



# Facial biostimulation with PRP activated with ozone resound on cellular redox balance, improves lipoatrophy and quality of life in HIV patients

[La bioestimulación facial con PRP activado con ozono repercute en el balance redox, mejora la lipoatrofia y la calidad de vida de pacientes VIH]

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## Abstract

**Context:** Pathogenic impact of high-grade local and systemic oxidative stress in antiretroviral treated HIV patients are recognized factor influencing in lipodystrophy, which is proposed can be ameliorate factor platelet rich plasma (PRP).

**Aims:** To determine the efficacy and safety of ozone and calcium activated PRP application in lipoatrophy- AIDS Cuban individuals.

**Methods:** Thirty HIV individuals enrolled in quasi experimental prospective study showed lipoatrophy grade from 1 to 3. A mean volume interval of 8 to 22.2 mL of PRP was injected in 5 interventions during a year. The clinical, chemical, oxidative stress and progression indexes determinations were performed prior to injections and at 6 and 12 months after. Also, questionnaires based on Short Form 36, Medical Outcomes Study HIV Health Survey were assessed. Different statistical analyses were done comparing baseline respect final values of variables.

**Results:** Beneficial improve of lipoatrophy grade ( $p < 0.05$ ) and stabilization in global indexes of damage and antioxidant status at the end of the study was demonstrated. The comparison revealed a significantly smaller damage and higher antioxidant status compared to baseline values ( $p < 0.05$ ). Non-significant modifications were observed in hematological and chemical indexes ( $p > 0.05$ ), respect quality of life 75% of three dimensions improved and depressive symptoms decreased. Non adverse reactions were observed during study period.

**Conclusions:** These results corroborate that beneficial amelioration of oxidative stress occurs in lipoatrophy AIDS patients during effective and safety PRP-ozone facial bioestimulation. Integral diagnosis would be worthwhile to conduct a more comprehensive study and manage of lipoatrophy.

**Keywords:** AIDS; HIV; lipoatrophy; oxidative stress; ozone; platelet-rich-plasma.

## Resumen

**Contexto:** El impacto patogénico del estrés oxidativo local y sistémico en pacientes VIH tratados con antirretrovirales ha sido reconocido como un factor que influye en la lipoatrofia, la que puede reducirse con plasma rico en plaquetas (PRP).

**Objetivos:** Determinar la eficacia y la seguridad de la aplicación de PRP activado con ozono y calcio en la bioestimulación facial de la lipoatrofia de pacientes sida cubanos.

**Métodos:** Se realizó un estudio cuasi experimental prospectivo, con evaluación antes y después de realizar cinco aplicaciones de PRP con volúmenes de 8 a 22,2 mL durante un año en 30 pacientes VIH/Sida con grados de 1 a 3 de lipoatrofia. Se evaluaron indicadores hematológicos, hemoquímicos, de progresión y de estrés oxidativo. Las reacciones adversas y la calidad de vida de los pacientes fueron evaluadas según cuestionarios con modelos oficiales y el MOS 30 HIV, respectivamente. El análisis estadístico se realizó en correspondencia de las variables analizadas.

**Resultados:** La evaluación sistemática demostró la eficacia y la seguridad del procedimiento con una mejoría del grado de lipoatrofia ( $p < 0,05$ ) y estabilización de los índices de estrés oxidativo con aumento significativo del 75% de la calidad de vida en tres dimensiones ( $p < 0,05$ ). No se observaron modificaciones en las variables de laboratorio, ni reacciones adversas.

**Conclusiones:** El presente trabajo aporta evidencias sobre la estabilización del estrés oxidativo en pacientes sida cubanos con lipoatrofia tratados con PRP activado con ozono. El diagnóstico integral se propone de utilidad para conducir estudios que mejoren la comprensión y el manejo de la lipoatrofia.

**Palabras Clave:** estrés oxidativo; lipoatrofia; ozono; plasma-rico-en-plaquetas; SIDA; VIH.

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## INTRODUCTION

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The drastic increase in the number of people infected with human immunodeficiency virus (HIV) worldwide is overwhelming instead of efforts encouraged by diverse man communities and organizations obtaining more than 25 antiretroviral (ARV) drugs. The ARV can contribute mainly to extend life expectancy of people living with HIV. However, HIV cannot be cured by antiretroviral therapy (ART) because it persists in a transcriptionally silent form in long-lived CD4+ cells (Yang et al., 2018). Diverse authors have been contributed to observation that reactive oxygen species (ROS) production could be a consequence of inflammatory process perpetuation mediated by a number of cytokines, which stimulate the oxidase NADPH activation, thus producing superoxide radicals. The oxidative stress (OS) resulting could drive disulfide CD4 modification, necessary for HIV entry on host, CD4 T lymphocyte depletion and also viral replication causing a predisposition to opportunistic infections, comorbidities, malignancies and aging (Elbim et al., 2001; Halliwell and Gutteridge, 2007; Mandas et al., 2009; Kashou and Agarwal, 2011; Nakagawa et al., 2013; González et al., 2014; Colado et al., 2015; Ivanov et al., 2016; Vaidya et al., 2016; Teeraananchai et al., 2017). In addition, HIV infection, separately and in combination with highly active ARV is closely associated with OS (Williams et al., 2017). OS occurs when there is a dysfunction in the overall balance between the production of ROS and the antioxidant defense mechanisms affecting redox circuits and modulating transcription factor or not influencing cellular survival, adaptation or death response (Valko et al., 2007; Alfadda and Sallam, 2012).

Inflammatory reactions combined with the disruption of the organism's control mechanism in HIV could lead to a persistent pro-inflammatory state as, evidenced in a wide range of diseases that involve no-resolving or re-occurring reactivities during infection (Yang et al., 2007; Schieber and Chandel, 2014; Colado et al., 2015; Ivanov et al.,

2016). Mitochondrial toxicity produced by ARV contribute also to OS occurring in HIV (Nerurkar et al., 2001; Viengchareun et al., 2007; Starkov, 2008; De Pauw et al., 2009; Bocci et al., 2010; Apostolova et al., 2011). Consistent changes in redox responsive cascades and in the expressions of corresponding target genes may have a similar or even greater impact on senescence as the direct radical inflicted damage of cellular constituents (Valko et al., 2007; Schieber and Chandel, 2014; Masia et al., 2016).

Facial lipoatrophy (FLA) in HIV infection is undoubtedly influenced by combination ART, which produces metabolic alteration, OS and a stigmatizing aspect that cannot be hidden by clothes (Moyle and Carr, 2002; Caron-Debarle et al., 2010a; 2010b; Domingo et al., 2010; Deavall et al., 2012; Morse et al., 2012; Abduljalil et al., 2015). Diverse evidences indicate that FLA is irreversible and it is associated with quality of life (QOL) impairment, due to its detrimental effects on self-esteem (Martinez et al., 2001; Guaraldi et al., 2008; Villarroya et al., 2010; Kashou and Agarwal, 2011; Leclercq et al., 2013; Verolet et al., 2015). The effects of biodegradable fillers on QOL in HIV related FLA have been well documented, and numerous studies have shown that facial filling effectively improves QOL and lowers depression rates (Koutkia and Grinspoon, 2004; Moyle et al., 2006; Mallewa et al., 2008; van Rozelaar et al., 2014).

The better comprehension about interrelation of viral, drug and host factors during infection is necessary for the rational development of interdisciplinary clinical interpretation and effective intervention (Silvana and Hepel, 2011; Nakagawa et al., 2013; van Rozelaar et al., 2014; Finkelstein et al., 2015). Considering backgrounds, the aim of the study was to evaluate efficacy and safety of platelet rich plasma activated with ozone (PRP) application in HIV Cuban patients with FLA. In addition, progression, redox and follow up clinical biomarkers were evaluated before and after treatment during a year.

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## MATERIAL AND METHODS

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### Chemicals

The following chemicals were obtained from Sigma (St. Louis, MO, USA): sodium phosphate, trichloroacetic acid (TCA), hydrogen peroxide, FeCl<sub>3</sub>, 2,4-dinitrophenylhydrazine, sulphuric acid, acetic acid, disodium salt of ethylenediaminetetraacetic acid (EDTA, 99%), sorbitol L, reduced glutathione and chloramine T. Also glutathione reductase, N-ethylmaleimide, malondialdehyde bis [dimethyl acetal], 3,3'-bis(N,N-di(carboxymethyl)-aminomethyl)-o-cresolsulfone-phatein, sodium salt, pyrogallol were from Sigma, St. Louis, M.O., USA. LPO-586 kit was obtained from Calbiochem (La Jolla, C.A., USA), Roche reagents and kits for Hitachi analyzer were used (Germany), TM CD3 CD4 and other reagents and kits for cell count were from PARTEC GmbH, Münster, Germany and PCR-NASBA was from Biomerieux, France.

### Experimental procedure

#### *Study design, standard protocol approvals and patient consents*

A quasi experimental study was designed enrolling 30 HIV-AIDS positive individuals with FLA. All the patients were selected from the outpatients clinic at the Institute "Pedro Kourí" (IPK) Hospital for HIV. They all gave written informed consent to take part in the study after verbal and written explanation of the methods and risks involved were given. The work was developed by a multidisciplinary group, including clinical experts in HIV/AIDS management. Procedures were previously reviewed and approved by the Institute "Pedro Kourí" Committee for Research on Human Subjects considering one year for inclusion. The study was in accordance with the principle of the Declaration of Helsinki concerning the Ethical Principles for Medical Research Involving Human Subjects (World Medical, 2013). The protocol was also approved by Traditional and Natural Medicine program of Cuban Ministry of Health (Code 1803009).

### *Patients*

Non-probabilistic convenient sampling was used in according to the assistance of patients to the specialized consult in tertiary Hospital. All subjects were assessed at the clinical visit. Anthropometry and laboratory tests were performed. Eligible patients were 18 years or older with FLA grades 1 to 3 according to the grading scale by Fontdevila et al. (2007). Exclusion criteria included earlier use of facial fillers, the presence of an inflammatory condition of the face, and the use of nonsteroidal anti-inflammatory agents within seven days before injection. Patients underwent an initial screening, which included the evaluation of their medical records, diet, and supplemental intake history, anthropometrics data (weight, height), and review of clinical lab results (complete blood count, platelet count and morphology, glucose, creatinine, urea, liver enzymes). This was done one month prior to recruitment and evaluation. Demographic and age data were processed by SIDATRAT (software package 2008). Subjects were classified according to gender, age, ethnicity, viral load and CD4+ T lymphocyte subset count.

### *Treatments*

The antiretroviral regimen consisted of a triple-drug combination allocated free, including two nucleoside reverse transcriptase inhibitors (RTI) and one protease inhibitors (PI), according to current guidelines (Adolescents poAGfAa, Adolescents, 2018). The antiretroviral drugs used in the different combinations were prescribed daily at the following doses: RTIs zidovudine 600 mg, lamivudine 300 mg, ritonavir 1200 mg or saquinavir 2400 mg. Patients also used concomitant prophylaxis for opportunistic infections. Eligible patients were treated with PRP. A single, specialized physician author (LvR) experienced in the use of fillers performed all injections. Using a butterfly blood collection set with a 21-gauge needle, approximately 20 mL of whole blood were collected in 9 mL test tubes. Utilizing an adaptation from a published platelet processing protocol (Everts et al., 2008; Cole et al., 2010; Lana et al.,

2014), each tube was then deposited into the cell concentrator Medifuge (Silfradent®, Italy). The centrifuge was then set for 14 min at 4 different gradients (CGF Mode). After the centrifuge process was complete, 3 fractions were extracted from each test tube; adult pluripotential cells, platelet rich (PRP) and platelet poor plasma (PPP). PRP was activated with an equal volume of 60 µg/mL ozone (O<sub>3</sub>) and after a 0.2 mL of calcium chloride solution at 10% for every 5 mL of PRP was added. PPP was heated on albumin heater device APAG (Silfradent®, Italy) up to 75°C. The 3 fractions were combined and homogenate to form an autologous gel to inject on pronounced nasolabial folds, depression of the cheeks and the atrophy of the fossa temporalis.

#### *Assessment of outcome*

At the baseline (t = 0) visit, patients were evaluated by clinical examination and standardized facial photography (to obtain LAF grade). Blood extraction was done to determine different indexes and quality of life questionnaires were applied. Clinical examination was performed at every follow-up visit, at 8, 16, 28, 40 and 52 weeks. Complaints, complications, and the effect of injections were evaluated. Patients were asked to fill out questionnaires at baseline, 6 months, and 1 year. Facial photography and laboratory determination were repeated at 6 months and at 1 year (t = 12 months). Also, PRP volume applied were computed.

#### *Flow cytometry analysis*

A study of T lymphocytes subsets CD3+/CD4+, CD3+/CD34+ in total blood was carried out. 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, program version 2.9.

#### *HIV-RNA plasma viremia (viral load)*

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 IU. NUCLISENS® EASYQ® is a specific iso-thermal method combining NASBA amplification and real-time detection using molecular beacon probes.

#### *Oxidative stress parameters*

Venous blood samples were taken from each fasted patient between 8.00 h and 10.00 h morning after informed consent was signed. Blood samples were collected by venipuncture into heparin-treated tubes and centrifuged to obtain serum. All redox parameters were determined by spectrophotometric methods using Zuzi Spectrophotometer from China model 4211/50. For assay of superoxide dismutase (SOD) and catalase (CAT) hemoglobin was extracted from hemolysate. 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.

#### *Reduced glutathione concentration*

Reduced glutathione (GSH) was used to generate standard curves. Serum GSH concentrations were measured by the kinetics assay using the glutathione reductase reaction (Tietze, 1974). Autoxidation of GSH to oxidized glutathione (GSSG) was prevented by addition of 0.05 µL N-ethylmaleimide to the samples at concentration 0.4 M.

#### *Malondialdehyde concentration*

Malondialdehyde (MDA) concentrations were analyzed with the LPO-586 kit. In this assay, stable chromophore production after 40 min of incubation at 45°C is measured at a wavelength of 586 nm. To ensure that no lipid oxidation occurs during the assay, BHT [0.01% (v/v) of a 2% stock solution in ethanol] and EDTA (1 mM final concentration) were added to the sample prior to assay development. Freshly prepared solutions of malondialdehyde bis [dimethyl acetal] assayed under identical conditions were used as reference standards. Concentrations of MDA in serum samples were calculated using the corresponding standard curve and values were expressed as nmol/g Hb (Ozdemirler et al., 1995).

### *Peroxidation potential (PP)*

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°C for 24 h. The PP was calculated by subtracting the MDA concentration at time 0 from the one obtained at 24 h (Ozdemirler et al., 1995; Bartosz, 2003).

### *Total hydroperoxide (HPO)*

HPO was measured based on the oxidation of ferrous ions to ferric ions by hydroperoxides under acidic conditions. Ferric ions bind with the indicator dye xylenol orange (3,3'-bis(N,N-di(carboxymethyl)-aminomethyl)-o-cresolsulfonate, sodium salt) to form a stable colored complex, which can be measured at 560 nm (Jiang et al., 1991).

SOD activities were assayed by a modified pyrogallol autoxidation method (Marklund and Marklund, 1974). CAT activity was measured according with the method of Clairbone (1986). Using a molar extinction coefficient of 43.6/M cm, the rate of the first 30 s was used to calculate the activity. Catalase activity was expressed as U/mg Hb.

### *Advanced oxidation protein products (AOPP)*

Serum AOPP was measured according to the methods of Witko-Sarsat et al. (1998). AOPP are the dityrosine containing protein cross linking products indicating the oxidized tyrosine residues of the plasma protein albumin, fibrinogen and lipoproteins. Determination involves oxidation principle of I<sup>-</sup> (from KI 1.16 M) to I<sup>-3</sup> by plasma AOPP under acidic condition and absorbance was read immediately at 340 nm. The values were expressed as mmol/L of chloramines-T equivalents and corrected by serum albumin concentrations.

### *Biochemical indexes*

Blood parameters such as hematocrit, hemoglobin, and erythrocyte sedimentation rate (ESR)

were screened by hematological counter ABX MICROS 60 (Horiba Medical, Japan). Others as triglycerides, creatinine, cholesterol and alanine aminotransferase and aspartate amino transferase activities were performed by standard procedures in HITACHI analyzer Cobas c311 (Roche, Germany), all in a specialized laboratory of IPK Hospital.

### *Quality of life*

Spanish-language version of the Medical Outcomes Study (MOS)---HIV Health Survey Questionnaire---adapted from the version used in Mexico and also applied in Cuba in 2005-2007. This questionnaire, aimed at people living with HIV, defines 11 health domains: general health perceptions, physical function, role function, social function, cognitive function, pain, mental health, energy/fatigue, health distress, QOL, and health transition and were applied to the 30 patients. It has been applied over world demonstrating its reliability and validity (Wu et al., 1997; Taylor et al., 2009; Aragonés-Lopez et al., 2012). Items and scales were scored following the MOS-HIV Health Survey Scoring Guidelines. Final scores ranged from 0 to 100, with a higher value indicating better health or less pain.

### **Statistical analysis**

For descriptive statistics of continuous variables, means and standard deviations were calculated, whereas categorical variables were expressed as proportions. The normality of variables was evaluated by the Kolmogorov-Smirnov test. Comparisons between baseline, values of interventions and final data were assessed using repeated measures ANOVA followed by a post hoc Newman Keuls methods. Comparison to HIV seronegative individuals (redox indexes) was done by T student test for independent samples. Univariate analyses for the MOS HIV subscales were done and overall scale were processed by t-tests. Statistical significance was defined as p<0.05. The SPSS software package version 20 was used for all statistical analyses.

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## RESULTS

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### Patients

The baseline characteristics of the 30 included subjects are showed in Table 1. Almost patients were on antiretroviral regimen consisted of a triple-drug combination, including two nucleoside reverse transcriptase inhibitors and one protease inhibitor, according to current guidelines. The mean value of treatment duration was 11 years. Accordingly to Fontdevila classification (Fontdevila et al., 2007) 8 patients were scored as grade 1, 12 patients were classified as grade 2 and 10 patients were grade 3.

### Treatment and evaluations

Patients received 5 ozonized platelet-rich plasma (PRPO<sub>3</sub>) treatments in a period of a year (Fig. 1). As expected, the total volume (mL) of injected material was higher in patients with grade 3 and 2. It was reduced significantly at application 5 compared with volume used at application 3 and 1 ( $p < 0.05$ ) (Fig. 2). After 5 PRP applications FLA grade was modified significantly by 68% of patients ( $p < 0.05$ ) (11 patients were scored as grade 0, 7 patients were scored as grade 1, 5 patients were classified as grade 2 and 7 patients were grade 3) (Fig. 3). CD34 determinations in PRP previous to interventions demonstrate necessary cells to produce bioestimulation were available in concentrate.

Progression indexes evaluated as VL and LCD4 not modified significantly during application. The mean value of all biochemical, redox indexes and HIV progression markers evaluated are shown in Tables 2 and 3. All biochemical indexes evaluated remained on interval considered as physiological-reference (RI) but erythrocyte sedimentation rate (ESR) and triglycerides were out of RI. ESR modify not significantly at application 3 and 5 ( $p > 0.05$ ) and the final value was on RI. Otherwise, triglycerides do not modify and persist out of RI at application 5.

MDA (marker of lipid peroxidation) and HPO serum concentrations modified significantly respect baseline at application 3 and 5 ( $p < 0.05$ ). In

serum levels of AOPP not modification was observed. Serum GSH levels were significantly lower in aids individuals compared to HIV- control value ( $p < 0.05$ ). The activity of the erythrocyte antioxidant enzyme SOD and CAT were significantly higher in HIV groups respect HIV-control ( $p < 0.05$ ).

PP is a global index. It assay serum antioxidant capacity shows serum susceptibility to lipid peroxidation. Aids patients had PP significantly higher, suggesting reduced lipid-serum antioxidant capacity respect control value ( $p < 0.05$ ) (Table 3).

### Quality of life

All the dimensions improved significantly ( $p < 0.05$ ) after the five PRP intervention, mainly the dimensions of fatigue energy (77.05 *vs.* 100), mental health (75.64 *vs.* 100), health transition (71.59 *vs.* 100), physical function (86.50 *vs.* 100) and role function (88.10 *vs.* 100). The overall QOL index shows an improvement in 71% of the patients (Fig. 3). The extent to which FLA ameliorate occur differently according severity but improved significantly over time in relation to QOL modification.

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## DISCUSSION

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HIV infection is characterized by severe immunodeficiency, a consequence of numerical and functional CD4+ T cell depletion (Milazzo et al., 2010; Nakagawa et al., 2013). The patients enrolled in this study showed stabilization in CD4+ T cell count as a consequence of ARV treatment and 68% of them attained VL undetectability, consistent with an improvement in immune-virological parameters.

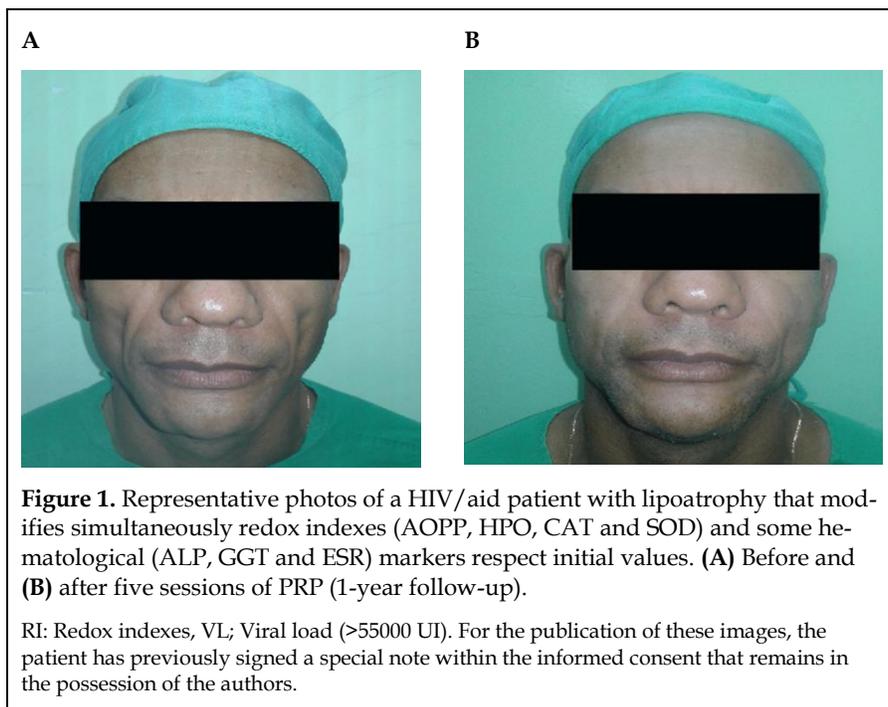
The multidrug antiretroviral regimens intervention based on a combination of reverse transcriptase and protease inhibitors, have been improved the clinical outcome of HIV-1 infection indicated by an important decline in aids mortality but they almost contribute to oxidative metabolism adding risk of molecular damage and facial lipoatrophy is a related consequence (Domingo et al., 2010; Villarroya et al., 2010; Deavall et al., 2012; Ivanov et al., 2016).

**Table 1.** Age, gender, ethnicity and treatment duration of participants attended in IPK at 2015-2017.

		Patients
N		30
Age, years (median $\pm$ SD)		49.53 $\pm$ 7.67
Gender (n, %)	Male	29 (96.6)
	Female	1 (3.3)
Ethnicity (n, %)	White	14 (46.6)
	Mixed race	12 (40.0)
	Black	4 (13.3)
Duration of HIV infection treatment mean (years)		11 $\pm$ 3.0
FLA Grade (n, %)	I	8 (26.8)
	II	12 (40.0)
	III	10 (33.0)

Source: Clinical History deposited in Medical Register Department. SD: standard deviation; FLA: facial lipoatrophy; HIV: human immunodeficiency virus; IPK, 2015: Period of consults in Institute of Tropical Medicine Pedro Kourí was from January to August 2015.

No significant differences were detected in comparison between variables for the different groups ( $p < 0.05$ ).



**Table 2.** Hematologic and chemical indexes data in the FLA study.

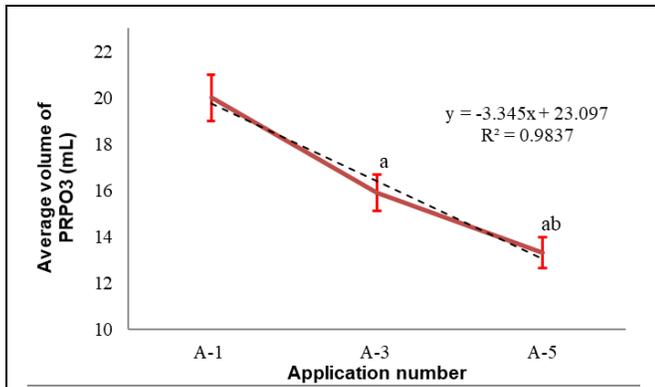
Indexes	Reference Intervals (RI)	Baseline (mean ± SD)	A-3 (mean ± SD)	A-5 (mean ± SD)	Out of RI (%)
Hemoglobin	11.00 - 16.00 g/L	14.02 ± 1.78	13.52 ± 3.10	14.35 ± 1.82	0
Hematocrit	0.35 - 0.50	0.42 ± 0.04	0.43 ± 0.04	0.40 ± 0.12	0
Erythrocytes sedimentation rate	0 - 15 mm/h	23.65 ± 21.01*	16.45 ± 11.09*	12.20 ± 6.64	31
Number of leucocytes	4.00 - 10.00 × 10 <sup>9</sup> /L	6.59 ± 1.58	6.05 ± 1.41	6.30 ± 1.74	0
Granulocytes	45.00 - 76.00 %	55.76 ± 12.26	58.54 ± 9.30	59.53 ± 8.68	0
Lymphocytes	17.00 - 48.00 %	34.68 ± 10.16	33.91 ± 8.69	33.67 ± 7.99	0
Monocytes	3.00 - 15.00 %	6.67 ± 1.94	6.21 ± 1.50	7.07 ± 2.05	0
Platelets	150 - 350 × 10 <sup>9</sup> /L	234.70 ± 83.95	240.20 ± 42.72	249.30 ± 39.56	0
Creatinine	70.70 - 150.20 µmol/L	81.71 ± 15.64	89.55 ± 26.50	85.11 ± 16.41	0
Uric Acid	208 - 430 µmol/L	305.6 ± 105.20	322.20 ± 89.90	292.60 ± 102.40	0
Albumin	35 - 52 g/L	46.04 ± 3.84	44.11 ± 9.23	43.37 ± 6.93	0
Alanine aminotransferase	0 - 50 U/L	38.67 ± 22.21	37.45 ± 20.47	35.00 ± 15.24	0
Aspartate aminotransferase	0 - 45 U/L	36.00 ± 19.05	34.09 ± 13.64	30.38 ± 4.82	0
Cholesterol	2.59 - 5.18 mmol/L	4.98 ± 1.03	4.91 ± 0.87	5.18 ± 1.16	0
Triglycerides	0.678 - 1.86 µmol/L	2.71 ± 1.56*	3.60 ± 3.04*	2.62 ± 2.17*	65
Lactate dehydrogenase	200 - 400 UI/L	208.00 ± 50.57	209.80 ± 47.59	217.50 ± 104.50	0
Total Proteins	60 - 80 g/L	77.59 ± 6.96	76.25 ± 5.19	76.10 ± 5.36	0

SD: standard deviation; RI: reference interval; A-3 PRP: application 3; A-5 PRP: application 5. \*Represents index value is out of reference interval (RI). Not significant differences were found in comparison of application indexes values respect baseline (n=30).

**Table 3.** Redox indexes and HIV progression markers data in FLA study.

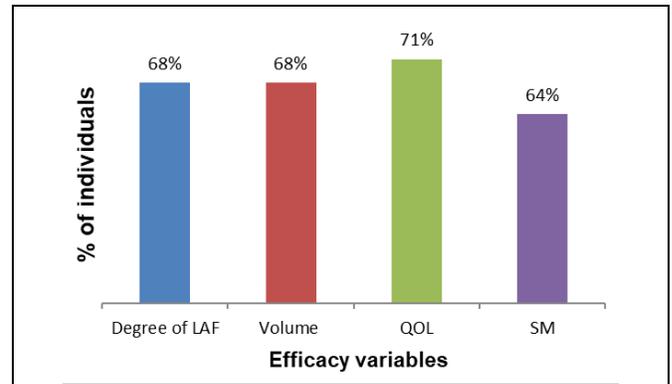
Indexes	HIV seronegative (healthy control)	Aids patients with FLA and ART baseline (mean ± SD)	Application 3 (mean ± SD)	Application 5 (mean ± SD)	Change (%)
MDA (nmol/g Hb)	3.28 ± 0.35	10.02 ± 1.37 <sup>a</sup>	6.96 ± 1.38 <sup>ab</sup>	7.18 ± 1.42 <sup>ab</sup>	75
HPO (µM)	116.70 ± 3.45	199.60 ± 20.48 <sup>a</sup>	155.80 ± 19.48 <sup>ab</sup>	158.50 ± 20.48 <sup>ab</sup>	50
AOPP (µM cloramina T)	13.70 ± 2.51	28.04 ± 8.84 <sup>a</sup>	24.75 ± 3.18 <sup>a</sup>	24.06 ± 3.10 <sup>a</sup>	-
SOD (U/mg Hb min)	2.82 ± 0.69	4.36 ± 1.54 <sup>a</sup>	4.55 ± 1.32 <sup>a</sup>	4.33 ± 0.97 <sup>a</sup>	-
CAT (U/mg Hb min)	144.50 ± 22.29	500.90 ± 72.61 <sup>a</sup>	292.40 ± 25.57 <sup>ab</sup>	288.90 ± 31.83 <sup>ab</sup>	70
GSH (µM/g Hb)	1215.00 ± 207.40	347.10 ± 32.30 <sup>a</sup>	586.10 ± 71.53 <sup>ab</sup>	570.20 ± 99.09 <sup>ab</sup>	80
PP (µM)	6.81 ± 0.29	12.51 ± 1.54 <sup>a</sup>	8.32 ± 0.85 <sup>ab</sup>	8.89 ± 0.98 <sup>ab</sup>	73
LT CD4+ (cell/mL)	1312.00 ± 248	467.70 ± 189.40 <sup>a</sup>	450.90 ± 173.10 <sup>a</sup>	428.30 ± 150.40 <sup>a</sup>	-
CV (copies/mL)	-	21.39 ± 65.26	48.50 ± 76.40	73.31 ± 104.30	-

HIV: human immunodeficiency virus; FLA: facial lipoatrophy patients; ART: antiretroviral therapy; SD: standard deviation; PP: peroxidation potential; CAT: catalase; SOD: superoxide dismutase; HPO: hydroperoxide; MDA: malondialdehyde; GSH: glutathione; AOPP: advanced oxidation protein' product; LTCD4: T CD4 + lymphocyte absolute count; VL: viral load. <sup>a</sup>Represents significant differences respect to HIV seronegative group (p<0.05); <sup>b</sup>Represents significant differences respect to Aids patients with FLA and ART baseline (p<0.05), n=30.



**Figure 2.** Average values of the volume of PRPO<sub>3</sub> used in the first, third and fifth applications for all patients (n=30).

PRPO<sub>3</sub>: Ozonized platelet-rich plasma; A-1: first application; A-3 third application; A-5: fifth application. Statistical test used: Friedman. <sup>a</sup>Represents significant differences (p <0.05) respect the first application. <sup>b</sup>Represents significant differences (p <0.05) respect the third application.



**Figure 3.** Percentage of individuals (n=30) with improvement in FLA grade, decrease in applied volume and improvement in quality of life after the fifth application of ozonated platelet-rich plasma.

FLA: facial lipoatrophy; QOL: quality of life; volume: applied volume of ozonated platelet-rich plasma; SM: simultaneous beneficial modification.

Actually, there are controversial data that substantiate the association of OS and persistent inflammation in several human diseases including HIV infection and ARV treatment (Mandas et al., 2009; Kashou and Agarwal, 2011; Masia et al., 2016). It has been previously shown that the HIV-infected Cuban population have significantly lower antioxidant concentrations than non-HIV individuals and these values could modify by ARV (González et al., 2014).

It is known that the therapy with ARV in some individuals may affect mitochondrial morphology and function and the activation of the P450 cytochrome enzyme system, which in turn increases ROS in circulation. In addition, ART is associated with metabolic disorders that increase oxidative stress in infected individuals (De Pauw et al., 2009; Apostolova et al., 2011; Deavall et al., 2012).

Abnormally high levels of prooxidant species as a consequence of chronic immune system activation by both HIV infection and ART could lead to a decline of antioxidants defense molecules and cumulative damage of cellular components generating augmented lipid peroxidation products and altered oxidized proteins responsible for damage of cells and tissues (Kashou and Agarwal, 2011;

Colado et al., 2015; Masia et al., 2016). Almost redox implicated enzymes and molecules are physiologically endogenous generated and they are involved in detoxification and general metabolism (Valko et al., 2007; Schieber and Chandel, 2014).

Persistent or chronically OS have a dramatic impact on immunological, clinical and nutritional status in HIV infection (Moyle and Carr, 2002; Halliwell and Gutteridge, 2007; Caron-Debarle et al., 2010b; Colado et al., 2015). Previous reports address that OS increase could be related to viral replication in HIV infection and also implicated on CD4+ T cell apoptosis. ROS could modulate and activate nuclear transcription factors, which ultimately lead to viral gene expression of HIV contributing to HIV-related opportunistic infections or malignancies (Kashou and Agarwal, 2011; Colado et al., 2015; Ivanov et al., 2016). In the present study, at baseline evaluation, the concentrations of antioxidants were also low respect RI, lipid and protein oxidation indexes were higher too in aids group. The reliable redox markers altered respects to healthy control were PP, CAT, GSH, MDA and HPO. CAT alterations could indicate altered HPO as is observed. Depletion of GSH and antioxidant capacity evaluated as PP could be related to its

consumption by increased chronic generation of ROS. Respect hematologic and chemical indexes only ESR and TRI shown alterations respect RI and both are unspecific indexes. Between baseline and application 3 and 5 not differences were observed related redox indexes contrasting previous studies related to HIV patients treated with ARV where values arises significantly (Mandas et al., 2009; Gil et al., 2011; Mgbekem et al., 2011; Sharma, 2014; Abduljalil et al., 2015; Tasca et al., 2017).

Alterations of lipid metabolism are common complications of HIV disease related to the inflammatory response and chronic HIV infection, also it could be related to ARV, which is probably mediated by cytokines, may in itself be proatherogenic (Abduljalil et al., 2015). How OS and its modulation are involved in these various steps is object of revision and investigation (Milazzo et al., 2010; Morse et al., 2012; Saeidnia and Abdollahi, 2014). However, reports concerning longitudinal and cross-sectional studies evaluate oxidative stress as a toxic effect and contributing factor to aids and non-aids associated disease (González et al., 2014; Ivanov et al., 2016; Jiang et al., 1991). Lipoatrophy is associated to ART mitochondrial toxicity involving oxidative stress. The redox attenuation could influence oxidative damage to molecules and disease evolution. Different interventions to resolve long lasting lipoatrophy have been suggested including biostimulation with permanent or semi-permanent fulfilling with autologous or synthetic preparations where PRP is an alternative with advantages related to easy procedure and economic aspect (Cole et al., 2010; van Rozelaar et al., 2014).

PRP is nowadays widely applied in different clinical scenarios, such as orthopedics, ophthalmology and healing therapies, as a growth factor pool for improving tissue regeneration. Studies into its clinical efficiency are encouraged for characterization of the biological responses (Anitua et al., 2004; Mehta and Watson, 2008; Dohan-Ehrenfest et al., 2012). Activation with ozone preclude a ROS generation improving growth factor release and also ozone products could induce hormesis response in microenvironments modulating redox status.

<http://jppres.com/jppres>

Medical ozone increased the capacity of the antioxidant endogenous system or resist oxidative injury, producing as a result a decrease in the damage to biomolecules (lipids and proteins) as well as oxidative mediators levels (Bocci and Borrelli, 2015). In order to clarify whether there was any relationship between the redox markers and the clinical and quality of life outcome simultaneous analyses were done.

Not modification of oxidative indexes permits to identify 25 patients with beneficial response. None of patients showed VL or LCD4+ alteration respect baseline. Twenty-one patients presented significantly improvement in QOL values and 19 presented reducing of PRP volume used and also improved FLA qualified by Fontdevila grade (Fontdevila et al., 2007). Simultaneous analyses noted that 64% of group (18/30 patients) present beneficial effects without ARV medical interference effects or hepatic, renal not metabolic toxicity. Previous works evaluated beneficial and secure indexes related to other filler alternative with similar percent (van Rozelaar et al., 2014) also PRP follow up in HIV patients with ulcer showed beneficial aspect and security (Cieslik-Bielecka et al., 2018).

Taking into account that causes of morbidities are complex and multifaceted, the recognition of molecular and cellular concert involved are crucial. A causal relationship between some elements such as oxidative macromolecules modifications, immunological status and viral load has emerged but the mechanism by which these molecular and biochemical events occur remain to be established.

The OS evaluations will therefore become potential useful to characterize infection, antiviral combinations effects, as well as the usefulness of alternative therapies for counteracts oxidative damage (Silvana and Hepel, 2011; Saeidnia and Abdollahi, 2014; Preedy and Watson, 2018).

Some contributions have been exploring mechanistic aspect of PRP for comprehensive understanding of effect on site of application and on metabolism (Anitua et al., 2004; Everts et al., 2006). Despite these concerns, substantial progress has been made toward an integrative understanding to

delineate mechanism considering OS and prooxidant species as potential key participants in pathology development.

HIV infection is considered at the moment as a chronic illness, according to that quality of life (QOL) assessment can serve as a health outcome and may also allow clinicians and other health workers to identify any reductions in QOL potentially related to short- and long-term therapy or intervention (Taylor et al., 2009; Veroleto et al., 2015).

In this sense, significant difference evidenced in some indexes related in first to PRP application and also to QOL proved its association to HIV infection evolution and its treatment. Gaining in knowledge of specific indexes and their relation to other factors, investigators will be provided with additional opportunities to impact on both quality of life and related diseases in humans and other species.

To our knowledge, this is the first study using autologous PRP activated with ozone as filling agents in HIV patients with FLA. Also, the measurement tool in evaluating the effect of treatment combines diverse biomarker offering integral observation.

Although important impact of PRP application on FLA grade of HIV studied patients occurred, a low percentage of patients never achieve remission or improved. Previous studies also reported similar results related to chronic wasting process (Koutkia and Grinspoon, 2004; Mallewa et al., 2008; van Rozelaar et al., 2014) Understanding the interplay of viral, drugs and host factors in FLA is critical for the rational effective intervention in future.

This study shows that Cubans receiving ART suffering lipoatrophy are reduced QOL. On average, the scores were lower than previous study in Cuba where involved patients initiate ART therapy (Aragones-Lopez et al., 2012). Also, it was lower than values on others countries for which data are available on all 11 dimensions and overall scale measured by the MOS-HIV (Taylor et al., 2009; Aragones-Lopez et al., 2012). We observed several

significant differences related to 6 dimensions of QOL questionnaires showing less pain, more cognitive functioning and better physical health after 1 year of 5 PRP applications. Those results are similar to previous one reporting increasing QOL after treatment of FLA with others fillers (van Rozelaar et al., 2014). People who have AIDS have been reported to have lower scores in all areas compared with those who have not yet advanced to AIDS. It is highly likely that the PRP treatment has improved the QOL of patients including on protocol based on integral management and follow up.

This study validates previous publications showing that HIV patients with FLA benefit greatly from facial treatment.

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## CONCLUSIONS

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The present study contributes to evidences that OS evaluated in blood plasma by several parameters occurs in Cuban HIV patients treated with ART whose presented lipoatrophy. It is possible that the concentration of this cumulative damage reported had direct impact on functional efficiency and cell functioning. Metabolic abnormalities as altered redox indexes remain an important part of complications in HIV infection. Their etiology, including roles for both non-HIV and HIV viral-related effects and treatment-associated factors, requires ongoing investigation. These complications could be implicated in patients' active clinical status and long-term consequences. Management options are encouraged to clarify its biological impact. Therapeutic interventions as PRP activated with ozone may provide substantial benefits including improvement of patient's quality of life. These conclusions are also methodologically important for the follow-up and manage of infected individuals.

It is likely that a combination of therapeutic agents targeting multiple signal transduction pathways will be needed for maximum therapeutic benefits. Under a sustained OS, significant damage may occur to cell structure and functions and also redox driven process are stimulated modulating different stages of inflammation.

## Limitations

No randomization was implemented during the design of this study. As our study was a prospective cohort study, it was not possible to blind either the investigators or patients to treatment. Sample size was also limited in our study according with patients included in specialized consult.

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## CONFLICT OF INTEREST

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The authors declare no conflict of interest.

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## REFERENCES

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- Abduljalil M, Liman H, Umar R, Abubakar M (2015) Glycaemic and lipid profiles in HIV positive patients on Antiretroviral therapy in Sokoto state, Nigeria. *IIOABJ* 6(2): 7-14.
- Adolescents (2018) P. o. A. G. f. A. a. Guidelines for the use of antiretroviral agents in adults and adolescents living with HIV.
- Alfadda AA, Sallam RM (2012) Reactive oxygen species in health and disease. *J Biomed Biotechnol* 2012: 936486.
- Anitua E, Andia I, Ardanza B, Nurden P, Nurden AT (2004) Autologous platelets as a source of proteins for healing and tissue regeneration. *Thromb Haemost* 91(1): 4-15.
- Apostolova N, Blas-Garcia A, Esplugues JV (2011) Mitochondria sentencing about cellular life and death: A matter of oxidative stress. *Curr Pharm Des* 17(36): 4047-4060.
- Aragones-Lopez C, Perez-Avila J, Smith Fawzi MC, Castro A (2012) Quality of life of people with HIV/AIDS receiving antiretroviral therapy in Cuba: A cross-sectional study of the national population. *Am J Public Health* 102(5): 884-892.
- Bartosz G (2003) Total antioxidant capacity. *Adv Clin Chem* 37: 219-292.
- Bocci V, Borrelli E (2015) How to solve the problem of chronic oxidative stress? *Bioenergetics* 4: 123.
- Bocci V, Zanardi I, Travagli V (2010) Ozonation of human HIV-infected plasmas for producing a global vaccine: How HIV-patients may help fight the HIV pandemia. *Virulence* 1(3): 215-217.
- Caron-Debarle M, Boccara F, Lagathu C, Antoine B, Cervera P, Bastard JP, Vigouroux C, Capeau J (2010a) Adipose tissue as a target of HIV-1 antiretroviral drugs. Potential consequences on metabolic regulations. *Curr Pharm Des* 16(30): 3352-3360.
- Caron-Debarle M, Lagathu C, Boccara F, Vigouroux C, Capeau J (2010b) HIV-associated lipodystrophy: from fat injury to premature aging. *Trends Mol Med* 16(5): 218-229.
- Cieslik-Bielecka A, Skowronski R, Jedrusik-Pawlowska M, Pierchala M (2018) The application of L-PRP in AIDS patients with crural chronic ulcers: A pilot study. *Adv Med Sci* 63(1): 140-146.
- Clairborne A (1986) Catalase activity. *Handbook of Methods for Oxygen Radical Research*. R. Green-Wald. Boca Ratón: CRC Press. pp. 283-284.
- Colado AN, Victorino VJ, Morimoto HK, Reiche EM, Panis C (2015) Redox-driven events in the human immunodeficiency virus type 1 (HIV-1) infection and their clinical implications. *Curr HIV Res* 13(2): 143-150.
- Cole BJ, Seroyer ST, Filardo G, Bajaj S, Fortier LA (2010) Platelet-rich plasma: where are we now and where are we going? *Sports Health* 2(3): 203-210.
- De Pauw A, Tejerina S, Raes M, Keijer J, Arnould T (2009) Mitochondrial (dys)function in adipocyte (de)differentiation and systemic metabolic alterations. *Am J Pathol* 175(3): 927-939.
- Deavall DG, Martin EA, Horner JM, Roberts R (2012) Drug-induced oxidative stress and toxicity. *J Toxicol* 2012: 645460.
- Dohan-Ehrenfest DM, Bielecki T, Mishra A, Borzini P, Inchingolo F, Sammartino G, Rasmusson L, Everts PA (2012) In search of a consensus terminology in the field of platelet concentrates for surgical use: platelet-rich plasma (PRP), platelet-rich fibrin (PRF), fibrin gel polymerization and leukocytes. *Curr Pharm Biotechnol* 13(7): 1131-1137.
- Domingo P, Cabeza MC, Pruvost A, Salazar J, Gutierrez M, Mateo MG, Domingo JC, Fernandez I, Villarroya F, Munoz J, Vidal F, Baiget M (2010) Relationship between HIV/highly active antiretroviral therapy (HAART)-associated lipodystrophy syndrome and stavudine-triphosphate intracellular levels in patients with stavudine-based antiretroviral regimens. *Clin Infect Dis* 50(7): 1033-1040.
- Elbim C, Pillet S, Prevost MH, Preira A, Girard PM, Rogine N, Hakim J, Israel N, Gougerot-Pocidalo MA (2001) The role of phagocytes in HIV-related oxidative stress. *J Clin Virol* 20(3): 99-109.
- Everts PA, Brown Mahoney C, Hoffmann JJ, Schonberger JP, Box HA, van Zundert A, Knape JT (2006) Platelet-rich plasma preparation using three devices: implications for platelet activation and platelet growth factor release. *Growth Factors* 24(3): 165-171.
- Everts PA, Devilee RJ, Brown-Mahoney C, van Erp A, Oosterbos CJ, Stellenboom M, Knape JT, van Zundert A (2008) Exogenous application of platelet-leukocyte gel during open subacromial decompression contributes to

- improved patient outcome. A prospective randomized double-blind study. *Eur Surg Res* 40(2): 203–210.
- Finkelstein JL, Gala P, Rochford R, Glesby MJ, Mehta S (2015) HIV/AIDS and lipodystrophy: implications for clinical management in resource-limited settings. *J Int AIDS Soc* 18: 19033.
- Fontdevila J, Berenguer J, Prades E, Pujol T, Guisantes E, Serra-Renom J, Gatell J, Martínez E (2007) Validation of a simple classification for facial lipoatrophy in HIV-infected adults. *Antivir Ther* 12(Suppl 2): L31.
- Gil L, Tarinas A, Hernández D, Riverón B, Pérez D, Tápanes R, Capo V, Pérez J (2011) Altered oxidative stress indexes related to disease progression marker in human immunodeficiency virus infected patients with antiretroviral therapy. *J Biom Aging Path* 1(1): 8–15.
- González I, Gravier R, Calas V, Reyes A, Pérez D, Hernández D, Bermúdez Y, Gil L, León O (2014) Oxidant/antioxidant status in subjects with HIV infection in different clinical conditions. *Biomed Aging Pathol* 4(3): 235–242.
- Guaraldi G, Murri R, Orlando G, Squillace N, Stentarelli C, Zona S, Garlassi E, Martinez E (2008) Lipodystrophy and quality of life of HIV-infected persons. *AIDS Rev* 10(3): 152–161.
- Halliwell B, Gutteridge J (2007) *Free Radicals in Biology and Medicine*, Oxford University Press.
- Ivanov AV, Valuev-Elliston VT, Ivanova ON, Kochetkov SN, Starodubova ES, Bartosch B, Isagulians MG (2016) Oxidative Stress during HIV Infection: Mechanisms and Consequences. *Oxid Med Cell Longev* 2016: 8910396.
- Jiang ZY, Woollard AC, Wolff SP (1991) Lipid hydroperoxide measurement by oxidation of Fe<sup>2+</sup> in the presence of xylenol orange. Comparison with the TBA assay and an iodometric method. *Lipids* 26(10): 853–856.
- Kashou H, Agarwal A (2011) Oxidants and antioxidants in the pathogenesis of HIV/AIDS. *Open Reprod Sci J* 3: 154–161
- Koutkia P, Grinspoon S (2004) HIV-associated lipodystrophy: pathogenesis, prognosis, treatment, and controversies. *Annu Rev Med* 55: 303–317.
- Lana J, Andrade-Santana M, Dias-Belangero W, Malheiros-Luzo A (2014) *Platelet-Rich Plasma*, Springer-Verlag Berlin Heidelberg.
- Leclercq P, Goujard C, Duracinsky M, Allaert F, L'Henaff M, Hellet M, Meunier JP, Carret S, Thevenon J, Ngo Van P, Pialoux G (2013) High prevalence and impact on the quality of life of facial lipoatrophy and other abnormalities in fat tissue distribution in HIV-infected patients treated with antiretroviral therapy. *AIDS Res Hum Retroviruses* 29(5): 761–768.
- Mallewa JE, Wilkins E, Vilar J, Mallewa M, Doran D, Back D, Pirmohamed M (2008) HIV-associated lipodystrophy: a review of underlying mechanisms and therapeutic options. *J Antimicrob Chemother* 62(4): 648–660.
- Mandas A, Iorio EL, Congiu MG, Balestrieri C, Mereu A, Cau D, Dessi S, Curreli N (2009) Oxidative imbalance in HIV-1 infected patients treated with antiretroviral therapy. *J Biomed Biotechnol* 2009: 749575.
- Marklund S, Marklund G (1974) Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem* 47(3): 469–474.
- Martinez E, Garcia-Viejo MA, Blanch L, Gatell JM (2001) Lipodystrophy syndrome in patients with HIV infection: quality of life issues. *Drug Saf* 24(3): 157–166.
- Masia M, Padilla S, Fernandez M, Rodriguez C, Moreno A, Oteo JA, Antela A, Moreno S, Del Amo J, Gutierrez F, CoRis B (2016) Oxidative stress predicts all-cause mortality in HIV-infected patients. *PLoS One* 11(4): e0153456.
- Mehta S, Watson JT (2008) Platelet rich concentrate: basic science and current clinical applications. *J Orthop Trauma* 22(6): 432–438.
- Mgbekem M, John M, Umoh I, Eyong E, Ukam N, Omotola B (2011) Plasma antioxidant micronutrients and oxidative stress in people living with HIV. *Pak J Nutr* 10(3): 214–219.
- Milazzo L, Menzaghi B, Caramma I, Nasi M, Sangaletti O, Cesari M, Zanone Poma B, Cossarizza A, Antinori S, Galli M (2010) Effect of antioxidants on mitochondrial function in HIV-1-related lipoatrophy: a pilot study. *AIDS Res Hum Retroviruses* 26(11): 1207–1214.
- Morse CG, Voss JG, Rakocevic G, McLaughlin M, Vinton CL, Huber C, Hu X, Yang J, Huang da W, Logun C, Danner RL, Rangel ZG, Munson PJ, Orenstein JM, Rushing EJ, Lempicki RA, Dalakas MC, Kovacs JA (2012) HIV infection and antiretroviral therapy have divergent effects on mitochondria in adipose tissue. *J Infect Dis* 205(12): 1778–1787.
- Moyle G, Carr A (2002) HIV-associated lipodystrophy, metabolic complications, and antiretroviral toxicities. *HIV Clin Trials* 3(1): 89–98.
- Moyle GJ, Sabin CA, Cartledge J, Johnson M, Wilkins E, Churchill D, Hay P, Fakoya A, Murphy M, Scullard G, Leen C, Reilly G, Uk RG (2006) A randomized comparative trial of tenofovir DF or abacavir as replacement for a thymidine analogue in persons with lipoatrophy. *AIDS* 20(16): 2043–2050.
- Nakagawa F, May M, Phillips A (2013) Life expectancy living with HIV: recent estimates and future implications. *Curr Opin Infect Dis* 26(1): 17–25.
- Nerurkar PV, Shikuma CM, Nerurkar VR (2001) Sterol regulatory element-binding proteins and reactive oxygen species: potential role in highly-active antiretroviral therapy (HAART)-associated lipodystrophy. *Clin Biochem* 34(7): 519–529.
- Ozdemirler G, Mehmetcik G, Oztezcan S, Toker G, Sivas A, Uysal M (1995) Peroxidation potential and antioxidant

- activity of serum in patients with diabetes mellitus and myocard infarction. *Horm Metab Res* 27(4): 194–196.
- Preedy V, Watson R (2018) *HIV/AIDS: oxidative stress and dietary antioxidants*. London, United Kingdom: Elsevier/Academic Press.
- Saeidnia S, Abdollahi M (2014) Role of micronutrients and natural antioxidants in fighting against HIV; a quick mini-review. *Res J Pharmacogn* 1(4): 49–55.
- Schieber M, Chandel NS (2014) ROS function in redox signaling and oxidative stress. *Curr Biol* 24(10): R453–462.
- Sharma B (2014) Oxidative stress in HIV patients receiving antiretroviral therapy. *Curr HIV Res* 12(1): 13–21.
- Silvana A, Hepel M (2011). *Oxidative Stress: Diagnostics, Prevention, and Therapy*. Washington DC.
- Starkov AA (2008) The role of mitochondria in reactive oxygen species metabolism and signaling. *Ann N Y Acad Sci* 1147: 37–52.
- Tasca KI, Caleffi JT, Correa CR, Gatto M, Tavares FC, Camargo CC, Sartori A, Biasin M, de Souza LDR (2017) Antiretroviral therapy initiation alters the redox system of asymptomatic HIV-infected individuals: A longitudinal study. *Oxid Med Cell Longev* 2017: 9834803.
- Taylor TN, Dolezal C, Tross S, Holmes WC (2009) Reliability and validity of two HIV/AIDS-specific quality of life instruments adapted for use in HIV-positive Zimbabweans. *AIDS Care* 21(5): 598–607.
- Teeraananchai S, Chaivooth S, Kerr SJ, Bhakeecheep S, Avihingsanon A, Teeraratkul A, Sirinirund P, Law MG, Ruxrungtham K (2017) Life expectancy after initiation of combination antiretroviral therapy in Thailand. *Antivir Ther* 22(5): 393–402.
- Tietze F (1974) Enzymatic method for quantitative determination of nanogram amounts of total and oxidized glutathione. *Anal Biochem* 27: 502–522.
- Vaidya K, Kadam A, Nema V (2016) Anti-retroviral drugs for HIV: Old and new. *Res Austin J HIV/AIDS* 3(2): 1026.
- Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 39 (1): 44–84.
- van Rozelaar L, Kadouc h JA, Duyndam DA, Nieuwkerk PT, Lutgendorff F, Karim RB (2014) Semipermanent filler treatment of HIV-positive patients with facial lipoatrophy: long-term follow-up evaluating MR imaging and quality of life. *Aesthet Surg J* 34(1): 118–132.
- Verolet CM, Delhumeau-Cartier C, Sartori M, Toma S, Zawadynski S, Becker M, Bernasconi E, Trelu LT, Calmy A, Lipo Group Metabolism (2015) Lipodystrophy among HIV-infected patients: A cross-sectional study on impact on quality of life and mental health disorders. *AIDS Res Ther* 12: 21.
- Viengchareun S, Caron M, Auclair M, Kim MJ, Frachon P, Capeau J, Lombes M, Lombes A (2007) Mitochondrial toxicity of indinavir, stavudine and zidovudine involves multiple cellular targets in white and brown adipocytes. *Antivir Ther* 12(6): 919–929.
- Villarroya F, Domingo P, Giralt M (2010) Drug-induced lipotoxicity: lipodystrophy associated with HIV-1 infection and antiretroviral treatment. *Biochim Biophys Acta* 1801(3): 392–399.
- Williams AA, Sitole LJ, Meyer D (2017) HIV/HAART-associated oxidative stress is detectable by metabolomics. *Mol Biosyst* 13(11): 2202–2217.
- Witko-Sarsat V, Friedlander M, Nguyen-Khoa T, Capeillere-Blandin C, Nguyen AT, Canteloup S, Dayer JM, Jungers P, Druke T, Descamps-Latscha B (1998) Advanced oxidation protein products as novel mediators of inflammation and monocyte activation in chronic renal failure. *J Immunol* 161(5): 2524–2532.
- World Medical (2013) World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* 310(20): 2191–2194.
- Wu AW, Revicki DA, Jacobson D, Malitz FE (1997) Evidence for reliability, validity and usefulness of the Medical Outcomes Study HIV health survey (MOS-HIV). *Qual Life Res* 6(6): 481–493.
- Yang D, Elnor SG, Bian ZM, Till GO, Petty HR, Elnor VM (2007) Pro-inflammatory cytokines increase reactive oxygen species through mitochondria and NADPH oxidase in cultured RPE cells. *Exp Eye Res* 85(4): 462–472.
- Yang H, Wallace Z, Dorrell L (2018) Therapeutic targeting of HIV reservoirs: How to give T cells a new direction. *Front Immunol* 9: 2861.

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Contribution	Gil-del Valle L	Acosta Suarez MA	Rabeiro Martinez CL	Gravier- Hernández R	Hernández González- Abreu MC	Bermúdez- Alfonso Y	Rosa-Font M	Campos-Díaz J	Hernández- Requejo D	Martínez-Sánchez G	Weiser MT
Concepts or ideas	x	x	x	x	x	x	x	x	x	x	x
Design	x	x	x								
Definition of intellectual content	x	x								x	
Literature search	x		x							x	x
Clinical studies	x	x									
Data acquisition	x	x	x	x	x	x	x	x	x		
Data analysis	x	x								x	
Statistical analysis	x		x								
Manuscript preparation	x	x	x							x	
Manuscript editing	x	x								x	
Manuscript review	x	x	x	x	x	x	x	x	x	x	x

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