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Original Article

Medical ozone therapy in HIV patients: Evaluation of two treatment cycles

[Ozonoterapia médica en pacientes con VIH: Evaluación de dos ciclos de tratamiento]

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Abstract

Context: Human immunodeficiency virus (HIV) infection and antiretroviral therapy (ART) induce oxidative stress, highlighting the need for bio-oxidative alternatives.

Aims: To evaluate the effects of two consecutive cycles of medical ozone therapy in HIV-seropositive patients and compare the results of both cycles using clinical indicators and redox biomarkers.

Methods: A quasi-experimental study was conducted following a research protocol, including 25 HIV-seropositive patients undergoing ART, who received medical ozone therapy via rectal insufflation (volumes of 150–300 mL in a stepwise manner) in 12 sessions over one month, with two cycles spaced three months apart. The initial and final evaluation included hematological and biochemical laboratory tests, absolute T-CD4+ lymphocyte count, viral load, and seven plasma redox balance indicators. Final variable values were compared with baseline values and a supposedly healthy individual group (SHIG). Adverse reactions were recorded.

Results: ART showed beneficial effects in 78% of cases, with no significant differences in hematological and biochemical variables ($p>0.05$) between the treated group at the beginning and end of the study. Significant differences ($p<0.05$) were observed in the mean values of malondialdehyde concentration, reduced glutathione, hydroperoxides, advanced oxidation protein products, and peroxidation potential at the end of the study compared to baseline values and SHIG.

Conclusions: The beneficial effects of two cycles of medical ozone therapy combined with ART demonstrated a 74% simultaneous modification in reducing oxidation of proteins and lipids and an increase in cellular immune indicators in the studied patients. No hematological or blood toxicity, adverse events, or drug interactions were observed.

Keywords: antioxidant status; HIV; oxidative stress; oxidative damage; ozone.

Resumen

Contexto: La infección por el virus de la inmunodeficiencia humana (VIH) y la terapia antirretroviral (TAR) inducen estrés oxidativo, lo que resalta la necesidad de alternativas biooxidativas.

Objetivos: Evaluar los efectos de dos ciclos consecutivos de ozonoterapia médica en pacientes VIH seropositivos y comparar los resultados de ambos ciclos mediante indicadores clínicos y biomarcadores redox.

Métodos: Se realizó un estudio cuasiexperimental siguiendo un protocolo de investigación, incluyendo a 25 pacientes VIH seropositivos en TAR, quienes recibieron ozonoterapia médica mediante insuflación rectal (volúmenes de 150-300 mL de forma escalonada) en 12 sesiones durante un mes, con dos ciclos separados por tres meses. La evaluación inicial y final incluyó pruebas de laboratorio hematológicas y bioquímicas, recuento absoluto de linfocitos T-CD4+, carga viral y siete indicadores plasmáticos del balance redox. Los valores finales de las variables se compararon con los valores basales y con un grupo de individuos supuestamente sanos (SHIG). Se registraron las reacciones adversas.

Resultados: La TAR mostró efectos beneficiosos en el 78% de los casos, sin diferencias significativas en las variables hematológicas y bioquímicas ($p>0,05$) entre el grupo tratado al inicio y al final del estudio. Se observaron diferencias significativas ($p<0,05$) en los valores medios de concentración de malondialdehído, glutatión reducido, hidroperóxidos, productos proteicos de oxidación avanzada y potencial de peroxidación al final del estudio, en comparación con los valores basales y la SHIG.

Conclusiones: Los efectos beneficiosos de dos ciclos de ozonoterapia médica combinados con TAR demostraron una modificación simultánea del 74% en la reducción de la oxidación de proteínas y lípidos, así como un aumento de los indicadores inmunitarios celulares en los pacientes estudiados. No se observó toxicidad hematológica ni sanguínea, eventos adversos ni interacciones farmacológicas.

Palabras Clave: daño oxidativo; estado antioxidante; estrés oxidativo; ozono; VIH.

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Abbreviations: AHT: arterial hypertension; AIDS: acquired immunodeficiency syndrome; ALP: alkaline phosphatase; APPO: advanced products of protein oxidation; AR: adverse reactions; ART: antiretroviral therapy; ARVs: antiretroviral drugs; cART: combination antiretroviral therapy; CAT: catalase; ESR: erythrocyte sedimentation rate; GGT: gamma-glutamyl transferase; GSH: reduced glutathione; HIV: human immunodeficiency virus; HPO: hydroperoxides; IPK: "Pedro Kouri" Institute of Tropical Medicine; MDA: malondialdehyde; MINSAP: Cuban Ministry of Public Health; MSM: men who have sex with men; NF-κB: nuclear factor kappa B; NO: nitric oxide; OI: opportunistic infections; OIs: opportunistic infections; OS: oxidative stress; PAHO: Pan-American Health Organization; PP: peroxidation potential; RI: reference interval; ROS: reactive oxygen species; SHI: supposedly healthy individuals; SHIG: supposedly healthy individual group; SOD: superoxide dismutase; VL: viral load.

INTRODUCTION

Human immunodeficiency virus (HIV) is the causative agent of acquired immunodeficiency syndrome (AIDS), first identified in 1983 (Cordeiro, 2008). This disease is characterized by a significant depletion of CD4+ T lymphocytes (T-CD4+) in the immune system, leading to an immunodeficient state evidenced by the onset of opportunistic infections (OIs) and associated malignant tumors (Cobas-Planchez et al., 2022).

Currently, HIV represents a global health issue due to its high prevalence, associated morbidity, and social impact. According to the Joint United Nations Programme on HIV/AIDS (UNAIDS, 2023), since the onset of the pandemic, 85.6 million people have been infected with HIV, and 40.4 million have died from related diseases. By the end of 2022, an estimated 39 million people worldwide were living with this disease, with 1.3 million new cases and 630,000 deaths associated with the condition (Carter et al., 2024). In Cuba, by the end of 2023, 32,000 people were living with HIV, with an estimated 6,000 deaths since the epidemic began in 1986 (PAHO, 2023). The implementation of a comprehensive and multisectoral care program by the Ministry of Public Health (MINSAP) focused on HIV prevention and treatment has placed Cuba among the countries in the Americas with the lowest prevalence (4 per 1,000 inhabitants), with approximately 1,500 cases diagnosed annually (Verdasquera Corcho et al., 2018).

Antiretroviral drugs (ARVs) have radically transformed HIV from a potentially fatal disease into a chronic condition. The reduction in HIV-related morbidity and mortality has been particularly pronounced since 1996 with the implementation of "*combination antiretroviral therapy*" (cART), which uses various ARV classes to significantly enhance effectiveness and reduce potential resistance (Lozano and Domingo, 2011; PAHO, 2023).

Since current ARVs cannot completely eradicate the infection, their primary goals are to reduce plasma viral load (VL), increase T-CD4+ count to restore immune function, prevent OIs and other associated complications, inhibit viral transmission, and improve patients' quality of life, enabling them to lead healthier and longer lives. However, several factors can significantly reduce ARV effectiveness. Key challenges include drug resistance, poor therapeutic adherence, and drug toxicity. One of the primary reasons for cART modification or discontinuation is toxicity, which may lead to adverse effects such as liver dysfunction, neutropenia, anemia, metabolic disorders, and cardiovascular diseases (Deavall et al., 2012; Hernández Espejel, 2022; Santos Corraliza and Fuentes Martin, 2006).

HIV pathogenesis is strongly linked to chronic inflammation and oxidative stress (OS), as evidenced by high plasma levels of pro-inflammatory cytokines and overproduction of reactive oxygen species (ROS) in seropositive individuals (Fang, 2011; Phaniendra et al., 2015; Soto Febles et al., 2002). This disruption in the cellular redox balance results from a chronic decline in endogenous antioxidant systems, leading to biomolecular damage, functional loss, and cell death (Curieses Andrés et al., 2023; Mañón Rossi et al., 2016). An oxidative environment promotes viral replication by stimulating transcription factors such as nuclear factor *kappa* B (NF-κB), which enhances HIV transcription, accelerating disease progression. Furthermore, OS contributes to the gradual and irreversible depletion of T-CD4+ cells due to increased apoptosis signaling (Dysangco et al., 2017; Kobayashi et al., 2016). This redox imbalance in seropositive patients may be further exacerbated by ARV toxicity. Mitochondrial dysfunction and metabolic disturbances are adverse reactions that contribute to ROS generation, thereby maintaining OS (Deavall et al., 2012; Palipoch and Koomhin, 2015; Sies and Jones, 2020). Given the chronic nature of HIV and the need for lifelong cART, implementing alternative therapies to mitigate OS in these patients is imperative (Lee, 2018). The use of medical ozone in clinical practice has significantly increased in recent decades, supported by numerous scientific studies (Dardes et al., 2017; Jafari-Oori et al., 2022; Michaudel et al., 2020; Tahmasebi et al., 2021; Yu et al., 2021) demonstrating its efficacy in treating various inflammatory, infectious, degenerative, cardiovascular, oncological, and metabolic diseases. The growing interest in ozone therapy stems from its broad pharmacological properties, including anti-inflammatory effects, antiseptic action, improved peripheral circulation, and modulation of the immune system and redox balance (Takon, 2018; Viebahn-Haensler and León-Fernández, 2023).

Due to its beneficial properties, high efficacy, and good tolerance, ozone therapy is emerging as a promising clinical treatment.

A previous study evaluated the effects of one cycle of medical ozone therapy combined with ARVs in HIV/AIDS patients. The goal of this combined therapy was to beneficially modulate plasma OS and assess treatment safety. Results indicated that rectal ozone insufflation enhances the antioxidant system, significantly reducing oxidative damage in patients. Moreover, it does not interfere with ARV pharmacological effectiveness; instead, it promotes an increase in T-CD4+ counts while demonstrating renal, hepatic, and hematological safety (Rabeiro-Martinez et al., 2023). Based on these findings, the present study aims to evaluate the effects of two consecutive cycles of medical ozone therapy via rectal insufflation in HIV-seropositive patients and compare the results of the first cycle using clinical indicators and redox biomarkers.

MATERIAL AND METHODS

Clinical study, design and conditions

A prospective quasi-experimental study was conducted with pre- and post-measurement following two cycles of medical ozone application. Patients received two cycles of ozone treatment via rectal insufflation. Each cycle consisted of 12 treatments administered over a four-month period between 2022 and 2024 (Fig. 1).

Adult patients diagnosed with HIV under antiretroviral therapy (ART), who were being treated at the "Pedro Kouri" Institute of Tropical Medicine (IPK) Hospital in Havana, Cuba, were selected.

The study was conducted in accordance with the state regulations of Good Clinical Practices for human trials in Cuba, as required by the Center for State Control of Drug Quality 2000, and the Declaration of the World Medical Assembly of Helsinki, updated at the 64th General Assembly in Fortaleza, Brazil, October 2013. As well as the Good Clinical Practice guidelines of the International Council for Harmonisation (ICH E6).

The protocol was reviewed and approved by the Specialized Scientific Commission and the Research Ethics Committee of IPK (Opinion CEIPK 012-20). All patients were previously assessed by their regular physician at IPK Hospital, who determined their eligibility for the study based on the established inclusion and exclusion criteria. They also determined the exclusion of any patient and/or discontinuation of the protocol if any exit criteria appeared. Individuals included in the study were informed of its characteristics, as well as the potential benefits and risks of the treatment, and signed an informed consent document. In each consultation, patients were informed of their laboratory test results. This research was part of a project associated with Program 5 of Transmissible Diseases of MINSAP with identification code: 2105025.

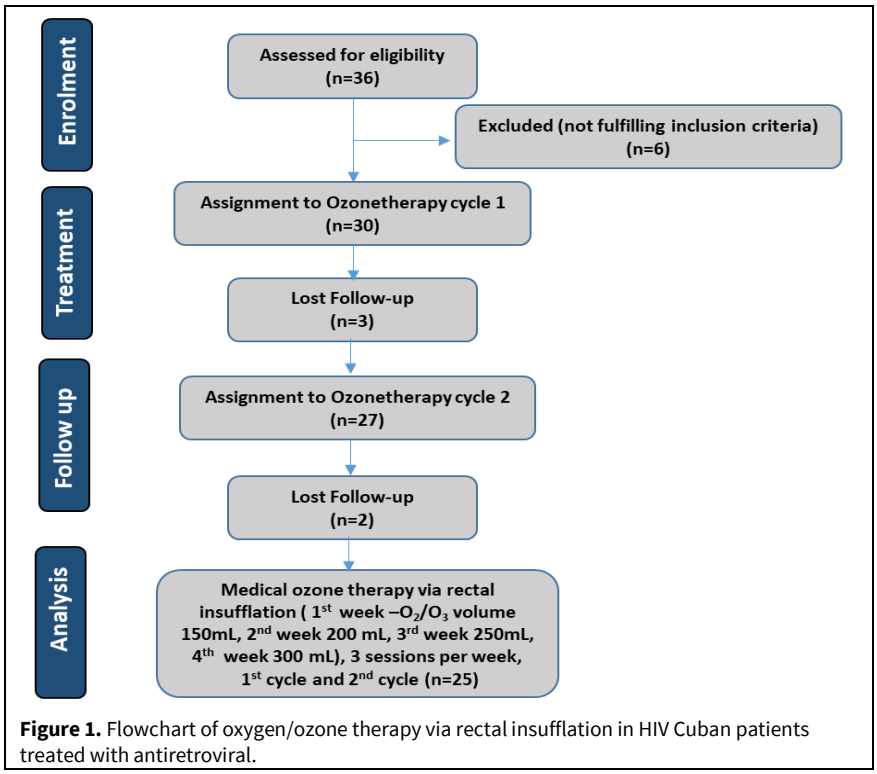

Figure 1. Flowchart of oxygen/ozone therapy via rectal insufflation in HIV Cuban patients treated with antiretroviral.

The doses of medical ozone applied in the study were justified based on their use in previous trials with other indications and satisfactory results, demonstrating low toxicity and good clinical efficacy associated with the treatment (Borroto, 2021). During the study, essential conditions were considered and ensured, as well as the availability of medical and scientific professionals trained in the detection, reporting, and appropriate treatment of adverse events and disease complications (García Milián et al., 2018; Santos Muñoz et al., 2018).

The research was conducted with a total of 25 patients, and for data analysis, 25 supposedly healthy individuals (SHI) matched in age, sex, and skin color to the ART treatment group were included to form a reference group related to redox state indicators and T-CD4+ lymphocyte count.

The preparation and application of medical ozone were carried out in the IPK operating room by a licensed nurse and a medical specialist, ensuring full compliance with biosafety standards.

Patients received two cycles of 12 ozone therapy treatments via rectal insufflation (3/week: Monday, Wednesday, and Friday) at 15 mg/L in volumes of 150-300 mL, gradually increasing according to the following scheme: 1st week (15 mg/L, 150 mL), 2nd week (15 mg/L, 200 mL), 3rd week (15 mg/L, 250 mL), and 4th week (15 mg/L, 300 mL). These volumes were drawn from the ozone generator using a syringe. The ozone/oxygen mixture was obtained from a medical-grade ozone generator (Ozonobaric P® class IIb certified).

For medical ozone application, patients were advised to defecate beforehand to minimize fecal content in the rectum. With the patient in the Sims position, an ozone-inert polyethylene catheter (30-40 cm long), lubricated, was slowly inserted 10-15 cm. The gas was slowly administered using a silicone-coated 50 or 100 mL syringe every 1-2 minutes to avoid discomfort. A Klemmer clamp was used after each insufflation to prevent gas leakage. Subsequently, the patient rested for 15 minutes' post-insufflation to prevent gas expulsion and allow ozone reaction with the biological medium.

Evaluation of the effect and safety of medical ozone application

The main variables considered to measure the effect of the intervention were the follow-up indicators lymphocyte (LT) CD4+ (Hernández-Reyes, 2013) and viral load (VL) (González-Alba et al., 2011), and redox indicators like malondialdehyde (MDA) (Erdelmeier et al., 1998), reduced glutathione (GSH) (Tietze, 1969), hydroperoxides (HP) (McLemore et al., 1998), advanced products of protein oxidation (APPO) (Witko-Sarsat et al., 1998), peroxidation potential (PP) (Özdemirler et al., 1995), nitrate/nitrite ratio (NO3-/NO2-) (Granger et al., 1996), superoxide dismutase enzymatic activity (SOD) (Marklund and Marklund, 1974), and catalase (CAT) (Clairborne, 1985).

Safety was evaluated by monitoring hematological and hemochemical parameters: hemoglobin, hematocrit, erythrocyte sedimentation rate (ESR), platelet count, neutrophil count, lymphocyte count, and monocyte count. These indicators were determined using an automated hematology analyzer ABX MICROS 60 (France), which allowed the diagnosis of samples and concentrates of whole blood components (Hernandez-Reyes, 2013). The Westergren method was used to determine ESR. Hemochemical parameters evaluated included creatinine, alanine aminotransferase, aspartate aminotransferase, cholesterol, triglycerides, glucose, gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), and total bilirubin. These determinations were performed using an automated clinical chemistry analyzer COBAS c311, at the IPK clinical diagnostic laboratory. All procedures used are validated by the International Federation of Clinical Chemistry. The calibrator (C.f.a.s.) and controls (Precinorm U, Precipath L) were lyophilized, based on human serum, and processed according to the directives established by the European Community for the use of samples with biological risk. The reagents used were from the Roche DIAGNOSTICS system for hemochemistry and the Horiba Medical system for hematology. The IPK Clinical Diagnostic Laboratory Department's procedure manual was used for all these determinations. A physical examination was performed on patients before and after each intervention, determining the occurrence of adverse reactions (AR). These adverse events were documented according to established reporting models that captured their manifestations.

Statistical analysis

Qualitative variable data were represented as frequencies and percentages and compared using the Chi-square test. Descriptive statistical parameters for quantitative variables, such as mean and standard deviation, were determined. Normality assumptions (Shapiro-Wilk test) and variance homogeneity (Levene's test) were checked to define the use of parametric or non-parametric tests. The parametric comparison test was applied if there was normal distribution and homogeneity of variances, using ANOVA with a Tukey post-test or otherwise, the Kruskal-Wallis test with a Dunn post-test. Statistical signifi-

cance was considered for a p-value <0.05. For immunological, virological, redox, hematological, and hemochemical indicators, an individual analysis was performed to determine the percentage of study patients who showed changes during the study period. A simultaneous modification analysis was also performed to determine the percentage of patients who had increased or decreased certain redox state indicators and whether there were statistically significant differences compared to the initial study value. Statistical analysis was performed using SPSS Statistics version 22.

RESULTS AND DISCUSSION

Initially, a total of 36 patients were evaluated, of which six were not selected to participate in the study because they did not meet the established selection criteria. To evaluate the combined effect of ART and ozone therapy during two cycles, two groups of subjects were chosen, one control and one intervention. The research was conducted with a total of 25 HIV patients, and for data analysis, 25 supposedly healthy individuals (SHI) matched in age, sex, and skin color to the ART treatment group were included to form a reference group related to redox state indicators and T-CD4+ lymphocyte count. All included patients successfully completed the number of ozone therapy sessions scheduled in the two cycles (Fig. 1).

The variables age, sex, and skin color were compared in both groups, and no significant differences were observed (p>0.05). Therefore, the homogeneity of these characteristics in the two study groups allows us to discard the influence of these factors during the analysis. The general characteristics of the subjects included in the study are presented in Table 1. The predominant sex was male, and the most common skin color was white, with a representation of 79% and 74%, respectively. These data correspond to the demographic characteristics of this population group reported in the literature (Fuentes Beltrán et al., 2019). In Cuba, the infection prevails mainly among white males, as the main source of transmission of the disease is through men who have sex with men (MSM) (Ochoa et al., 2024).

MSM constitute a key population group, representing almost half of the new infections in the region (Boakye et al., 2024). According to reports from UNAIDS in Western and Central Europe and Latin America, one of the main forms of transmission is anal intercourse, which is more predominant among MSM (UNAIDS, 2023). A descriptive study conducted between 2017 and 2021 in Santiago de Cuba reported a high prevalence in males of 91.9%, of which 55% were MSM (Reyes Mediaceja et al., 2023). An article published in 2022 analyzed the main characteristics of a group of HIV/AIDS patients in the municipality of Guanabacoa, Havana, in the period between 1986 and 2019. The results obtained showed a trend in males (83%), white skin color (51.9%), and homosexuality (62.4%) among the studied patients (Cobas-Planchez et al., 2022).

Table 1. General characteristics of the study groups.

Variable		SHI	HIV patients
N		25	25
Age (years) (M ± SD)		47.22 ± 4.52	48.74 ± 11.6
Sex	Male	18	17
	Female	7	8
Skin color	White	14	15
	Mixed	6	4
	Afro-descendant	5	6
Comorbidities	DM	-	3
	HTA	-	6
	Hepatitis	-	2
	Kaposi's sarcoma	-	-
	Hepatitis B virus	-	1
Time of diagnosis (years) (M ± SD)			15.79 ± 8.21
ART time (years) (M ± SD)			13.0 ± 5.94

Source: Data taken from the patients' medical records. Data are represented as mean ± standard deviation (M ± SD) for age, time of diagnosis, time of ART, and frequencies for sex, skin color, and comorbidities. Statistical tests for qualitative variables was Chi square, and for quantitative variables was t-Student for independent samples were used. N: number of individuals per group; SHI: supposedly healthy individuals; DM: diabetes mellitus; AHT: arterial hypertension; TAR: antiretroviral therapy; VHB: Hepatitis B virus.

The ages of the patients in the present study ranged from 26 to 66 years, and they presented arterial hypertension (AHT) as the most prevalent comorbidity. The age range of HIV patients in Cuba is broad; according to MINSAP (2019), the highest incidence is among men between 20 and 54 years of age. In recent years, there has been an increase in the age groups susceptible to infection, increasing the risk in people over 50 years, according to reports from WHO and UNAIDS (PAHO, 2023). Regarding comorbidities, it was observed that almost half of the patients had another condition, of which 66.6% suffered from AHT. The incidence of cardiovascular diseases in the general population has increased significantly over the years, becoming the number one cause of death today. The main risk factors are smoking and AHT, which are often more frequent in the HIV population. In addition, these patients have lipid and carbohydrate metabolism alterations due to ART and the negative effect of the infection on the endothelium, which further increases the risk of AHT (UNAIDS, 2023). In a retrospective and observational study by Pérez-Bastán (2020), the incidence of comorbidities was compared between an HIV group vs. the general population, observing a higher prevalence in cardiovascular conditions such as AHT, with 14%. Although this non-communicable disease has a high incidence in this population group, there are others, such as opportunistic infections (OI), that, in many countries, show a higher prevalence (UNAIDS, 2023). This is the case in many Latin American countries, as reflected in a review article on comorbidity data in Ecuador, which shows a higher incidence of cerebral toxoplasmosis, bacterial pneumonia, and pulmonary tuberculosis (Tumbaco-Quirumbay et al., 2021).

Regarding the time of HIV diagnosis, it ranged from 2 to 29 years, with an average time of 15 years. The majority of the included patients (68%) had known their serological status for more than 14 years. These results are similar to those obtained in an epidemiological study in São Paulo, Brazil, in which it was observed that most individuals had more than 10 years of diagnosis (Pimentel et al., 2024). These rates may be related to the early detection of the disease.

ART should be initiated in all patients with HIV infection, with or without symptoms, and regardless of the number of LT-CD4+. An exception is made for patients who maintain undetectable VL sustainably without ART (elite controllers). In this case, there is not enough information to assess the beneficial effect of ART, so a recommendation cannot be established (PAHO, 2023).

Currently, most patients with adequate adherence to ART treatment have a better quality of life, and those who have received ART for at least six months and have an LT-CD4+ count above 500 cells/mL have an improvement in survival, very close to that of the general population. Despite this decrease in HIV-related mortality, there is an increase in the proportion of deaths from other causes. In addition, with increased longevity, there has been an increase in comorbidities related to chronic non-communicable diseases such as diabetes mellitus, hypertension, dyslipidemia, and heart and kidney diseases, among others (Smith et al., 2013).

In the present study, patients had an average disease diagnosis time of 16 years, with a standard deviation of 8 years, in a range of 1 to 29 years, and an average time receiving ART of 13 years, with a standard deviation of 6 years, in a range of 1 to 21 years (Table 1). This time was similar to that presented in the study by Mendez et al. (2022), related to the profile and adherence to ART of people living with HIV/AIDS, which reported that most respondents (54.2%) had 10 or more years of HIV/AIDS infection, and 44.4% had more than 10 years of ART use.

The number of LT-CD4+ and VL constitute validated markers for monitoring the progression of HIV-caused disease. The former is related to the coordination capacity of the immune system response, and the latter to the quantification of viral RNA in plasma; the evaluation of both has a predictive character in the assessment of patients infected with this virus and, above all, for its usefulness in monitoring ART treatment (UNAIDS, 2023). Table 2 shows the mean LT-CD4+ values in patients before starting medical ozone treatment (T_0). These showed statistically significant differences ($p<0.05$) compared to the SHI values. However, when analyzing the behavior over time, the results showed a non-significant increase ($p>0.05$) after the application of medical ozone treatment (T_1) compared to T_0, but significant with the SHI group ($p<0.05$), preserving the initial immunological capacity and a significant modification after the second cycle T_2.

Regarding VL, it remained undetectable throughout the treatment. This shows that the application of medical ozone did not modify the effectiveness of ART in these patients. Previous studies support the use of ART with a beneficial influence on the number of LT-CD4+, reporting a stabilization or increase of this marker (González-Blanco et al., 2018). In Cuba, Hernandez-Reyes (2013) analyzed progression markers in elderly HIV/AIDS patients with ART, obtaining, at one year of treatment, a decrease in VL and an

increase in the number of LT-CD4+, regardless of the regimen used. In the present study, a significant increase in the number of LT-CD4+, similar to the study by Hernandez-Reyes (2013), was observed. Jointly, the analysis of the two progression markers shows that medical ozone does not interfere with the therapeutic effect of the ARVs used in the study. It is important to note that the treatment used managed to maintain an undetectable VL during treatment, which resulted in a potentiating effect on the number of LT-CD4+ cells.

In the evaluation of the general condition of the patients, the quantification of hematological parameters was used, and no statistically significant differences were detected ($p>0.05$) at T_0, T_1, or T_2 (Table 3). Both values for each variable were within the reference interval, except for ESR, which was higher in the case of T_0, showing a subsequent decrease in T_1.

Although the patients had AIDS, hematological function was not altered, as only ESR was higher than the RI values. This indicator is a non-specific marker that can be altered by various conditions, including infections, neoplasms, inflammatory and autoimmune diseases, and kidney damage (Simeon et al., 2024). The latter is one of the most frequent factors in this condition (Lungu et al., 2024). ESR has generally been observed with very high values (>100 mm/h) related to wear and tear from opportunistic infections or other comorbidities (Chandran et al., 2020).

The analyses of hematological values continue to be useful in the diagnosis and monitoring of different chronic pathologies, sepsis, malignant tumors, anemias, and leukosis, among other pathologies associated with the elevation of their levels (Valero-Cedeño et al., 2020).

Table 2. Mean values of the parameters related to progression markers in the studied groups.

Marker	SHI (M ± SD)	HIV patients	
		T_0 (M ± SD)	T_2 (M ± SD)
LT- CD4+ (cel/mL)	1283 ± 225	514.83 ± 206.51* [a]	730.21 ± 213.15* [ab]
Viral load (UI)	-	Undetectable	Undetectable

Source: Data taken from the patients' medical records. Data are represented as mean ± standard deviation (M ± SD) of n = 25/group. Statistical tests applied were ANOVA parametric variables and Tukey's post-test. For longitudinal studies in the case of parametric variables, t-Student's test for dependent samples. [a]Represents significant differences ($p<0.05$) between the LTCD4 values of HIV patients at the different extractions with respect to the ISS. [b]Represents significant differences ($p<0.05$) with T_0.

LT-CD4+: lymphocytes T-CD4+, SHI: supposedly healthy individuals, T_0: sample collection before treatment with medical ozone, T_2: sample after two cycles of medical ozone application.

Table 3. Mean values of hemochemical parameters in patients treated with medical ozone.

Variable	RI	T_0 (M ± SD)	T_1 (M ± SD)	T_2 (M ± SD)
Creatinine (µmol/L)	70.7-150.2	88.30 ± 21.61	81.78 ± 26.17	92.0 ± 16.74
Alanine aminotransferase (U/L)	0-50	21.80 ± 10.18	26.35 ± 19.29	28.12 ± 19.30
Aspartate aminotransferase (U/L)	0-45	22.61 ± 8.82	24.06 ± 6.64	28.94 ± 10.90
Cholesterol (mmol/L)	2.59-5.18	4.54 ± 0.92	4.28 ± 0.82	4.50 ± 0.90
Triglycerides (µmol/L)	0.678-1.86	1.55 ± 0.75	1.44 ± 0.73	1.26 ± 0.47
Lactate dehydrogenase (U/L)	200-400	249.56 ± 108.20	201.29 ± 68.80	216.78 ± 30.86
Glucose (mmol/L)	3.33-6.10	5.28 ± 1.42	5.33 ± 1.15	5.04 ± 0.68
Gamma-glutamyl transferase (U/L)	0-55	37.01 ± 24.39	32.16 ± 18.79	31.61 ± 20.24
Alkaline phosphatase (U/L)	53-128	113.5 ± 69.68	105.8 ± 53.66	103.4 ±45.69
Total bilirubin (µmol/L)	3.42-20.52	9.49 ± 4.41	23.9 ± 56.61	6.94 ± 3.48

Source: Data taken from the patients' medical records. Data are represented as mean ± standard deviation (M ± SD) of n = 25/group. Statistical tests applied were ANOVA parametric variables and Tukey's post-test, non-parametric variables Kruskal-Wallis and Dunn's post-test.

RI: reference interval, T_0: sample collection before treatment with medical ozone, T_1: sample after a cycle of medical ozone application, T_2: sample after two cycles of medical ozone application.

Table 4. Mean values of hemochemical and redox parameters in patients treated with medical ozone.

Variable	RI	T_0 (M ± SD)	T_1 (M ± SD)	T_2 (M ± SD)
Hemoglobin (g/L)	11.0-16.0	12.38 ± 4.76	12.43 ± 3.99	12.63 ± 5.13
Hematocrit (L/L)	0.35-0.50	0.437 ± 0.042	0.43 ± 0.03	0.437 ± 0.418
ESR (mm/h)	0-15	16.88 ± 12.99 *	13.47 ± 10.77	14.63 ± 9.13
Platelets (× 10^9/L)	150-350	251.10 ± 75.68	239.58 ± 55.23	241.53 ± 50.43
Monocytes (%)	3.0-15.0	6.50 ± 2.48	6.73 ± 1.83	4.20 ± 2.86
Lymphocytes (%)	17.0-48.0	37.46 ± 5.50	34.90 ± 8.49	32.61 ± 14.67
Neutrophils (%)	45.0-76.0	55.42 ± 5.45	55.01 ± 16.43	57.33 ± 7.73
Redox index	**SHI**	**T_0 (M ± SD)**	**T_1 (M ± SD)**	**T_2 (M ± SD)**
MDA (nmol/g Hb)	2.22 ± 0.19	3.87 ± 0.48 [a]	2.81 ± 0.36 [ab]	2.48±0.40 [ab]
HPO (µM)	117.4 ± 3.10	145.27 ± 14.88 [a]	123.61 ± 6.68 [ab]	106.42±3.50 [abc]
APPO (µM chloramine T)	13.59 ± 2.34	20.92 ± 2.62 [a]	15.70 ± 1.63 [ab]	13.92±0.93 [b]
NO−3/NO−2 (µM)	63.91 ± 4.80	44.28 ± 3.58 [a]	55.10 ± 4.39 [ab]	59.33±2.54 [bc]
GSH (µM/g Hb)	1163.00 ± 167.00	478.31 ± 81.20 [a]	676.30 ± 79.74 [ab]	729.37 ± 79.54 [abc]
CAT (U/mg Hb min)	149 ± 19.70	220 ± 27.08 [a]	138.06 ± 16.99 [b]	115.0 ±10.95 [abc]
SOD (U/mg Hb min)	3.04 ± 0.72	4.11 ± 0.82 [a]	2.94 ± 0.61 [b]	2.46 ± 0.38 [ab]
PP (µM)	6.84 ± 0.31	11.00 ± 0.84 [a]	8.98 ± 1.01 [ab]	7.33 ± 0.60 [ab]

Source: Data taken from the patients' medical records. Data are represented as mean ± standard deviation (M ± SD) of n = 25/group. Statistical tests applied were ANOVA parametric variables and Tukey's post-test, non-parametric variables Kruskal-Wallis and Dunn's post-test. *Values outside the reference interval; [a]Represents significant differences ($p<0.05$) with the SHI; [b]Represents significant differences ($p<0.05$) with T_0; [c]Represents significant differences ($p<0.05$) with T_1.

RI: reference interval, ESR: Erythrocyte sedimentation rate, T_0: sample collection before treatment with medical ozone, T_1: sample after a cycle of medical ozone application, T_2: sample after two cycles of medical ozone application, SHI: supposedly healthy individuals, MDA: malonyldialdehyde, HPO: hydroperoxides, APPO: advanced products of protein oxidation, NO−3/NO−2: nitrate and nitrite ratio, GSH: reduced glutathione, CAT: catalase enzyme activity, SOD: superoxide dismutase enzymatic activity and PP: peroxidation potential.

García Vázquez et al. (2021), when analyzing the clinical, immunological, and virological implications and relating them to elevated ESR values in HIV/AIDS patients, stated that it was not necessary to determine ESR in the monitoring of HIV-positive patients, as it did not seem to be a good marker of the deterioration of their clinical, immune, or viral situation. On the other hand, Menendez-Capote et al. (2012) used the global lymphocyte count, hemoglobin levels, and ESR in the monitoring of ART in a group of HIV/AIDS patients in Angola and found a statistically significant relationship with the number of LT-CD4+, concluding that this variable could be used as an adequate parameter in the initiation and monitoring of ART in low-income countries, as it presented high specificity and positive predictive value when compared with other standardized variables of immunological deterioration (Menendez-Capote et al., 2012).

In the present study, hematological indicators, including ESR at T_1 and T_2, were not modified, so the combined use of ART and medical ozone did not interfere with the functionality and proportion of blood cells.

When analyzing the hemochemical variables, which are reflected in Table 4, it was detected that GGT and ALP at T_0 were higher than RI in 44% of patients, which shows alterations in liver damage markers. At T_1, no statistically significant differences were observed in these variables ($p>0.05$), although a decrease in the mean values of all variables was observed. When analyzing the values with RI, it was appreciated that these are within the intervals considered normal. These hemochemical indicators are usually recommended as follow-up tests in individuals who use different drugs, including those involved with new therapeutic options. This allows for the evaluation of their effect on general metabolism and performing the necessary benefit-risk balance for decision-making according to the results (Lozano and Domingo, 2011). Several authors recognize that HIV and ART can cause dyslipidemia in infected people, aspects that can affect the quality of life and treatment adherence and constitute risk factors for other degen-

erative diseases (Achila et al., 2022; Lopera-Rodríguez and López-Quiceno, 2021).

Elevated levels of serum liver enzymes (alanine aminotransferase, ALP, and GGT) are the expression of alterations at the level of liver cells or bile ducts. A predominance in the elevation of aminotransferases commonly indicates hepatocellular damage; however, an increase in ALP and GGT levels indicates cholestatic damage. Clinically, patients with cholestasis present itching, fatigue, and, in severe forms, jaundice, which is detected by elevated serum bilirubin levels. However, in the early stages of liver damage, symptoms may be absent, and only increases in ALP or GGT indicate a cholestatic condition (Deavall et al., 2012).

The results of the present research did not show statistically significant differences ($p>0.05$) for any of the variables in the T_0 group with respect to T_1 or T_2. In the case of GGT and ALP, it is evident that medical ozone has a beneficial effect by decreasing the values of the indicators after treatment in 22% and 33%, respectively, of patients who initially presented this alteration. This result coincides with the report by Takon et al. (2018), who studied the hepatoprotective effects of medical ozone in patients with rheumatoid arthritis treated with methotrexate, observing a decrease in GGT levels to values close to RI, with the inclusion of medical ozone as combination therapy.

The monitoring of patients through clinical laboratory bioindicators and through disease progression markers, which include hematological and hemochemical investigations, constitutes an indispensable tool in decision-making for the initiation and active evolutionary monitoring of the disease, with great importance in medical practice (Catumbela et al., 2013).

In the present study, minimal alterations were observed in the evolution of a group of HIV patients treated with ART and medical ozone. There was no toxicological or pathological impact, which shows an alteration in the renal, hepatic, and metabolic safety of the combination. Compliance with follow-up guidelines through monitoring and appropriate clinical management, with an assessment of the benefit-risk balance in the prolonged use of drugs, constitutes an emerging issue in patient care, which was fulfilled in this study, according to the proposed protocol. The results presented show the usefulness of laboratory test monitoring in the evaluation of the evolution of HIV/AIDS infection and its treatment for the evaluation of the appropriate benefit-risk balance in the management of these patients. No adverse effects were reported in patients treated with medical ozone. The results of the analysis of ER indicators in the two groups are shown in Table 4. At T_0, compared to the SHI group, statistically significant differences were observed ($p<0.05$) in the means of SOD, CAT, and serum levels of GSH, MDA, APPO, PP, HPO, and NO. The means of MDA, GSH, PP, and NO at T_1 and T_2 showed statistically significant differences with SHI, and the means of MDA, HPO, APPO, GSH, CAT, SOD, PP, and NO of this same group with T_0. 50% and 56%, respectively, of patients who started combination therapy beneficially modified SOD and GSH values, 78% modified PP values, 86% NO values, 88% HPO values, 94% MDA and APPO values, and 100% modified CAT values, attributable to medical ozone.

When there is an endogenous stimulus, in this case, a pathogen like HIV, which sustains the production of ROS, this could affect the formation of cytotoxic products that influence cellular and systemic communication and damage (Lin, 2024). In HIV infection, a chronic inflammatory state occurs that potentiates the increase in viral replication, which would contribute to the weakening and wear of the immune system, activating the T cell apoptosis process (Mu et al., 2024).

All this is also related to the metabolism of some drugs that could affect both the therapeutic effect and the toxic effects (Foka and Mufhandu, 2023). In the case of HIV/AIDS patients, there is molecular and clinical evidence of a chronic inflammatory state related to sustained OS (Gil-del Valle et al., 2022; Gravier Hernández et al., 2022). In previous decades, during the natural course of infection (without ART), the associated morbidity and mortality were high worldwide (Boyd et al., 2020).

The results of the present study showed that the T_0 group was characterized by a redox imbalance, which could be influenced by the use of ARVs and the infection itself, as reported in certain previous studies (Gravier et al., 2014). However, with the use of the ozone-ART combination, a decrease in the values of some redox indicators related to damage and an increase in antioxidants was obtained, which could be attributed to ozone as a therapeutic complement with antioxidant activation properties (Viebahn-Haensler and León-Fernández, 2023).

In the HIV group at T_0, redox values were higher than the mean SHI values, except for NO, a product of imbalance caused by the virus and ART. A decreased secondary antioxidant capacity (GSH and PP) and increased oxidative damage (MDA, HPLO, APPO) were observed, both statistically significant ($p<0.05$) with respect to the SHI group. In antioxidant enzymes, values higher than those of the SHI group were observed. This aspect may be related to the sustained presence of oxidized metabolites and ROS

generated in the context of infection and ART, which chronically activate these ROS (Harshithkumar et al., 2024). In other reports of HIV patients, a decrease in these enzymes is evident (Monday et al., 2022). This could be related to the active presence of the pathogen in detectable amounts, which inhibits the expression process of the enzymes (Shytaj et al., 2020). In the case of the patients studied, there is a low undetectable VL, so it is assumed that there is also low replication, as it is inhibited by ART, and therefore, the expression of enzymatic proteins should be lower. Similar results have been observed in another study that carried out a comprehensive evaluation of redox biomarkers in HIV/AIDS patients. This showed statistically significant differences ($p<0.05$) between HIV patients and the control group (SHI) regarding SOD and CAT enzymatic activity and GSH and MDA serum levels during two years of follow-up (Gonzalez-Blanco et al., 2018).

In contrast, in the T_1 and T_2 groups, a decrease in some of the redox indicators was observed, except for the increase in NO, and similar to the SHI values for HPO, APPO, CAT, and SOD. As a consequence of the interaction of ozone with the biological medium and the production of low-concentration oxidized metabolites, stimulation of primary antioxidant mechanisms occurs (Viebahn-Haensler and León-Fernández, 2023). This influences the preservation or recycling of GSH, which increased at T_1 and T_2 compared to T_0. This influences the decrease in oxidative events in biomolecules, which manifest with low APPO, MDA, and HPO values. Probably, the O_2^- concentration decreases due to an increase in SOD1 or SOD2 (intranuclear and intracellular), and by not interacting with NO, its bioavailability increased, and the presence of $ONOO^-$ (not evaluated) should decrease, also influencing the low biomolecular oxidation observed at T_1 and T_2 by other indicators (MDA, HPO). As a consequence of the decrease in plasma oxidants, primary antioxidant capacity (inducible, extracellular) should decrease, which is reflected in the low SOD and CAT values and the increase in total antioxidant capacity (decrease in PP) (Lin, 2024). Similar results, in some aspects, were obtained in other studies with antioxidant supplementation with *Spirulina platensis* for three months at a dose of 5 g/d in 73 women infected with HIV, observing an increase in GSH and total antioxidant capacity in serum, as well as a decrease in MDA, HPLO, and APPO oxidation indicators (Gil-del Valle et al., 2018). The antioxidant/oxidant imbalance that occurs in HIV infection contributes to the increase in oxidative biomolecular deterioration of proteins, lipids, carbohydrates, and nucleic acids, as well as the decrease in antioxidant capacity and the alteration of antioxidant enzyme activity (Marrocco et al., 2017). These studies demonstrate the modulation of OS by bio-oxidative agents with an increase in global antioxidant capacity and a decrease in oxidative damage to biomolecules, such as lipids. Therefore, the combination of ART with bio-oxidative or antioxidant alternatives can influence the beneficial modulation of the redox state.

An integral analysis of redox indicators (MDA, HPO, APPO, NO, GSH, CAT, SOD, and PP) significantly modified at T_1 and T_2 was performed (Fig. 2). It was found that 74% of patients with ozone-ART simultaneously modified oxidative damage indicators (MDA, HPO, APPO, and NO), antioxidants (GSH, CAT and SOD) and CD4 count (Fig. 3). Similar results were observed in a study conducted in South Africa, which analyzed the effect of red palm oil on OS reduction in HIV patients co-infected with TB, as this oil had antioxidants with the ability to reduce oxidation and influence disease progression (Oguntibeju et al., 2010).

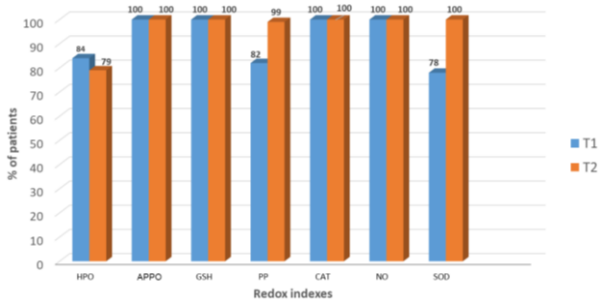

Figure 2. Global analysis of redox indicators that changed significantly in T1 and T2.

MDA: malondialdehyde; HPO: hydroperoxides; APPO: advanced products of protein oxidation; NO: nitric oxide; GSH: reduced glutathione; CAT: enzymatic activity of catalase; SOD: enzymatic activity of superoxide dismutase; and PP: peroxidation potential. T1: sample collection after the start of the application of medical ozone; T2: sample collection after the completion of the second ozone cycle.

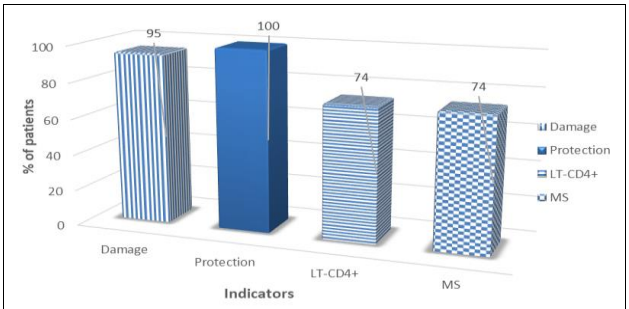

Figure 3. Percentage of simultaneous changes in HIV patients after two ozone cycles.

Damage: indicators of oxidative damage; Protection: indicators of antioxidant protection; LT-CD4+: number of CD4+ T lymphocytes; MS: simultaneous modification of the indicators; T2: sample collection after the second ozone cycle has concluded.

During treatment and follow-up, none of the participants were hospitalized. After rectal insufflation sessions, patients reported a feeling of stomach full-

ness and hunger after therapy. In both cases, the sensations disappeared over time, and treatment was not required. Regarding safety, no renal, hepatic, or blood toxicity was detected, no drug interaction with ART was observed, nor were there severe or life-threatening ARs.

The present study has some limitations, including a limited number of patients who were included due to the required inclusion criteria. The group of patients presented other chronic comorbidities such as AHT, also contributing to redox alteration, so the stimulation period (as a single initial cycle of rectal insufflation is called) results from induction, which in chronic conditions requires several interventions to influence the antioxidant system globally and sustainably (Borroto, 2021).

In the included patients, different ART combinations were administered, so some may have been more exposed to oxidation if they used non-nucleoside reverse transcriptase inhibitors (more mitochondrial toxicity, more ROS) or protease-boosted inhibitors, or integrase strand transfer inhibitors (less mitochondrial toxicity, less ROS). The information obtained was from a real HIV group. Although the group was homogeneous with respect to SHI in terms of sex, age, and skin color, this HIV group presented diverse characteristics representative of the Cuban HIV population (in terms of sex, skin color, different diseases, and ART combinations).

CONCLUSION

ART showed adequate effectiveness in HIV/AIDS patients during the study period without therapeutic interaction with medical ozone. Medical ozone produces a beneficial modulation of oxidative damage indicators with an increase in antioxidant capacity, with two application cycles, and was safe for renal, hepatic, and hematological systems without adverse events during the study period in these patients.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Concepts or ideas	x		x								x	x		x	
Design		x	x								x	x		x	
Definition of intellectual content		x		x										x	
Literature search	x					x	x		x	x			x	x	x
Clinical studies	x	x	x	x	x										
Experimental studies	x	x	x			x	x	x	x	x	x	x	x	x	
Data acquisition	x	x						x			x	x		x	
Data analysis		x											x	x	
Statistical analysis	x													x	
Manuscript preparation	x	x									x		x	x	x
Manuscript editing			x								x	x			x
Manuscript review	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

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