 5.315. Patients above this threshold had higher in-hospital mortality and longer hospital stays, findings attributed to increased inflammation. Xu *et al*. [11], in a retrospective study including 14,639 patients with sepsis, reported that patients with elevated RAR had significantly higher 28-day and 90-day mortality rates.

In line with these findings, our study demonstrated that RAR values decreased following ozone therapy, supporting the conclusion that ozone therapy exerts anti-inflammatory effects.

Strengths and Limitations

Our study is one of the few studies on ozone therapy conducted in our country. Furthermore, our prospective study makes a significant contribution to the literature. The limitation of our study is that it is a single-center study. More prospective studies with a larger number of patients should be conducted on ozone therapy.

CONCLUSION

In conclusion, ozone therapy was found to reduce spinal pain and thereby decrease patient morbidity. Furthermore, by comparing pre- and post-treatment RAR values, our study demonstrated that ozone therapy contributes to the reduction of chronic inflammation. Ozone therapy may therefore be considered as an adjunctive treatment to mitigate inflammation in chronic diseases. However, further studies are warranted to clarify and strengthen the role of ozone therapy in the management of chronic inflammatory conditions. Further large-scale randomized controlled trials are needed to improve upon the results of our study.

Ethics Approval and Consent to Participate

This study was approved by the Balıkesir Atatürk City Hospital Scientific Research Ethics Committee. (Decision No: 2024/09/09; date: 19/09/2024). All procedures were conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from all individual participants included in the study.

Data Availability

All data generated or analyzed during this study are included in this published article. The data that support the findings of this study are available on request from the corresponding author, upon reasonable request.

Authors' Contribution

Study Conception: MBK, MAN, İBYK; Study Design: MBK, MAN, İBYK; Supervision: MBK, İBYK; Funding: MBK, MAN; Materials: MBK, MAN; Data Collection and/or Processing: MBK, İBYK; Statistical Analysis and/or Data Interpretation: MBK, MAN, İBYK; Literature Review: MBK, İBYK; Manuscript Preparation: MBK, MAN; and Critical Review: MBK, MAN.

Conflict of Interest

The author(s) disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The author(s) disclosed that they did not receive any grant during the conduction or writing of this study.

Acknowledgments

We would like to thank the staff of the Traditional

and Complementary Medicine Center at Balıkesir Atatürk City Hospital.

Generative Artificial Intelligence Statement

The author(s) declare that no artificial intelligence-based tools or applications were used during the preparation process of this manuscript. The all content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

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