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Research Article

Medical Ozone Advantage effects as Complementary Treatment to the Antiretroviral Therapy in HIV Patients

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Abstract

Background/Aim: Oxidative stress (OS) has been demonstrated previously in HIV. Medical ozone (O3) has shown clinical efficacy in different diseases where OS is pathophysiological involved. This study characterizes the O3 effects as complementary treatment to antiretroviral therapy (ART) in HIV Cuban patients.

Material and Methods: Prospective quasi-experimental study was carried out, with blood extraction before and after the application of one O3 cycle by rectal insufflation during 5 weeks in 20 HIV patients under ART. The clinical response of the patients was evaluated by comparing the final values of the virological, immunological, hematological, biochemical and OS indexes respect to the baseline values, also were compared to a supposedly healthy volunteer (SHV).

Results: No significant differences were observed in the hematological and biochemical parameters evaluated ($p>0.05$) in the treated group at baseline and end of the study, with the exception of erythrocyte sedimentation rate. At initial significant differences ($p<0.05$) were observed for malondialdehyde, advanced oxidation protein product (AOPP), peroxidation potential, hydroperoxide (HPO), glutathione, catalase (CAT), superoxide dismutase (SOD) and nitric oxide with respect to SHV. After the O3 cycle the AOPP, HPO, CAT and SOD median values, did not show significant differences ($p>0.05$) respect to SHV. All the redox parameters improved significantly ($p>0.05$) at 8th weeks in relation to the baseline values of the group, also CD4+ T cells increased ($p<0.05$).

Conclusions: The beneficial effects of medical ART-ozone combination, in terms of OS, were demonstrated in the studied patients, without hematologic or blood toxic influence, no pharmacological interactions and non-adverse reactions was observed.

Keywords: antiretroviral therapy; medical ozone; oxidative stress; HIV/aids

1.Introduction

Therapies that use medical ozone as a basis for the resolution and improvement of different pathophysiological conditions have been recognized for their benefits. These medical benefits are related to the biological effects that ozone has in regulating oxygen metabolism, modulating the biological redox balance and the immune system, as well as its antimicrobial properties [1, 2].

Pandemic of human immunodeficiency virus and acquired immunodeficiency syndrome (HIV/aids) continues to be a major global public health issue, having claimed 40.4 million (32.9-51.3 million) lives so far.[3] There is currently no cure for HIV infection and there were an estimated 39.0 million people living with HIV at the end of 2022 [4]. In Cuba, it is estimated that the total number of cases diagnosed with HIV until end 2022 end is 38 688, of this total number of cases 31 112 are alive [5]. HIV mainly affects the immune system cells, disturbing their normal function. In this way, peoples infected with the virus have a higher risk of contracting opportunistic infections, certain types of cancer or presenting

other clinical manifestations than people with a healthy immune system [6]. The introduction of antiretroviral therapy (ART) have decreased dramatically HIV-infected patients morbidity and mortality [7].

Evidences show that HIV infection triggers pronounced oxidative stress in both laboratory models and the context of *in vivo* infection [8, 9]. HIV induces oxidative metabolism by deregulation of redox pathways with escalation of reactive oxygen species (ROS) production and by inducing mitochondrial dysfunction [10]. In the other hand the ART series of studies reported an increase in oxidative tone additional to the persistent redox imbalance associated with HIV infection, manifested by an increase in oxidants and a decrease in antioxidant serum levels [11].

Oxidative stress is in fact closely related with the developed of several human diseases, such as inflammation, diabetes, arteriosclerosis, autoimmune disorders, skin diseases, hypertension, cancer, infectious diseases, among others[12-15] and the maintenance of a proper redox balance seems to be a critical step in order to improve the clinical outcome [1, 16].

Administration of rectal ozone insufflation is a procedure with efficacy and safety have been scientifically proven[17]. However, no study investigating the clinical and redox characterization at the end of one cycle of ozone treatment in HIV patients has yet been performed. Taking into account the good clinical response achieved in several disease treated with more than one ozone cycle, the aim of this study was to assess the effect a single ozone cycle application (by rectal insufflation) as a complementary treatment to ART used in HIV/aids patients.

2.Material and methods

2.1 Study design

A prospective quasi-experimental study was carried out, with measurements before and after the application of one ozone cycle in HIV/aids patients. The study was approved by both institutional and national review committees (Scientific Committee and Ethics Committee of the "Pedro Kourí" Institute (IPK), Cuban Ministry of Public Health (code number: 2105025), in compliance with the principles of the Declaration of Helsinki (2013 version) and the Good Clinical Practice Guidelines of the International Conference on Harmonization. All patients gave their informed consent to enrolment after receiving adequate information concerning the study (characteristics, benefits and possible side effects). Before enrolment, all participants attended a training program to familiarize them with the study objectives and treatment plans.

2.1.1 Inclusion criteria: Adult individuals (≥18 and ≤60 years) of any gender with an HIV/aids diagnosis who have been receiving ART for five or more years were included. In addition, patients with absolute CD4+ T cells number ≥50 cell/mL and HIV viral load ≤50000 copies/mL were considered for inclusion.

2.1.2 Exclusion criteria: Individuals receiving another investigational product, pregnant or lactating, uncontrolled hyperthyroidism, analytically proven glucose-6-phosphate-dehydrogenase deficiency, acute infection at the time of uptake, oncological pathologies or psychiatric illnesses involving incompetence of the subject were excluded.

2.1.3 Exit criteria: Individuals who express their desire to leave the study, non-compliance with treatment and clinical follow-up in consultation, appearance of any of the exclusion criteria, appearance of serious adverse reactions related to treatment, death of the patient or deterioration of the clinical condition that, in investigator opinion, prevents further administration of study treatment. Patients who discontinue treatment will form part of the study and will be taken into account when analyzing the final data.

The study was assessed in the period from January 2021 to May 2022. The participants assisted to specialized clinic of IPK Hospital, where were included into the study. Non-probabilistic convenience selection was applied to identify the patients based on the inclusion criteria. In addition, a group of 20 supposedly healthy volunteers (SHV) age/gender related to HIV/aids group was recruited to consider it as physiological values for redox state indicators in relation 1-1 to HIV patients.

The patients receiving one cycle of treatment with 12 ozone applications by rectal insufflation (3 times a week) using concentration of 15 mg/L and volumes increasing from 150 mL to 300 mL according to following schedule:[18]

Cycle of medical ozone: 1st week (15 mg/L, 150 mL: dose 2,25 mg); 2nd week (15 mg/L, 200 mL: dose 3,00 mg); 3rd week (15 mg/L, 250 mL: dose 3,75 mg); 4th week (15 mg/L, 300 mL: dose 4,50 mg).

Rectal insufflation was done using ozone resistant syringes and siliconized rectal catheter, according the procedure described in the Madrid Declaration [18]. The preparation and application of the ozone/oxygen mixture was carried out in a clinical environment at the IPK Hospital, by the nurse and the specialized physician in angiology and vascular surgery, with full compliance about biosafety standards. Medical professionals were instructed to report all adverse reactions, whether described in relation to the studied products or not. The ozone/oxygen mixture was generated from an ozone generator for medical use (Ozonobaric P class IIb certification, SEDECAL, Spain), which contain a build-in spectrophotometer to get precise ozone concentration.

2.1.4 Outcome measures

The main variables that were taken into account to measure the effect of the intervention were the infection follow-up indicators CD4+ T cells count and HIV viral load[19]. The redox indicators were using also to characterize systemic antioxidant capacity and damage degree as accrual target of ozone[20]. Safety was evaluated by monitoring some hematological and biochemical parameters and also appearance of side effect. Physical examination was performed on patients before and after each intervention, searching the possible evidence of side effects.

2.2 Quantification of laboratory parameters

All laboratory indexes were obtained using validated analytical techniques for diagnosis in humans according to international recommendations[21]. Reference values were established in a healthy supposedly population considered as a control. Venous blood collection was performed before starting ozone treatment (considered first at baseline) and 8 weeks (one cycle of treatment according to de Madrid Declaration)[18] after ozone initial treatment.

2.2.1 Quantification of HIV infection progression markers (22)

CD4+ T cells count: for each T lymphocyte subsets TM CD3 CD4 were used. These analyses were performed on a Cyflow Space Cytometer (PARTEC GmbH, Münster, Germany) by FloMax 2014 version 2.9.4 program.

HIV viral load: was determined following the manufacturer's recommendations of the Biomerieux polymerase chain reaction ultrasensitive assay (PCR-NASBA, France) with the lower limit of quantification of 50 copies/mL. NUCLISENS® EASYQ® is a specific isothermal method combining NASBA amplification and real-time detection using molecular beacon probes.

2.2.2 Quantification of hematological and biochemical parameters

2.2.2.1 Hematological parameters such as hematocrit, hemoglobin, erythrocyte sedimentation rate (ESR) and platelets number were screened by hematological counter ABX MICROS 60 (Horiba Medical, Japan).

2.2.2.2 Biochemical parameters such as triglycerides, creatinine, cholesterol, glucose, alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase (GGT), alkaline phosphatase (AP), and total bilirubin were performed by standard procedures in HITACHI analyzer Cobas c311 (Roche, Germany), all in a specialized laboratory of IPK Hospital.

2.2.2.3 Quantification of redox state indicators

For assay of Superoxide dismutase (SOD) and Catalase (CAT) erythrocytes lysate was uses and results were expressed by mg of hemoglobin. For the rest of analysis, 3 mL of serum were employed. Serum samples were frozen at -70 °C and protected from light exposure until analyses were carried out. [20]

All redox parameters were determined by spectrophotometric methods using Zuzi Spectro-photometer from Japan.

Serum reduced glutathione (GSH) was determined for spectrophotometry after the reaction with 5.5'-Dithiobis (2-nitrobenzoic acid) (DTNB). All of the non-protein sulfhydryl groups are in the form of reduced glutathione. DTNB is a disulfide chromogen that is readily reduced by sulfhydryl compounds to an intensely yellow compound. The observance of the reduced chromogen is measured at 412 nm and is directly proportional to the GSH concentration. GSH (Sigma, St. Louis, M.O., USA) was used to generate standard curves[23].

Malondialdehyde (MDA) concentrations were analyzed with the LPO-586 kit obtained from Calbiochem (La Jolla, C.A., USA). In this assay, stable chromophore production after 40 min of incubation at 45 °C is measured at a wavelength of 586 nm by Pharmacia Spectrophotometer. Concentrations of MDA in serum samples were calculated using the corresponding standard curve and values were expressed as nmol g^{-1} Hb [24].

For the determination of the susceptibility to lipid peroxidation, serum samples were incubated with a solution of cupric sulfate (final concentration of 2 mM) at 37 ºC for 24 h. Then, MDA concentration was determined at 587 nm using the method described previously. Peroxidation Potential (PP) was calculated by subtracting the MDA concentration at time 0 from the one obtained at 24 h[25].

Total hydroperoxide (HPO) was measured by Bioxytech H_2O_2-560 kitCat.21024 (Oxis internacional Inc. Portl and, USA). The assay is based on the oxidation of ferrous ions to ferric ions by hydroperoxides under acidic conditions. In this assay peroxide first reacts with sorbitol (which provides sensitivity enhancement), converting it to a peroxyl radical, which in turn initiates Fe^{2+} oxidation to Fe^{3+}. Ferric ions bind with the indicator dye xylenol orange (3.3'-bis(N,N-di(carboxymethyl)-aminomethyl)-o-cresolsulfone-phatein, sodium salt) to form a stable colored complex which can be measured at 560 nm[26].

Superoxide dismutase (SOD) activity was measured by the method suggested by Marklund. This method utilizes the inhibition of auto-oxidation of pyrogallol by SOD. In this method the autoxidation of pyrogallol was investigated in the presence of EDTA at pH 7.9 the reaction is inhibited to 99% by superoxide dismutase [27].

Catalase (CAT) activity was measured according with the method of Clairbone. The initial absorbance decrease rate at 240 nm was monitored at 30 °C. One unit of this enzyme is defined as the activity to consume 1 µmol of hydrogen peroxide per minute. Using a molar extinction coefficient of 43.6 M^{-1} cm^{-1}, the rate of the first 30 s was used to calculate the activity. Catalase activity was expressed as U mg^{-1} Hb [28].

Advanced oxidation protein products (AOPP) serum was measured according to the methods of Witko-Sarsat *et al*.[29]. Chloramine-T solution that absorbs at 340 nm in the presence of potassium iodide was used to generate calibration curves. Absorbance of the reaction was read at 340 nm against a blank containing phosphate-buffered saline. The values were expressed in chloramine T equivalents and corrected by serum albumin concentrations.

Nitrite/nitrate levels as a measure of Nitric Oxide (NO) were determined by the Griess reaction after first converting nitrates to nitrites using cadmium activation [30].

2.3 Treatment follow-up

Adverse reactions are defined by the WHO as noxious and unintended responses to drugs at standard doses. The adverse reactions surveillance was assessed by nurses and physicians that were specialized in pharmacovigilance following the symptoms and sensations after ozone application. In the case that adverse reactions was identified the model 33-36-03 of Cuban Ministry of Public Health had to be fulfilled and sent to principal investigator and hospital pharmacotherapy committee.

2.4 Statistical analysis

The OUTLIERS preliminary test for detection of error values was initially applied. Multiple comparison tests were used (t student, Wilcoxon, ANOVA and Kruskal-Wallis test) to analyze data. Results are presented as means ± standard deviation of the mean and categorical variables were expressed as proportions. The normality of variables was evaluated by the Kolmogorov-Smirnov test. The level of statistical significance used was at least $p<0.05$. In addition, an individual analysis was made reporting the percentage of patient that evidences the significant modification in each variable. The IBM® SPSS® Statistics version 22.0 and GraphPad Prism 5 were used for all statistical analyses.

3.Results

Were evaluated 25 patients, of whom 20 were included in the study as they met the selection criteria established in the research protocol. All the patients included completed the number of ozone therapy sessions programed.

General characteristics of the patients involved in the study are showed in (Table 1). Men were the predominated gender as well as white were the skin color of most frequency. Patients between 40 and 60 years of age and with comorbidity of arterial hypertension predominated. Average time of HIV diagnostic and antiretroviral treatment was prevalent ten years. The

SHV and treated groups showed no differences (p>0.05) for the variables age, gender and skin color.

Demographic data		**SHV**	**HIV patients**
Age (years)		47.2 ± 4.5	42.6 ± 11.0
Gender	Male	15	16
	Female	5	4
Skin color			
	White	14	14
	Brown	4	3
	Black	2	3
Comorbidities		-	9
Hypertension		-	3
DM		-	1
Hepatitis		-	4
Kaposi's sarcoma		-	1
Bronchial Asthma		-	1
Years since diagnosis		-	15.3 ± 9.2
Years exposure to ART		-	11.8 ± 6.5

Table1: Results are presented as means ± standard deviation, n=20**.** No significant differences were found (p>0.05) by statistical test (T test for no related samples). SHV, supposedly healthy volunteers; DM, diabetes mellitus; HIV, human immunodeficiency virus; ART, antiretroviral therapy.

Table 1: Clinical characteristics of patients included in the study.

The mean values of CD4+ T cells in the patients before starting the treatment with medical ozone (week 0) was 381.9 ± 208.9 cell/mL (range 63-791 cell/mL), while eight weeks after of the application with medical ozone (weeks 8) the value obtained was 455.6 ± 215.3 cell/mL (range 159-828 cell/mL) showed significant increase (p<0.05) compared at week 0. Regarding the HIV viral load, it remained undetectable throughout the treatment.

Hematologic indexes obtained before and after the intervention are shown in (Table 2). When analyzing these values, it was identified that the majority were within the reference interval and without significant differences between week 0 and weeks 8. Nine patients had values of ESR higher than the reference interval (RI) at week 0, subsequently observing a significant decrease (p<0.05) of them at weeks 8, that´s were within the reference interval.

Indexes	**Reference interval**	**W_0**	**W_8**
Hemoglobin (g/L)	11.0-16.0	14.42 ± 1.38	14.39 ± 1.04
Hematocrit (%)	0.35-0.50	0.43 ± 0.04	0.44 ± 0.03
ESR (mm/h)	0-15	16.88 ± 12.99 [a]	13.47 ± 10.77[b]
Platelets (x 10^9/L)	150-350	246.2 ± 76.64	228.1 ± 58.01
Monocytes (%)	3.0-15.0	7.128 ± 2.303	7.194 ± 1.909
Linfocytes (%)	17.0-48.0	34.02 ± 8.436	36.44 ± 9.870
Granulocytes (%)	45.0-76.0	58.80 ± 8.141	59.02 ± 10.58

Table 2: Results are presented as means ± standard deviation, n=20. [a] Means values out of the reference interval, [b] Represents significant differences (p<0.05) between extractions (T test for related samples, Wilcoxon test). W_0, sample in week 0 before treatment with medical ozone; W_8, sample 8 weeks after treatment with medical ozone; ESR, erythrocyte sedimentation rate.

Table 2: Hematological indexes in patients treated with medical ozone before and after treatment with medical ozone.

The biochemical parameters analysis presented in (Table 3), permit to identify that only the mean values corresponding to the GGT and AP parameters at week 0 were higher than the RI. No significant differences were observed between any of the parameters before and after medical ozone treatment.

Indexes	**Reference interval**	**W_0**	**W_8**
Alanine aminotransferase (U/L)	0-50	30.23 ± 16.60	26.61 ± 17.74
Aspartate aminotransferase (U/L)	0-45	32.54 ± 19.45	26.43 ± 8.766
Creatinine (µmol/L)	70.7-150.2	81.59 ± 17.28	78.29 ± 24.75
Cholesterol (mmol/L)	2.59-5.18	3.45 ± 1.91	2.18 ± 2.29
Triglycerides (µmol/L)	0.678-1.86	1.534 ± 1.044	0.940 ± 0.753
Lactate dehydrogenase (U/L)	200-400	214.1 ± 52.76	189.7 ± 27.28
Glucose (mmol/L)	3.33-6.10	3.252 ± 2.373	3.220 ± 2.464
Gamma glutamyl transferase (U/L)	0-55	60.39 ± 56.11[a]	46.76 ± 34.59
Alkaline Phosphatase (U/L)	53-128	135.3 ± 59.27[a]	122.6 ± 66.19
Total Bilirubin (µmol/L)	3.42-20.52	8.342 ± 2.812	7.978 ± 5.431

Table 3: Results are presented as means ± standard deviation, n=20. [a] Means values out of the reference interval. No significant differences were found (p>0.05) by statistical test. W_0, sample in week 0 before treatment with medical ozone; W_8, sample 8 weeks after treatment with medical ozone.

Table 3: Biochemical indexes in patients treated with medical ozone before and after treatment with medical ozone.

The results of the analysis of the RE indicators in the two groups are shown in (Table 4). At week 0 with respect to the HSV group, differences ($p<0.05$) were observed for the means of SOD, CAT and serum levels of GSH, MDA, AOPP, PP, HPO and NO.

The means of MDA, GSH, PP and NO at week 8 presented significant differences respect HSV and the means of MDA, HPO, AOPP, GSH, CAT, SOD, PP and NO of the same group at week 0.

Redox indexes	SHV	W_0	W_8	%
GSH (μM/g Hb)	1163 ± 167	539 ± 150[a]	726 ± 129[a,b]	56
CAT (U/mg Hb*min)	149.0 ± 19.7	230.3 ± 28.0[a]	140.0 ± 19.8[b]	100
SOD (U/mg Hb*min)	3.04 ± 0.71	4.00 ± 0.95[a]	3.03 ± 0.70[b]	50
PP (μM)	6.84 ± 0.32	10.71 ± 1.13[a]	8.48 ± 0.95 [a,b]	78
MDA (μM)	2.22 ± 0.19	3.78 ± 0.52[a]	2.64 ± 0.34 [a,b]	94
HPO (μM)	117.4 ± 3.10	140.30 ± 17.46[a]	120.90 ± 6.02[b]	88
AOPP (mM chloramine T)	13.59 ± 2.34	20.47 ± 3.03[a]	14.47 ± 1.89 [b]	94
NO (μM)	63.91 ± 4.80	44.75 ± 4.69[a]	55.00 ± 4.20 [a,b]	86

Table 4: Results are presented as means ± standard deviation, n=20. [a] Represents significant differences ($p<0.05$) with the SHV group, [b] Represents significant differences ($p<0.05$) between extractions W_0 and W_8 (ANOVA test and Kruskal-Wallis test). SHV, supposedly healthy volunteers; W_0, sample in week 0 before treatment with medical ozone; W_8, sample 8 weeks after treatment with medical ozone; MDA, malondialdehyde; AOPP, advanced oxidation protein product; PP; peroxidation potential; HPO, hydroperoxide; GSH, glutathione; CAT, catalase; SOD, superoxide dismutase; NO, nitric oxide; %, percentage of individuals presenting the modifications of this variable.

Table 4: Redox indexes in patients treated with medical ozone.

3.1 Treatment follow-up

During the treatment and monitoring, none of the participants was hospitalized. After rectal insufflation sessions, it was referred to, by patients, the sensation of full stomach and feeling of hunger after therapy. In both cases, the sensations disappeared over time and no treatment was required.

4.Discussion

Currently, most HIV patients with adequate adherence to ART achieve a life expectancy close to that of the general population [4]. However the increased incidence of hepatic, cardiovascular, renal and neurological disease occurs in HIV patients compared with general population [31].

Combined application of ART and medical ozone (ART-O3) enhanced the clinical state of the treated patients. This improvement was evidenced by the increase in the CD4+ T cells number and protective redox marker, also accompanied by the decrease in indicators of oxidative damage and some hematological (ESR) and biochemical (GGT and AP) parameters. These effects seem to be a result of the fact that medical ozone have as therapeutic target the redox balance and anti-inflammatory (immunomodulation), as demonstrated in other clinical trials[32-34].

Studies have linked oxidative stress with many aspects of HIV pathogenesis, including stimulation of HIV replication, functional and numerical impairment of CD4+ T cells, disordered immune response, and ART toxicity [35, 36].

Viral load and CD4+ (21)T cells count are fundamental parameters in the clinical follow-up of HIV patients because they are both predictive character. Viral load is an indicator of ART efficacy while CD4+ T cells count is a reliable indicator of immune status [37]. Increase in the CD4+ T cells number was observed in 70% of the patients in this study. However, the mean value of LT-CD4+ at 8 weeks not was sufficient to reach the RI established 500-1500 cell/mL for healthy individuals. Bocci *et al*. evaluated efficacy of medical ozone in seven HIV/aids patients and report a significant increase in CD4+ T cells number and any variation in viral load [38]. In other study of safety and efficacy with the use of ozone-treated blood in the therapy of HIV/aids treatment-naïve patients, the authors did not observe significant changes in the CD4+ T cells count or viral load during any phase of the study [39]. The application of medical ozone showed its efficacy to maintain stability in the virological response and even improved the immune status of the patients studied, contributing to the pharmacological response of ART, this also was reported by Cespedes *et al*. [40].

At week 0 nine patients had values of ESR higher than the RI (range 16-52 mm/h), subsequently observing a decrease in eight of these patients at 8 weeks to enter within the RI to SHV. ESR is an unspecified biomarker, which can be altered by various conditions including infections, inflammatory autoimmune diseases, renal damage and neoplasms. The increase in ESR in these conditions is attributed to increased production of acute phase proteins and releases of proteins by the causative organism into the circulation [41]. Hence, ESR can be used as sensitive index of plasma protein changes, which result from inflammation or tissue damage in HIV. A chronically elevated ESR reflects immune activation, correlating well with cytokines, including tumor necrosis factor-alpha and interleukin-6, a finding documented both in other chronic inflammatory conditions [42, 43]. Decrease of tumor necrosis factor-alpha and interleukin-6 in osteoarthritis models as an effect of medical ozone application was also reported [44].

GGT and AP parameters at week 0 were higher than the RI in 30% and 65% of the patients respectively. Elevated levels of serum liver enzymes (Alanine aminotransferase, AP and GGT) are an expression of alterations at the level of liver cells or bile ducts. A predominance in the increase of AP and GGT levels indicates cholestatic damage [45]. The present investigation evidenced that medical ozone has a beneficial effect by decreasing the values of GGT and AP after treatment in 66% and 69% respectively of patients who presented this alteration initially. Oru *et al.* observed a decrease in GGT levels to values close to RI with the application of medical ozone, as a combined therapy in patients with rheumatoid arthritis [46].

This study showed that at week 0 the patients presented a marked redox imbalance. This imbalance was characterized by a decreased secondary antioxidant capacity (GSH and PP) and increased oxidative damage (MDA, HPO, AOPP), both significant with respect to the SHV group. In the case of the antioxidant enzymes SOD and CAT, higher values were observed than in the SHV group, this aspect may be related to the sustained presence of oxidized metabolites and ROS generated in the context of HIV infection [47] and ART that activate them chronically[48].

Antioxidant enzymes SOD and CAT are part of the antioxidant endogenous system and regulate ROS generation to prevent damage to cellular macromolecules. SOD is responsible to convert superoxide radicals into hydrogen peroxide (H_2O_2), while CAT performed H_2O_2 neutralization. The effect of HIV/HIV proteins on the cellular SOD and CAT levels is debatable [49]. Several groups reported a decrease in SOD and CAT activities in plasma of the HIV patients [50-52], while others found an increase in these enzymes [53, 54].

With the application of ozone treatment at week 8 all these indicators of oxidative damage (with the exception of NO) decreased significantly with respect to week 0 and in the case of HPO and AOPP levels similar to the SHV group were reached. Some authors propose that these oxidative markers of damage (HPO, AOPP, MDA and NO) constitutes an additional predictor of mortality, independent of established HIV-associated predictors such as CD4+ T cells count and HIV viral load [55]. On the other hand, elevated MDA levels have been correlated with frailty in HIV patients [56].

Medical ozone promoted the stimulation of primary antioxidant mechanisms such as glutathione reductase and SOD enzymes[57] not evaluated in the study. This influenced the preservation or recycling of GSH, which increased at week 8 with respect to week 0. This has repercussions in the decrease of oxidative events in biomolecules that are manifested by low AOPP, MDA and HPO values. Probably the concentration of $O_2^{\bullet-}$ decreases due to the increase of SOD and as it does not interact with NO the bioavailability of this increased and the presence of ONOO (not evaluated) should decrease influencing also the low oxidation to biomolecules observed in week 8 (MDA, HPO). As a consequence of the decrease in plasma oxidants the 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) [58].

The mainly limitations of this study include: the incorporation of a limited number of patients, which is lower than in other studies. The group of patients studied had other chronic comorbidities such as hypertension, which also contributed to redox alteration. The patients studied were also administered different combinations of ART, so that some may be more exposed to oxidation.

4.1 Conclusions

Combined treatment ART-O3 had beneficial effect to HIV patients in under our experimental conditions. The application of medical ozone influenced different biological functions resulting in improved redox balance, immune response and no evidence of toxicity or side effects. Taking into account these findings, the application of a second medical ozone cycle to these patients are encouraged that could maintain increase the benefits achieved. The study should also be continued with the inclusion of a large number of patients. The observed effects of ozone as an adjuvant therapy in the management of redox status in HIV/ART patients merit further research and support futures randomized clinical trial.

Author Contributions

Conceptualization: C.L -R.M., L -G.V, M.A. -A.S., R. -G.H., M.C. -H.G.A., Y.-B.A., M.-R.F., T.-R.G-, N.-O.G. and G.-M.S; formal analysis: C.L -R.M., L -G.V, and G.-M.S.; methodology: C.L -R.M., L -G.V, M.A. -A.S., R. -G.H., M.C. -H.G.A., Y.-B.A., M.-R.F., T.-R.G- and N.-O.G.; initial management and HIV follow-up: C.L -R.M. and M.A. -A.S.; treatment with ozone therapy: B.C., and I.J.J.; writing—original draft: B.C., G.M.-S., Y.R.-F., F.R.-E., and P.S.-A.; writing—review, editing and approval of the final version: C.L -R.M., L -G.V, M.A. -A.S., R. -G.H., M.C. -H.G.A., Y.-B.A., M.-R.F., T.-R.G-, N.-O.G. and G.-M.S. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The authors declare that the funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results. All authors confirm that they had full access to all the data in the study and accept responsibility to submit for publication.

Declarations of interest: none

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