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Medical ozone modifies D-dimer, interleukin-6, lactic acid and oxidative stress levels: A possibility for the comprehensive treatment of COVID-19

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ABSTRACT

Background: SARS-CoV-2-induced inflammation in COVID-19 is mediated by cytotoxic and pro-oxidant effects that potentiate alveolar, endothelial and immune tissue damage. Objective: We investigated the effect of medicinal ozone administration on the oxidative stress markers; in addition to D-dimer, lactic acid and interleukin-6 as markers of endothelial injury and inflammation process. Methodology: Medicinal ozone with oligo metals was administered *in vivo* (major autohemotherapy) and *in vitro* (peripheral blood), to subsequently determine the levels of: H_2O_2, NO, GPx, CAT, TAP, TBARs, D-dimer, lactic acid and interleukin-6. Results: Medicinal ozone administration with oligo metals induced changes in oxidative stress markers both *in vitro* and *in vivo*. The H_2O_2 and TBARs levels decreased, in turn, NO levels increased (cardiovascular function marker). On the other hand, the levels of the antioxidant enzymes (GPx and CAT) show slightly increase, which indicates an antioxidant enzyme system regulation that counteracts the pro-oxidative effect of the infection. Furthermore, interleukin-6 levels decreased indicating the regulation of the systemic inflammatory process. Finally, lactic acid and D-dimer levels were decreased, establishing an improvement of energy metabolism and endothelial function respectively. Conclusion: The medicinal ozone administration induce decrease in the markers levels of oxidative stress, inflammation and cellular damage, improving the enzymatic antioxidant capacity and cellular metabolism with decrease plaque aggregation that contribute to reducing the risk of vascular endothelial damage. These benefits could be feasible to integrate in the treatment of endothelial injury in COVID-19 patients.

Keywords: oxidative stress; medicinal ozone; D-dimer; inflammation; interleukin-6; COVID-19.

RESUMEN

Antecedentes: La inflamación inducida por SARS-CoV-2 en COVID-19 está mediada por efectos citotóxicos y pro-oxidativos que potencializan el daño del tejido alveolar, endotelial e inmunitario. Objetivo: Investigamos el efecto de la administración de ozono medicinal sobre marcadores de estrés oxidativo; además del dímero-D, ácido láctico e interleucina-6 como marcadores del proceso de lesión endotelial e inflamación. Metodología: Se administró ozono medicinal con oligometales *in vivo* (autohemoterapia mayor) e *in vitro* (sangre periférica), para determinar los niveles de: H_2O_2, NO, GPx, CAT, TAP, TBARs, dímero-D, ácido láctico e interleucina-6. Resultados: La administración de ozono medicinal con oligometales indujo cambios en los marcadores de estrés oxidativo tanto *in vitro* como *in vivo*. Los niveles de H_2O_2 y TBARs disminuyeron, a su vez, los niveles de NO aumentaron (marcador de función cardiovascular); además los niveles de enzimas antioxidantes (GPx y CAT) mostraron un leve aumento, indicando una posible regulación del sistema antioxidante enzimático que contrarresta el efecto pro-oxidativo de la infección. Además, los niveles de interleucina-6 disminuyeron indicando la regulación del proceso inflamatorio sistémico. Finalmente, se redujeron los niveles de ácido láctico y dímero-D, estableciendo una mejora del metabolismo energético y de la función endotelial respectivamente. Conclusión: La administración de ozono medicinal induce la disminución en los niveles de marcadores de estrés oxidativo, inflamación y daño celular, mejorando la capacidad antioxidante enzimática y el metabolismo celular con disminución de la agregación plaquetaria que contribuyen a reducir el riesgo del daño endotelial vascular. Estos beneficios podrían ser factibles de integrar en el tratamiento de las complicaciones vasculares y de la lesión endotelial en pacientes con COVID-19.

Palabras clave: estrés oxidativo; ozono medicinal; dímero D; inflamación; interleucina-6; COVID-19.

INTRODUCCIÓN

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infects the host using the angiotensin-converting enzyme 2 receptor, which is expressed in different organs as well as in endothelial and immune cells, reason why it can cause endothelial tissue damage and immune system injury [1, 2]. It is currently suggested that vascular disorders in COVID-19 are due to the involvement of endothelial cells, as it has been shown that SARS-CoV-2 can directly infect engineered human blood vessel organoids *in vitro* and through across vascular beds potentiates tissue damage [1, 3, 4]. Therefore, the reduction of cellular toxic effects would make the COVID-19 disease more controllable. The SARS-CoV-2 induces a severe increase of inflammatory cytokines, reactive oxygen species and cell death induced by these cell events is a cause that can result in significant multi-organ damage, so the regulation of oxidative stress is essential [2, 5]. Thus, modulation of oxidative stress may be able to prevent the development

of severe disease symptoms in coronavirus patients, reduce the severity of their symptoms, and/or reduce the immuno-pathology of coronavirus infection on patients' health after the active phase of the infection is over.

Ozone (O_3) is a gas composed of 3 atoms of oxygen, including a stable pair (O_2) and a third unstable atom, which gives ozone its beneficial effects [6]. For medical purposes, concentrations of 10–30 μg/mL are commonly used and ozone therapy can be administered systemically by adding it to a sample of a patient's own blood and then refusing it, in what is termed ozonated autohemotherapy [7]. When blood is exposed to this gas mixture (O_2 – O_3), oxidation reactions are generated, which are responsible for its biological and therapeutic effects. Ozone therapy has many beneficial effects, including immune system modulation, improvement of microcirculation, anti-inflammatory action stimulation of oxygen metabolism, and promotion of tissue oxygenation [7, 8]. The main mechanism of ozone therapy on human physiology fits the concept of oxidative preconditioning. This concept has now been demonstrated at both the proteomic and genomic level, in *in vitro* studies and clinical trials [7, 9]. A calibrated oxidant stimulus by medicinal ozone can modulate the endogenous antioxidant system and aid in the control of different pathological conditions. The modulation of ozone at the Keap1/Nrf2/ARE pathway and the reduction inflammation markers (IL-1β and IL-6) are involved in the mechanism of ozone action [10]. This implies that the cytoprotective effect observed during the ozone treatment may impact the clinical conditions in COVID-19 patients.

Because redox modulation may be an alternative to mitigate endothelial injury associated with oxidative stress and inflammation COVID-19-induced. Therefore, the aim of this study was to evaluate the association between D-dimer, interleukin-6 levels and different biomarkers to assess the relationship between oxidative stress and tissue injury in patients with COVID-19 and type 2 diabetes mellitus (DM2), for a projection in the endothelial lesion established in COVID-19-patients.

METHODOLOGY

Study design and subjects for study

The study was performed in accordance with the 2000 declaration of Helsinki. It was approved by the research ethics committee of CMNBm (Folio: CB007), and informed consent was obtained from all participants. **Control patients (+) with endothelial dysfunction:** 3 diabetic and hypertensive patients; they presented metabolic uncontrol, stage C2 chronic venous disease and stage IIb peripheral arterial disease for the Fontaine classification. Laboratory studies showed the following alterations: glucose 287±21 mg/dL; urea 57.5±8.9 mg/dL; BUN 26.2±5.6 mg/dL; creatinine 1.1±0.2 mg/dL; triglycerides 541±19 mg/dL; cholesterol 263±29 mg/dL; lactic acid 3.9±0.4 mmol/L; D-dimer 0.65±0.06 mg/L. **COVID-19 patients:** 3 patients with confirmed

SARS-CoV-2 infection with the qPCR test. Presents hypoxemia saturating 80±5%, febrile, lung exploration found decreased vesicular murmur in both subscapular fields and crackling sounds, and with edema in both lower limbs. The hematic cytometry shows leukocytosis with a predominance of neutrophils, the blood gas shows pH 7.55±0.4, PO_2 35±2, PCO_2 28±1, HCO_3 24±1. A computed tomography scan of the thorax was performed, showing a large bilateral interstitial occupation in ground glass and a basal air bronchogram. **Process sample:** 4 mL of peripheral blood were obtained by venipuncture from all patients: control (healthy, 3 patients), with a history of endothelial dysfunction (positive control-DMII, 3 patients) and with COVID-19 (3 patients); They were incubated at 36 ºC for 1 hour, then divided into 4 different groups: No treatment (sample in the basal state; control), oxidative stress (H_2O_2, 400 µL to 4.8 µM as an inducer of oxidative stress), treatment 1 (H_2O_2 + O_3 , 21 mm, which corresponds to 10% of the total blood volume treated) and treatment 2 (H_2O_2 + O_3 with intravenous oligo metals). Subsequently, the hermetically covered tubes were incubated for 15 min in an inversion mixer; after incubation they were centrifuged and the following parameters were determined in the serum: H_2O_2, CAT, GPx, NO, TBARs and TAP

Ozone administration by autohemotherapy

Major autohemotherapy consists of the extraction of a certain blood volume, coming from the venous system, which will come into contact with a mixture of 95 % oxygen and 5 % ozone at a ratio of 1mL of blood to 1mL of the mixture mentioned. To achieve the objective of modulating the antioxidant systems of the hematopoietic tissue during major autohemotherapy, 21 µg/mL of medicinal ozone (modulating dose of the redox system) was used, a volume of peripheral venous blood corresponding to 10 % of blood volume with 3 mL of endovenous oligometals (Tracefusin®) and 500 mg of intravenous ascorbic acid. The therapy was performed on day 0, at 24 and 72 h, after the administration´s blood samples were obtained to determine the markers: H_2O_2, TBARs, CAT, GPx, NO, TAP, interleukin-6, lactic acid and D-dimer.

Biochemistry analysis

On days 0, 1 and 3 blood samples were obtained by venipuncture after an overnight fast. The blood was immediately centrifuged at 4000 rpm/10 min and the serum samples were separated into aliquots and frozen at -30 ºC until tested. Nine different tests for free radical, antioxidant capacity, lesion tissue and inflammation markers were utilized. All of them were assayed in microtiter plates using commercial enzymatic colorimetric kits (BioAssay system, Hayward CA): H_2O_2 (QuiantiChrom™ peroxide assay kit, DIOX-250), catalase (EnzyChrom™ catalse assay kit, ECAT-100), glutathione peroxidase (QuantiChrom™ peroxidase assay kit, D2PD-100), total plasma antioxidant capacity (QuantiChrom™ DTAC-100), nitric oxide (QuantiChrom™ nitric oxide assay kit, D2NO-

100), thiobarbituric acid reactive substances (QuantiChrom™ DTBA-100, interleukin-6 (IL-6 human, high sensitivity ELISA kit), Absorbance was measured in a spectrophotometer (ATI-Unicam 300, UK) and expressed according to each of its units. Lactic acid and D-dimer were analyzed by colorimetric and turbidimetric methods respectively in plasma samples mediated kit assay (lactate winner lab and D-dimer winner lab).

Statistical analyses

Results were expressed as mean values ± SEM. All data were statistically analyzed using one- or two-way analysis of variance (ANOVA) for repeated measures, followed by post hoc Bonferroni's test. All analytical procedures were performed using the scientific statistical software GraphPad Prism 5 (GraphPad Scientific, San Diego, CA, USA). Differences of $p<0.05$ were considered as statistically significant. On the other hand, the Shapiro-Whilks normality test was performed to determine the behavior of the variables, then the relationship between the variables H_2O_2, TAP, NO, TBARs, CAT and GPx was analyzed; and between the IL-6, D-dimer and lactic acid variables, by estimating with Pearson's multiple connection in the R studio pracma library

RESULTS

Modulation of oxidative stress markers by Ozone in the DM2 and COVID-19 samples

The oxidative stress modulation in blood was compared by the application of hydrogen peroxide (oxidative stress), ozone (O_3) and ozone with oligo metals (O_3 + OligoM) for the baseline, in a sample of healthy patients (control), diabetes mellitus type 2 and COVID-19 (Fig. 1). Graph 1A shows the levels of hydrogen peroxide (H_2O_2), and an increase in its concentration is observed in the three groups when hydrogen peroxide is applied as an inducing agent of oxidative stress, observing a greater significant difference between the control and COVID-19 groups (800±53 and 1127±145 respectively). The levels induced after treatment with H_2O_2 + O_3 (906±69) did not show a significant effect on the amount of H_2O_2 compared to the baseline group (800±53). While the effect of the administration of H_2O_2 + O_3 + OligoM did induce a significant decrease compared to baseline (698±50), and a greater extent to the problem groups, being more significant in the COVID-19 group (1127±145).

Concerning nitric oxide (Fig. 1B) we can observe that there is a modulation of the concentration in the three groups to administer the O_3 with oligo metals ($p<0.05$). In diabetes mellitus patients we can observe that there is a decrease in the concentration control (21±4 with respect to basal in DM2 group, 35±5), contrary to what occurs in the sample of COVID-19 (basal levels, 7±1) where an increase is observed (9±1), and between groups there is also a difference in the concentrations of both the baseline values and the values after treatment with ozone therapy.

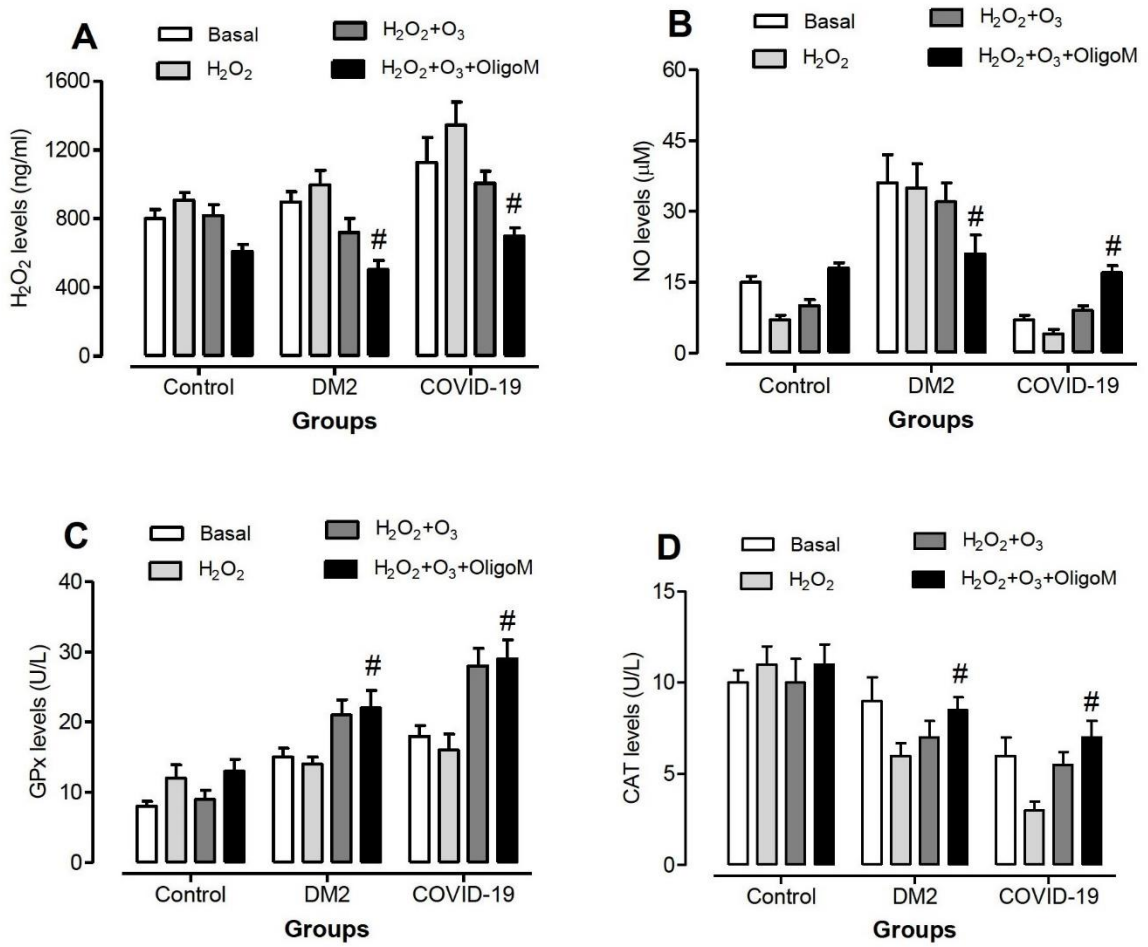

Figure 1. Oxidative stress modification by ozone- and ozone plus oligometals-induced in blood of patients with type-2 diabetes mellitus (DM2) or COVID-19. Levels of: A) Hydrogen peroxide (H_2O_2), B) Nitric oxide (NO), C) Glutathione peroxidase (GPx), and D) Catalase (CAT); determined at basal level (Control), after infusion of reactive oxygen species (H_2O_2), or treatments: H_2O_2 plus ozone (O_3), or H_2O_2 plus ozone and oligometals (O_3 + OligoM). Data expressing mean values ± SEM, n=3. Two-way ANOVA for repeated measures followed by Bonferroni's test for multiple comparisons. Statistical differences are denoted as *p<0.05 vs control group and #p<0.05 vs H_2O_2 group.

Regarding antioxidant enzymes, glutathione peroxidase (Fig. 1C) showed an increase due to treatment in its basal levels (DM2 15±1.3 and COVID-19 18±1.5), and with respect to pathologies, an increase was observed after treatment with ozone plus oligo metals, observing a greater increase in the group with COVID-19 (DM2 22±2.5 and COVID-19 29±2.7). Regarding catalase we can observe that for the control group (basal) a regulation was established in the activity of the enzyme that oscillates between 10-11±1.3. While in the groups of pathologies, an increase in activity was presented after treatment with ozone plus oligo metals (8.5±0.7 and 7.0±0.9), compared with the control of both groups respectively (DM2, 9±1.3 and COVID-19, 6±1.0), this effect being greater in the COVID-19 group

(Fig. 1D).

Ozone treatment ameliorated the oxidative stress-induced lesion in DM2 patients

Oxidative stress modulation through the application of ozone + oligo metals in type 2 diabetes mellitus patients (Figure 2). The modulation of oxidative stress markers by ozone therapy was observed, where a significant decrease was obtained in the concentration of hydrogen peroxide (Fig. 2A; 359.2±59.9 respect to DM2 group 909.3±35.10) and TBARs (Fig. 2E; 12.57±0.52 respect to DM2 group 15.40±0.96), which decreased after 24 h the application of treatment. Furthermore, NO levels (Fig 2B) in the DM2 group decreased (46.31±4.49 respect to control 82.12±5.21) while the treatment increased these levels at 24 h after treatment (68.54±2.03 compared to the DM2 group). In addition, it is observed that the treatment with ozone therapy induces an improvement in the antioxidant systems since a tendency is established to increase the activity of the enzymes: CAT (Fig. 2C; 10.59±0.59 respect to DM2 group 9.39±0.20), GPx (Fig. 2D; 15.79±0.60 respect to DM2 13.25±0.38), and the total antioxidant activity (Fig. 2F; 480.0±14.43 respect to DM2 423.3±11.67) as a reflection of the complete function of the antioxidant system.

The normality of the variables H_2O_2, TAP, NO, TBARs, CAT and GPx was checked using the Shapiro-Wilk test, which was significant (W = 0.702, p=<0.005). The multiple correlation test indicated that there is a strong correlation between almost all the variables, except with GPx (Table 1). While Fig. 3A and 3B show the correlation between the levels of hydrogen peroxide (H_2O_2) and the levels of the variables: TBARs (R^2= 0.93), nitric oxide (R^2= -0.92), TAP (R^2= -0.96), CAT (R^2= -0.86) and GPx (R^2= -0.48); negative R values but close to 1 were found, which implies a high relationship between these variables, except for GPx.

Ozone administration decreases D-dimer, lactic acid and IL-6 levels in COVID-19 patients

Blood levels of tissue damage and inflammation markers in the COVID-19 patient were modified by ozone therapy. Ozone was administered by autohemotherapy and a significant decrease was observed in the levels of D-dimer (Fig. 4A) at 24 h (0.81±0.07) and 72 h (0.52±0.06) compared to COVID-19 group (1.28±0.08), lactic acid (Fig. 4B) at 24 h (4.79±0.50) and 72 h (3.01±0.60) compared to COVID-19 group (5.29±0.63), and interleukin-6 (Fig. 4C) at 24 h (5.49 ±0.33) and 72 h (3.17±0.29) compared to COVID-19 group (6.14±0.39), after the ozone treatment, compared with the values shown before therapy and derived from the infection by the SARS-CoV-2 virus (Time 0 h in figures 4A, B and C).

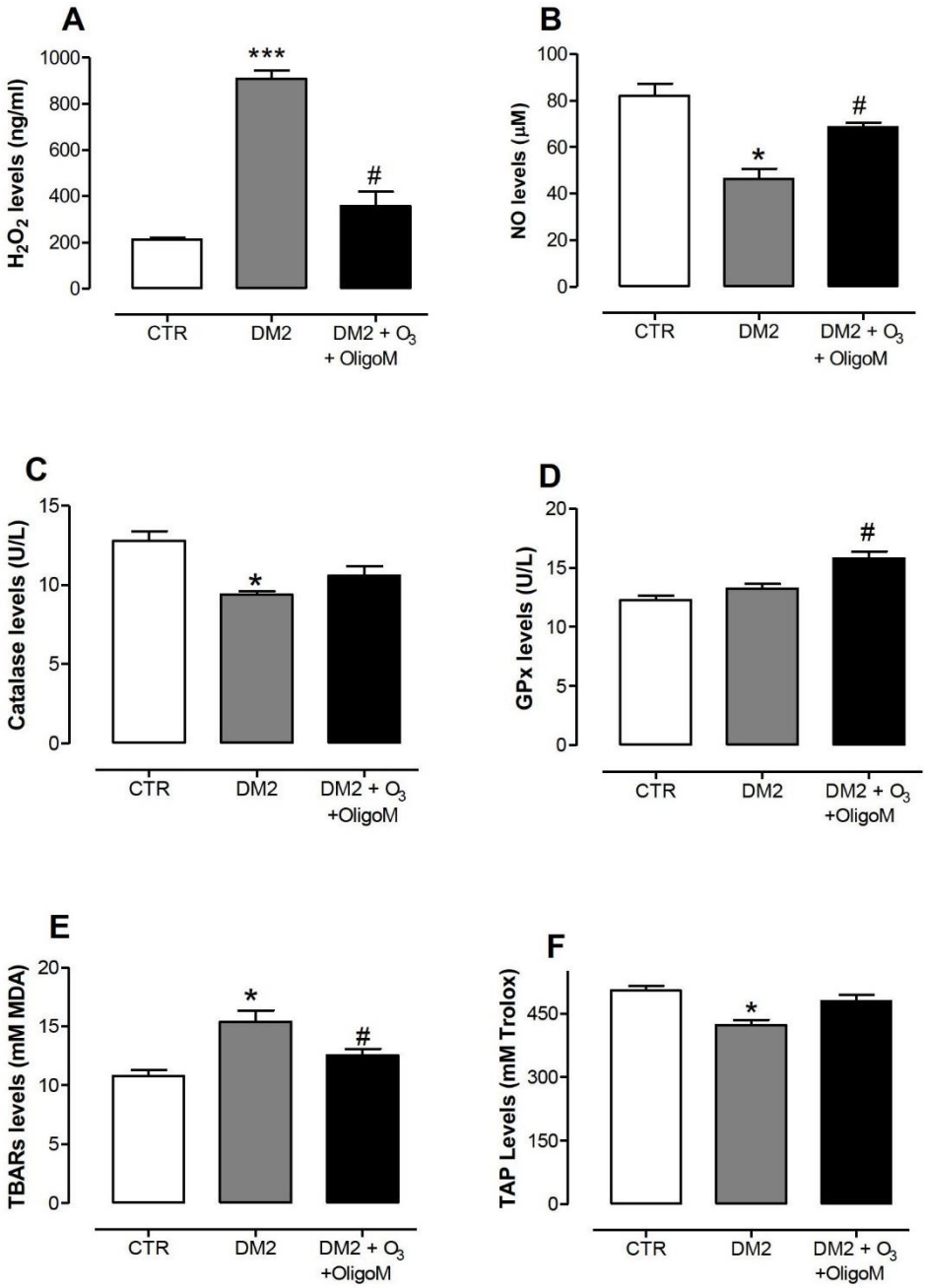

Figura 2. Oxidative stress modification by medicinal ozone- and ozone plus oligometals-administration induced in type-2 diabetes mellitus (DM2) patients. Levels of: A) Hydrogen peroxide (H_2O_2), B) Nitric oxide (NO), C) Catalase (CAT), D) Glutathione peroxidase (GPx), E) TBARs (Malondialdehyde; MDA), and F) Total antioxidant potential (TAP); determined at basal level (Control), after infusion of medicinal ozone plus oligometals (O_3 + OligoM). Data expressing mean values ± SEM of n=3 experiments. Student's-t tests for to analyze differences between groups. Statistical differences are denoted as *p<0.05 vs control group and #p<0.05 vs DM2 group.

Table 1. Correlation between H_2O_2 values with respect to the variables: TAP, NO, TBARs, CAT and GPx. The values of Person's multiple correlation are shown, indicating the values of negative or positive R according to the type of association between variables.

	H_2O_2	TAP	NO	TBARs	CAT	GPx
H_2O_2	1.000	-0.956	-0.925	0.930	-0.859	0.016
TAP	-0.956	1.000	0.992	-0.981	0.960	-0.289
NO	-0.925	0.992	1.000	-0.984	0.974	-0.342
TBARs	0.930	-0.981	-0.984	1.000	-0.928	0.271
CAT	-0.859	0.960	0.974	-0.928	1.000	-0.476
GPx	0.016	-0.289	-0.342	0.271	-0.476	1.000

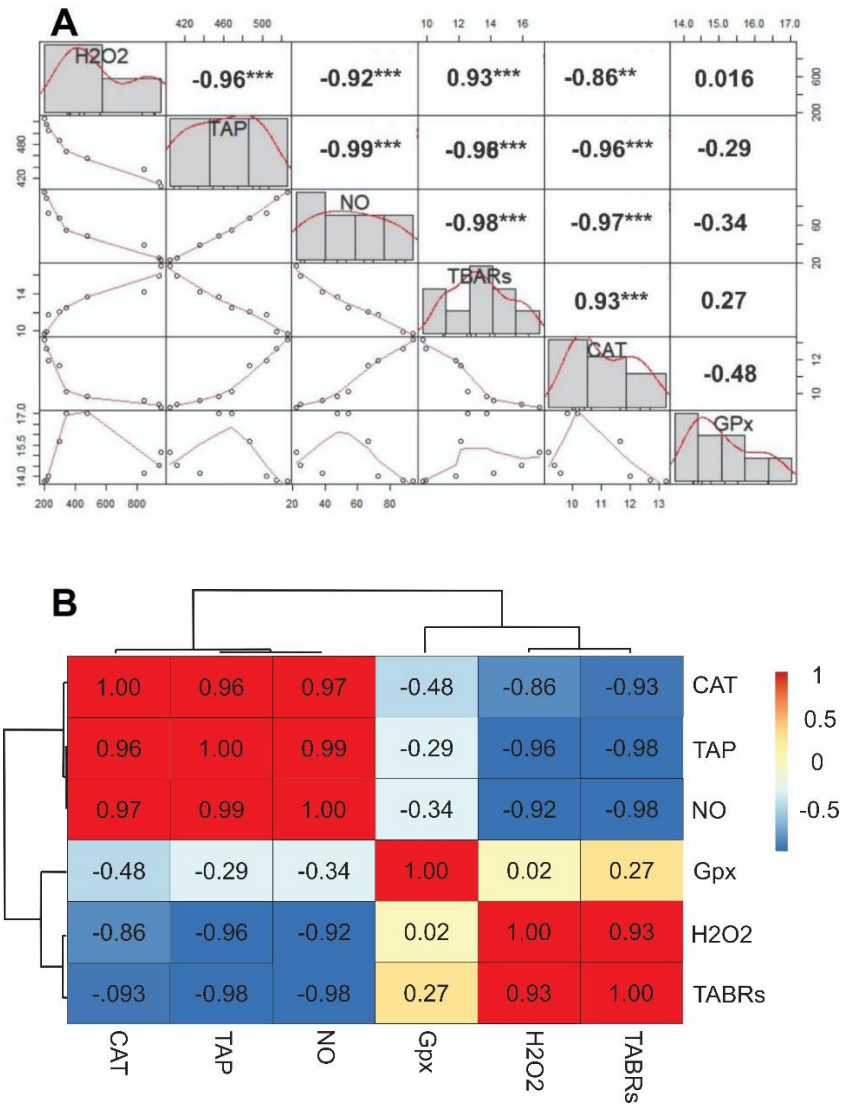

Figura 3. Associated of oxidative stress markers modification by medicinal ozone administration-induced in type-2 diabetes mellitus (DM2). A-B) Correlation analysis between the peroxide levels and the levels of the variables TBARs, NO, TAP, CAT and GPx, by Person's multiple correlation, indicating the negative or positive values of R.

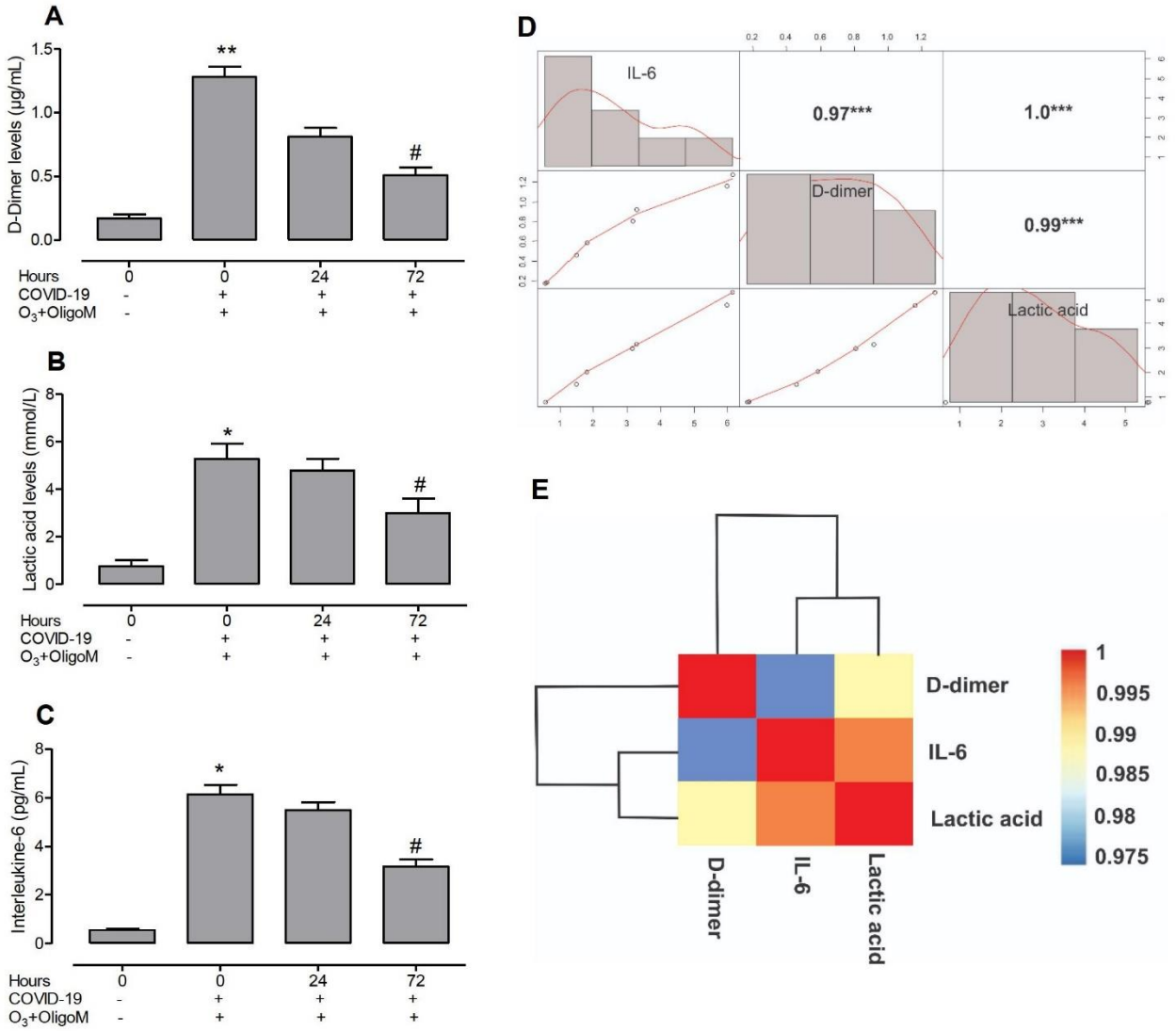

Figure 4. Medicinal ozone-induced decreased in D-dimer, lactic acid and interleukin-6 levels in COVID-19-patients. A) D-dimer, B) Lactic acid and C) IL-6 levels change by ozone-therapy plus oligo metals-induced in COVID-19 patients. Levels of D-dimer [µg/mL], lactic acid [mmol/L] and interleukin-6 [pg/mL] were determined at basal level (control) and COVID-19 patients before (0 h) and after infusion of ozone therapy plus oligo metals (24 and 72 h). D-E) Correlation analysis between the Il-6 levels and the levels of the variables D-dimer and lactic acid, by Person's multiple correlation, indicating the negative or positive values of R. Data expressing mean value ± SEM, n=3. Statistical differences are denoted as *p<0.05 vs control group and #p<0.05 vs COVID-19 group.

The normality of the IL-6, D-dimer and Ab variables was reviewed. Lactic through the Shapiro-Wilk test, which was significant (W = 0.844, p=0.001). The multiple correlation test indicated that there are positive correlations between the 3 variables (Table 2). On the other hand, the administration graphs between the levels of interleukin-6 with respect to the levels of D-dimer and lactic acid are presented (Fig. 4D-E). A high connection was found between the parameters with values of R^2 = 0.97 and R^2 = 0.99, respectively.

Table 2. Correlation table between IL-6 values with respect to the variables: D-dimer and lactic acid. The values of Person's multiple correlation are shown, indicating the values of negative or positive R according to the type of association between variables.

	Il-6	D-dimer	Lactic acid
IL-6	1	0.973	0.995
D-dimer	0.973	1.000	0.988
Lactic acid	0.995	0.988	1.000

DISCUSSION

The viral stimulation in COVID-19 is prone to elicit intensive immunological reactions and cytokine storm. In addition, overactivation of the immune system can produce numerous reactive oxygen species (ROS) including H_2O_2, $\cdot O_2^-$, ·OH, etc [5]. Increased ROS can induce oxidation of cellular proteins, membrane lipids and quickly destroy not only virus-infected cells but also normal cells in the lung and even endothelial, resulting in multiple organ failure [5, 11]. Therefore, a possible redox modulation therapy could be proposed to alleviate the cardiovascular cytotoxic events caused by COVID-19, so we report the effect of ozone therapy in patients with COVID-19 pneumonia and DM2 with endothelial dysfunction by autohemotherapy.

There is no currently available effective treatment for COVID-19 pneumonia. The pathogenesis of the virus is not fully understood, but the pathological picture in the lungs varies significantly in terms of diffuse alveolar damage and microcirculopathy leading to life-threatening hypoxia [7, 12]. Ozone has multiple beneficial properties that could be useful in the treatment of COVID-19 pneumonia [13]. Ozone can deliver sufficient energy and oxygen to the tissues through activating the pentose phosphate pathway, elevating 2,3-diphosphoglyceric acid content in erythrocytes, and stimulating erythrocyte oxygen metabolism. Furthermore, it improves the rheology and capillary action of the blood, which has been reported to be helpful for patients with ischemic vascular diseases [7, 14]. Additionally, ozone has an antiplatelet effect and increases the release of some prostacyclin such as PGI2, which are beneficial for patients with microthrombosis [15]. All these effects can help decrease the hypercoagulation phenomena observed in COVID-19 patients. Another important role played by ozone in COVID-19 is its immunomodulatory effects [16]. The inflammatory response is a hallmark of severe infection, and cytokine modulation is key to avoid patient deterioration. Ozone has potent anti-inflammatory properties through modulation of the NLRP3 inflammasome, which plays a crucial role in the initiation and persistence of inflammation in various diseases [17].

It is known that COVID-19 activates the renin-angiotensin-aldosterone system inducing

oxidative stress that eventually leads to a cytokine storm [18]. Furthermore, we have shown that SARS-CoV-2 is capable of generating damage to immune cells by inducing an inhibition in mitochondrial function, which would lead to increased oxidative stress and tissue damage [19]. Therefore, the modulation of oxidative stress is essential, and in this regard, ozone therapy can induce rapid activation of the transcriptional factors Nrf2, which is an important physiological mechanism in the control and regulation of the enzymatic antioxidant system inhibiting said oxidative and even inflammatory cytotoxic process [20].

Ozone therapy can also confer renal protection; the rate of kidney damage in COVID-19 patients is significant, and ozone modulates the accumulation of neutrophils locally, the expression of interleukin-6, tumor necrosis factor (TNF)-α, and albumin modified by ischemia in the kidneys, and increases local antioxidant capacity [7, 21]. Ozone can induce the release and modulation of interferons (IFNs) and related cytokines, such as IL-2, IFN-γ, and TNF-α, and colony-stimulating factors, and can also modulate and stimulate phagocytic function, which can have a very positive effect in COVID-19 infection [21, 22].

Ozone therapy is recommended to counter the disruptive effects of severe COVID-19 on lung tissues, especially if administered in the early stages of the disease, thereby preventing the progression of COVID-19 lung disease, as well as the favorable rheological and tissue perfusion properties to limit ischemic lung damage [23]. The therapeutic effect of ozone is maximized in the early phases of disease development when blood oxygenation is hampered by interstitial edema and alveolar fluid exudates, but only with a limited extension of lung tissue consolidation [23, 24]. For ozone therapy it has been postulated an anti-inflammatory effect especially in reducing pro-inflammatory cytokines and other authors have also observed a decrease of IL-6 levels after ozone treatment [25]. In our study, we observe this effect, with respect to the control group, on the reduction of IL-6 level, confirming its regulating effect on the inflammation process.

Finally, D-dimer levels is a classic hypercoagulability biomarker, useful in the diagnosis of thromboembolic events. There is an association between D-dimer levels with the development of atherothrombosis and cardiovascular complications in patients with diabetes, indicating that D-dimer can be useful in evaluating the risk of cardiovascular disease in these patients, as in other associated diseases [26]. Therefore, the decrease in D-dimer levels due to the administration of medicinal ozone is a fundamental aspect in the therapy against COVID-19, since thromboembolic events have been manifested in these patients [26, 27]. Likewise, and in support of vascular function, it has been reported that the ozone therapy favors the regeneration of the microcirculation and the gaseous exchanges (through the increase of the blood flow, a decrease of the blood viscosity and the platelet aggregation) and, at the same time, to reduce thrombotic and fibrotic processes [28].

Ozone has biological properties that could

allow its use as an alternative therapy in the different phases of SARS-CoV-2 infection. Ozone could stimulate the cellular and humoral immune systems, being useful in the early COVID-19 infection phase. Ozone improves gas exchange, reduces inflammation, and modulates the antioxidant system, so it would be useful in the hyper inflammation or cytokine storm phase, and in the hypoxemia and/or multi-organ failure phase [16, 29]. It has been observed how the markers of different biological processes are modified during the development of a pathology, in particular in COVID-19, it has been observed that there is an association between the progressive increase in markers of oxidative stress and inflammation, with an increase in levels of dimer-D and lactic acid as tissue damage, and that correlate with the gradual decrease of the enzymatic antioxidant system as well as the antioxidant function of the system (Fig. 5).

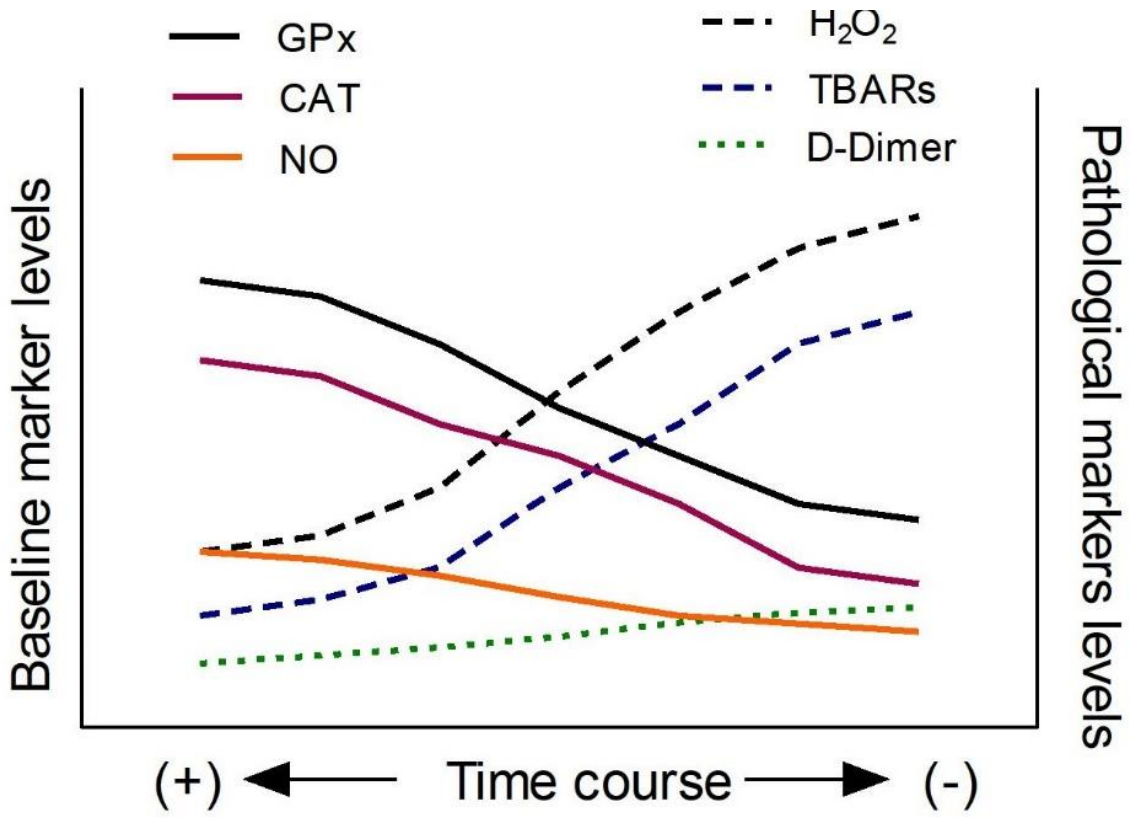

Figura 5. Modification of markers of oxidative stress, inflammation and cell damage in the course of COVID-19 disease. The image shows how the levels of oxidative markers (H_2O_2 and TBARs) decrease as the activity of antioxidant enzymes (CAT and GPx) increases. This can be correlated with the improvement of the antioxidant capacity of the system that tries to compensate for the oxidative effect generated by free radicals and that affect biomolecules. In addition to establishing that the improvement in markers such as nitric oxide correlates with the decrease in D-dimer and therefore in the functional recovery of the vascular system.

CONCLUSION

The administration of medicinal ozone generated an improvement of the redox system, as well as an inhibition of markers of inflammation and cell damage (D-dimer and lactic acid related to endothelial tissue injury), according to this, an improvement in the antioxidant capacity of the cardiovascular system would be established which system that reduces the risk of tissue damage. Therefore, ozone therapy could be effective in patients with COVID-19, and could be integrated into the treatment of vascular complications due to endothelial injury in these patients. Likewise, it is necessary to identify in which stages of the COVID-19 disease medicinal ozone can be used safely and effectively.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCIAS

[1]. Liu F, Han K, Blair R, Kenst K, Qin Z, Upcin B, *et al*., SARS-CoV-2 infects endothelial cells *in vivo* and *in vitro*. Front Cell Infect Microbiol. 2021; 11:1-13

[2]. Wang LL, Yang JW, Xu JF. Coronavirus (SARS-CoV-2) causes lung inflammation and injury. Clin Microbiol Infect. 2021; S1198-743X (21):00674-1.

[3]. Giordo R, Paliogiannis P, Mangoni AA, Pintus G. SARS-CoV-2 and endothelial cell interaction in COVID-19: molecular perspectives. Vasc Biol. 2021; 3(1):R15-R23.

[4]. Sardu C, Gambardella J, Morelli MB, Wang X, Marfella R, Santulli G. Is COVID-19 an endothelial disease? Clinical and basic evidence. Preprints. 2020: 2020040204

[5]. Cecchini R, Cecchini AL. SARS-CoV-2 infection pathogenesis is related to oxidative stress as a response to aggression. Med Hypotheses. 2020; 143:110102.

[6]. Borrelli E, Bocci V. Basic biological and therapeutic effects of ozone therapy in human medicine. Ozone Science and Technology - Encyclopedia of Life Support Systems (EOLSS), 2011.

[7]. Hernández A, Viñals M, Isidoro T, Vilás F. Potential role of oxygen-ozone therapy in treatment of COVID-19 pneumonia. Am J Case Rep. 2020; 21(e925849):1-6.

[8]. Díaz LJ, Macías AC, Menéndez CS. Modulator effect of ozone therapy over immune system activity. Rev Cubana Hematol Inmunol Hemoter. 2013; 29(2):1-12.

[9]. Bocci V. The Clinical Application of Ozonetherapy. Ozone. 2nd ed. 2010, 97-232.

[10]. Clavo B, Rodríguez-Esparragón F, Rodríguez-Abreu D, Martínez-Sánchez G,

Llontop P, Aguiar-Bujanda D, *et al*., Modulation of Oxidative Stress by Ozone Therapy in the Prevention and Treatment of Chemotherapy-Induced Toxicity: Review and Prospects. Antioxidants (Basel). 2019; 8(588):1-20.

[11]. Suhail S, Zajac J, Fossum C, Lowater H, McCracken C, Severson N, *et al*., Role of oxidative stress on SARS-CoV (SARS) and SARS-CoV-2 (COVID-19) Infection: A Review. Protein J. 2020; 39(6):644-656.

[12]. Iba T, Connors JM, Levy JH. The coagulopathy, endotheliopathy, and vasculitis of COVID-19. Inflamm Res. 2020; 69(12):1181-1189.

[13]. Izadi M, Cegolon L, Javanbakht M, Sarafzadeh A, Abolghasemi H, Alishiri G, *et al*., Ozone therapy for the treatment of COVID-19 pneumonia: A scoping review. Int Immunopharmacol. 2021; 92(107307):1-9.

[14]. Giunta R, Coppola A, Luongo C, Sammartino A, Guastafierro S, Grassia A, *et al*., Ozonized autohemotransfusion improves hemorheological parameters and oxygen delivery to tissues in patients with peripheral occlusive arterial disease. Ann Hematol. 2001; 80(12):745-8.

[15]. Yousefi B, Banihashemian SZ, Feyzabadi ZK, Hasanpour S, Kokhaei P, Abdolshahi A, *et al*., Potential therapeutic effect of oxygen-ozone in controlling of COVID-19 disease. Med Gas Res. 2022; 12(2):33-40.

[16]. Tommaso-Ranaldi G, Rocco-Villani E, and Franza F. Rationale for ozone-therapy as an adjuvant therapy in COVID-19: a narrative review. Med Gas Res 2020; 10(3):134-138.

[17]. Wiegman CH, Li F, Ryffel B, Togbe D, Chung KF. Oxidative stress in ozone-induced chronic lung inflammation and emphysema: A facet of chronic obstructive pulmonary disease. Front Immunol. 2020; 11(1957):1-13.

[18]. Guo J, Huang Z, Lin L, Lv J. Coronavirus Disease 2019 (COVID-19) and cardiovascular disease: A viewpoint on the potential influence of angiotensin-converting enzyme inhibitors/angiotensin receptor blockers on onset and severity of severe acute respiratory syndrome coronavirus 2 infection. J Am Heart Assoc. 2020; 7:9(7): 1-5.

[19]. De la Cruz-Enríquez J, Rojas-Morales E, Ruíz-García MG, Tobón-Velasco JC, Jiménez-Ortega JC. SARS-CoV-2 induces mitochondrial dysfunction and cell death by oxidative stress/inflammation in leukocytes of COVID-19 patients. Free Radic Res. 2021: 1-14.

[20]. Martínez-Sánchez G, Schwartz A, Donna VD. Potential cytoprotective activity of ozone therapy in SARS-CoV-2/COVID-19. Antioxidants. 2020; 9(389):1-12.

[21]. Hernández A, Viñals M, Pablos A, Vilás F, Papadakos PJ, Wijeysundera DN, *et al*., Ozone therapy for patients with COVID-19 pneumonia: Preliminary report of a prospective case-control study. Int Immunopharmacol. 2021; 90(107261):1-7.

[22]. Fernández-Cuadros ME, Albaladejo-Florín MJ, Peña-Lora D, Álava-Rabasa S, Pérez-Moro OS. Ozone (O_3) and SARS-CoV-2: Physiological bases and their therapeutic

possibilities according to COVID-19 evolutionary stage. SN Compr Clin Med. 2020: 1-9.

[23]. Sozio E, De Monte A, Sermann G, Bassi F, Sacchet D, Sbrana F, *et al*., CORMOR study Group. CORonavirus-19 mild to moderate pneumonia Management with blood Ozonization in patients with Respiratory failure (CORMOR) multicentric prospective randomized clinical trial. Int Immunopharmacol. 2021; 98(107874):1-8.

[24]. Tascini C, Sermann G, Pagotto A, Sozio E, De Carlo C, Giacinta A, *et al*., Blood ozonization in patients with mild to moderate COVID-19 pneumonia: a single centre experience. Intern Emerg Med. 2021; 16(3):669-675.

[25]. Tartari APS, Moreira FF, Pereira MCDS, Carraro E, Cidral-Filho FJ, *et al*., Anti-inflammatory effect of ozone therapy in an experimental model of rheumatoid arthritis. Inflammation. 2020; 43(3):985-993.

[26]. Naymagon L, Zubizarreta N, Feld J, van Gerwen M, Alsen M, Thibaud S, *et al*., Admission D-dimer levels, D-dimer trends, and outcomes in COVID-19. Thromb Res. 2020; 196:99-105.

[27]. Sakka M, Connors JM, Hékimian G, Martin-Toutain I, Crichi B, Colmegna I, *et al*., Association between D-dimer levels and mortality in patients with coronavirus disease 2019 (COVID-19): a systematic review and pooled analysis. J Med Vasc. 2020; 45(5):268-274.

[28]. Martínez-Sánchez G, Delgado-Roche L, Díaz-Batista A, Pérez-Davison G, Re L. Effects of ozone therapy on hemostatic and oxidative stress index in coronary artery disease. European Journal of Pharmacology, 2012. 691(1-3):156-62.

[29]. Gavazza A, Marchegiani A, Rossi G, Franzini M, Spaterna A, Mangiaterra S, *et al*., Ozone therapy as a possible option in COVID-19 management. Front Public Health. 2020; 8:4