Journal of Clinical & Cellular Immunology

Rivera-Rivera et al., J Clin Cell Immunol 2014, 5:6
http://dx.doi.org/10.4172/2155-9899.1000276

Research Article Open Access

Depression Correlates with Increased Plasma Levels of Inflammatory Cytokines and a Dysregulated Oxidant/Antioxidant Balance in HIV-1-Infected Subjects Undergoing Antiretroviral Therapy

Yainyrette Rivera-Rivera[1], Yashira García[1], Valerie Toro[2], Nydia Cappas[2], Pablo López[3], Yasuhiro Yamamura[3], and Vanessa Rivera-Amill[1*]

[1]*Department of Microbiology, Ponce Health Sciences University-School of Medicine/ Ponce Research Institute, USA*

[2]*Department of Clinical Psychology, Ponce Health Sciences University-School of Medicine/ Ponce Research Institute, USA*

[3]*AIDS Research Program, Ponce Health Sciences University-School of Medicine/ Ponce Research Institute, USA*

[*]**Corresponding author:** Vanessa Rivera-Amill, Department of Microbiology and RCMI AIDS Program, Ponce Health Sciences University-School of Medicine/ Ponce Research Institute, 395 Research Building Zona Industrial Reparada 2, Ponce PR 00716-2347, USA, Tel: 787- 840-2575; Fax: 787-841-5150; E-mail: vrivera@psm.edu

Received date: August 27, 2014, **Accepted date:** November 24, 2014, **Published date:** December 01, 2014

Abstract

Objective: Depression is the most common psychiatric diagnosis in the HIV/AIDS population and represents a risk factor for disease progression. Since HIV-1 infection is characterized by immunologic and metabolic disturbances, we want to study the effects of depression on different components related to pro-inflammatory and oxidative stress markers. We hypothesize that depression will lead to increased pro-inflammatory cytokine levels and altered antioxidant/oxidant balance.

Methods: We included males and females who were ≥21 years of age, whose HIV-1 sero-status was confirmed by Western Blot, and who were currently undergoing antiretroviral treatment. Patients completed the participation consent form, a socio-demographic survey, and the Patient Health Questionnaire-9 (PHQ-9) for depression assessment. We isolated the plasma from participants' blood samples for viral load analysis (RT-PCR), T-cell counts (flow cytometry), and hematological parameters. A cytokine magnetic bead panel was used to measure interleukin-15 (IL-15), interferon gamma-induced protein 10 (IP-10), IL-12 and granulocyte colony-stimulating factor (G-CSF) levels. We also performed assays to determine the antioxidant activity of superoxide dismutase (SOD) and catalase and to measure the lipid peroxidation levels using malondialdehyde (MDA) and 8-isoprostane assays. Statistical comparisons and correlations at 5% level of significance were determined.

Results: Our results show that subjects with mild/moderate to severe depression as assessed by PHQ-9 had a significantly decreased adherence to anti-retroviral treatment. Subjects with depression also had significantly lower levels of white blood cells (WBC) and platelets (PLT) than did the non-depressed group. The HIV^+ subjects with depression had increased levels of IL-15, IP-10, IL-12 p40/p70 and G-CSF compared to their non-depressed counterparts. The latter had increased MDA and 8-isoprostane levels.

Conclusions: Our results suggest that HIV^+ subjects with depressive symptoms have higher levels of inflammation and altered oxidant/antioxidant balance. Although the groups were small, this study strengthens the hypothesis that alterations in cytokines are associated with the mechanisms underlying depression symptoms.

Keywords: HIV; Depression; IL-15; IP-10; IL-12; p40/p70; G-CSF; Catalase; Superoxide dismutase; Malondialdehyde; 8-isoprostane

Introduction

Infection with the human immunodeficiency virus type 1 (HIV-1) is characterized by disruption in the normal functions of the immune and metabolic systems. At the immune system level, HIV-1 causes its deleterious effects in the host mainly through the progressive loss of $CD4^+$ T cells, eventually leading to the development of Acquired Immune Deficiency Syndrome (AIDS) [1]. The effects of HIV-1 are also evident at the metabolic level, specifically in terms of oxidant/antioxidant balance. Under normal conditions a homeostatic balance exists between the production and elimination of Reactive Oxygen Species (ROS). It is well known that many diseases involve the dysregulation of this system by the disruption of antioxidants and the overload of oxidants. HIV-1 infection promotes the shifting of the oxidant/antioxidant system, causing oxidative stress due to the depletion of the tissue antioxidant defense system [2-4].

Because of the availability of antiretroviral therapy, having the HIV-1 infection has changed from being a sure death sentence to a manageable, chronic disease [5]. Compared to the general population, the treated HIV-infected population is showing increased longevity but also is exhibiting greater development of some non-AIDS comorbid diseases. Mental health comorbidities among the population with infectious diseases are getting much importance in primary health care [6]. The medical burden of depression is increasing and studies have shown that people who are infected with HIV are more likely than the general population to develop depression, the most common psychiatric diagnosis in people living with HIV/AIDS [7-9]. HIV/AIDS causes psychological trauma and adverse effects to the nervous system leading to the development of mania, depression, and other cognitive disorders [10]. In the USA, the estimated prevalence of

depression is 2-10 times higher in HIV/AIDS patients than it is in the general population [9]. According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-V), depression is classified as a mood disorder characterized by the presence of a severely depressive mood for at least 2 weeks. Some of the most important changes in patients who suffer from major depression are changes related to appetite, weight, sleep patterns, and psychomotor behavior, and those symptoms can both increase and decrease among patients [11]. Interestingly, immune system function changes are present in major depression too [12], and it has been suggested that these immune changes may be more apparent in depressed patients infected with HIV-1 since their immune systems are already compromised [12]. Recently, Song and Wang reviewed the correlation between pro-inflammatory cytokines, free radical species, and oxidants in the etiology of depression [13,14]. Erlandson and colleagues demonstrated that development of impaired physical function and frailty are associated with markers for immune activation such as Interleukin-6 (IL-6), high sensitive C Reactive Protein (hs-CRP) and Tumor Necrosis Factor alpha (TNF-α), and inflammation [15]. Another study revealed that depressed patients had significantly increased production of oxidative stress, autoimmune and inflammatory markers against Lipopolysaccharide (LPS) than do the controls [16]. Furthermore, decreased intracellular levels of free Glutathione (GSH) and increased oxidized GSH were found to be correlated with increased levels of pro-inflammatory cytokines and Malondialdehyde (MDA) in macrophages from HIV-infected patients compared to controls [17].

As there is strong evidence for the dysregulation of inflammatory response and oxidative imbalance in HIV-1 as well as in depressive disorders, in the present study we wanted to assess markers related to the immunological, pro-inflammatory, and oxidant/antioxidant balance in HIV-1 infected subjects with and without depressive symptoms. The findings of this study led us to hypothesize that the successful management of depression would lead to increased antiretroviral treatment adherence and overall enhancement/re-establishment of physical and immunological status in individuals diagnosed with HIV/AIDS.

Materials and Methods

Ethics statement

Before our obtaining any information or biological samples, patients completed an informed consent to participate, were briefed on the study, and were encouraged to ask questions. The intervention was designed to take place on the same day as the patients' routine medical visit, to minimize any discomfort. The Institutional Review Board at Ponce School of Medicine and Health Sciences (PSMHS) approved the study protocol.

Participants

A total of 23 participants from the Ryan White HIV/AIDS Program (Part A) were recruited at Special Health Clinic, Juana Díaz, Puerto Rico. This program is administered by the U.S. Department of Health and Human Services and provides funds to those communities that do not have sufficient health care coverage or financial resources for the treatment of HIV disease. In our study, we included males and females who were ≥21 years of age, whose HIV sero-status was confirmed by Western Blot and who were currently undergoing antiretroviral treatment. We excluded any participant with a history of a significant pre-existing neurological brain disease, including Alzheimer's disease, stroke, seizure disorder, or traumatic brain injury; a chronic psychiatric illness involving psychosis (e.g., schizophrenia), according to DSM-V criteria; end-stage disease (life expectancy<12 months); and evidence of opportunistic CNS infections (toxoplasmosis, progressive multifocal leukoencephalopathy, neoplasm). We also excluded those who had sero-converted to negative on HIV testing and were no longer on antiretroviral drugs.

Behavioral assessment

A professional therapist from the Clinical Psychology Program (PSMHS) interviewed each patient to obtain sociodemographic data (age, gender, town of origin, time since HIV diagnosis, antiretroviral treatment, method of infection, and psychological treatment) and information related to alcohol and/or drug abuse and depressive symptoms. This was accomplished through the administration of the Patient Health Questionnaire-9 (PHQ-9); treatment adherence was determined through the administration of the Immunological Status Report.

PHQ-9

The PHQ-9 is a 9-item questionnaire used for the assessment of depressive symptoms in primary care settings [18]. This questionnaire evaluates the presence of depressive symptoms over the 2 weeks prior to the test's being filled out. Each of the items can be scored from 0 (not at all), to 3 (nearly every day). The general score can range from 0 to 27 and is interpreted as follow: a score of 0 to 4 means that the subject has minimal or no symptoms, 5 to 9 signifies mild depression, 10 to 14 signifies moderate depression, 15 to 19 signifies moderately severe depression, and 20 to 27 signifies that the subject has severe depression. Its validity and reliability as a diagnostic measure, as well as its utility in assessing depression severity and monitoring treatment response are well-established [18-22].

Treatment adherence

The percentage of treatment adherence was measured by patient self-reporting using the Immunological Status Report. This instrument was developed by the Health Psychology Program's team. The level of adherence was calculated by dividing the number of doses taken by the number of prescribed doses over the 2 weeks previous to the visit.

Blood collection

Trained personnel from the clinic drew blood samples for the assessment of immunological, blood chemistry, pro-inflammatory and oxidative stress parameters. Samples were collected from overnight-fasted participants by venipuncture and using blood collection tubes containing heparin. Blood samples were centrifuged at 1000 × g for 10 minutes at 4°C. Plasma was isolated and stored at -80°C until assayed.

Viral load and $CD4^+$ T-cell counts

Viral load analyses were done by trained personnel from the Immunology Reference Laboratory at PSMHS using the COBAS AmpliPrep/TaqMan HIV-1 v2.0 Test, according to manufacturer's protocol.

CD4+ T-cell counts

Analyses were done by trained personnel from the Immunology Reference Laboratory at PSMHS using the BD Multitest CD3/CD8/CD45/CD4 and analyzed in a BD FACSCalibur™ system according to the manufacturer recommendations.

Plasma level of cytokines

Human cytokines and chemokines were quantified simultaneously using a panel of 39 fluorescent-labeled magnetic beads based technology from MILLIPLEX® MAP (MILLIPLEX HCYTMAG-60K-PX39, Biosource International Inc., Carlsbad, CA, USA), according to the manufacturer's instructions. Results were obtained with the MAGPIX® with xPONENT software program (xPONENT 4.1) and analyzed with the MILLIPLEX Analyst (EMD Millipore, Chicago, IL) using a standard curve derived from recombinant cytokine and chemokine standards.

Superoxide dismutase

Superoxide Dismutase (SOD) activity was measured using the Superoxide Dismutase Assay Kit (Cayman Chemical Company, Ann Arbor, MI), according to the manufacturer recommendations. Briefly, 200 µl of diluted radical detector and 10 µl of standards or samples (diluted 1:5 with sample buffer) were added to their corresponding well. To initiate the reaction, 20 µl of diluted xanthine oxidase was added as quickly as possible to each well and the plate was shaken for a few seconds. At this point the plate was covered and then incubated in an orbital shaker for 20 min at room temperature. Absorbance was read at a wavelength of 450 nm, using a plate reader.

Catalase

Catalase activity was measured using the Catalase Assay Kit (Cayman Chemical Company, Ann Arbor, MI), according to the manufacturer recommendations. Briefly, 100 µl of diluted assay buffer and 30 µl of methanol were added to each well being used. Then, 20 µl of standard, catalase control, or plasma samples (diluted 1:5 with sample buffer) were added to the corresponding well. To initiate the reaction, 20 µl of diluted hydrogen peroxide was added and the plate was covered and incubated in an orbital shaker for 20 min at room temperature. To terminate the reaction, 30 µl of diluted potassium hydroxide and then 30 µl of Catalase Purpald (Chromagen) was added to each well. The plate was covered and incubated for 10 min at room temperature in an orbital shaker. Finally, 10 µl of catalase potassium periodate was added to each well, after which the plate was covered and incubated for 5 minutes at room temperature in an orbital shaker. Absorbance was read at a wavelength of 540 nm, using a plate reader.

Malondialdehyde

Malondialdehyde (MDA) levels for lipid peroxidation were measured using the TBARS Assay Kit (Cayman Chemical Company, Ann Arbor, MI), according to the manufacturer recommendations. Five mL polypropylene screw-cap tubes were used to prepare standards and samples. Briefly, 100 µl of SDS solution and 4 mL of color reagent were added to 100 µl of standard and plasma samples. Samples were boiled for 1 hour, incubated on ice for 10 minutes, and centrifuged for 10 minutes at 1,600 × g at 4°C. One hundred and fifty µl (in duplicate) of each sample was loaded in a 96-well plate. Absorbance was read colorimetrically at a wavelength of 540 nm, using a plate reader.

8-Isoprostane

Free 8-isoprostane fraction was measured using the 8-Isoprostane EIA Kit (Cayman Chemical Company, Ann Arbor, MI). Plasma was collected and stored at -80°C with 0.005% Butylated Hydroxytoluene (BHT) until assayed. Briefly, 100 µl and 50 µl of EIA buffer were added to Non-Specific Binding (NSB) and maximum binding (B_0) wells, respectively. Then, 50 µl of standards (starting with 8) and samples were added to their corresponding wells. Fifty µl of AchE tracer and 50 µl of 8-isoprostane EIA antiserum were added to the corresponding wells and the plate was incubated for 18 hours at 4°C. The second day, wells were washed and rinsed with wash buffer prior to the addition of Ellman's reagent to each well. Five µl of tracer were added to the total activity well. The plate was covered from the light and incubated for 90-120 min at room temperature. Absorbance was read at a wavelength of 405 nm, using a plate reader until the B_0 wells reached 0.3 absorbance units (A.U.).

Statistical analyses

Data were analyzed by means of descriptive statistics (including percentages, means, and standard deviations–SD). The data were expressed as mean ± SD. Difference between groups was assessed using a 2-sided Wilcoxon–Mann–Whitney test and correlation analyses with a 2-sided Spearman test. Results with a $p = 0.05$ were considered statistically significant.

Results

Clinical and demographic parameters for study participants

The information depicted in Table 1 presents the demographic and clinical characteristics for the subjects in the HIV-positive cohort. The mean age of the 23 subjects included in these analyses was 53.1 years old (range, 40-69) for subjects with no depression symptoms and 48.5 years old (range, 41-61) years for subjects with mild-moderate to severe depression symptoms. All of the subjects lived in Puerto Rico, and 70% of them were male. Based on the assessment of depression symptoms using the PHQ-9, 10 subjects showed no-depressive symptoms, while 13 exhibited mild-moderate to severe depression symptoms. On average, subjects had a self-reported combination antiretroviral treatment (CART) adherence of 90% (SD = 13%).

However, subjects experiencing depressive symptoms reported a significantly decreased adherence to the antiretroviral treatment. There were no statistical differences in mean CD4+ T-cell counts and HIV viral load between the two groups. A total of 35% of the subjects had an undetectable HIV viral load. More than 50% of the subjects in both groups were infected through heterosexual contact (Table 1). A total of 14 subjects were also con-infected with Hepatitis C Virus (HCV). Although there was no statistical difference in the distribution of HCV co-infection or viral load between HIV+ non-depressed and depressed subjects, we observed higher levels of alaline transaminase (ALT) in HIV/HCV co-infected subjects with depression symptoms (Table 1).

Parameter	Non-depressed	Depressed (Mild-Moderate/Severe)	p value
Number of subjects (M/F)	10 (6/4)	13 (10/3)	-
Age[a]	53.1 (40-69)	48.5 (41-61)	0.208[c]
Assessment of depression[b]	1.9 ± 1.29	11.2 ± 4.71	< 0.0001[c*]
CD4$^+$ T cells (current) (cells/µl)[b]	466.5 ± 212.95	461.4 ± 252.2	0.879[c]
Viral load (copies/mL)a	196 (20 - 1,116)	4,616.7 (20 - 41,223)	0.624[c]
% undetectable	20%	26%	
HCV co-infection	40%	69%	0.102[d]
HCV viral load (IU/mL)[a]	2.78 x 10^6 (7.93 x 10^4 – 5.02 x 10^6)	2.03 x 10^6 (2.84 x 10^5 – 6.10 x 10^6)	1.00[c]
ALT (IU/L)[a]	25.0 (17-36)	61.0 (7-169)	0.042[c*]
AST (IU/L)[a]	33.3 (15-51)	53.3 (13-114)	0.649[c]
Self-reported adherence (%)[b]	96.0% ± 0.02%	85.4% ± 19.5%	0.041[c*]
Transmission (%)	60.0% Heterosexual	53.8% Heterosexual	1.00[d]

[a]Values are expressed as mean (range)

[b]Values are expressed as mean ± standard deviation

[c]Differences between groups were assessed with 2-sided Wilcoxon-Mann-Whitney test

[d]Differences between groups were assessed with 2-sided Fisher's Exact Test

[*]$p < 0.05$, statistically significant difference

ALT: Alanine Transaminase; AST: Aspartate Aminotransferase

Table 1: Demographic and Clinical Characteristics of HIV Positive Subjects in the Study.

Parameter	Non-depressed[a]	Depresseda(Mild-Moderate/Severe)	p value[b]
WBCs (cells/mL)	6.4 x 10^3 ± 1.9 x 10^3	4.38 ± 1.7 x 10^3	0.023[*]
Lymphocytes	2.09 x 10^3 ± 0.48 x 10^3	1.70 x 10^3 ± 0.83 x 10^3	0.277
(%)	(35 ± 13.81)	(37.68 ± 9.10)	-0.31
Monocytes	0.41 x 10^3 ± 0.11 x 10^3	0.38 x 10^3 ± 0.16 x 10^3	0.702
Neutrophils	3.69 x 10^3 ± 1.84 x 10^3	2.14 x 10^3 ± 0.77 x 10^3	0.018[*]
Eosinophils	0.16 x 10^3 ± 0.10 x 10^3	0.18 x 10^3 ± 0.13 x 10^3	0.917
Basophils	0.05 x 10^3 ± 0.02 x 10^3	0.03 x 10^3 ± 0.02 x 10^3	0.049[*]
PLT (cells/mL)	272.2 x 10^3 ± 102.38 x 10^3	198.67 x 10^3 ± 79.91 x 10^3	0.048[*]
Hb (g/dL)	13.91 ± 2.19	13.06 ± 1.77	0.508
HCT (%)	40.68 ± 6.14	38.45 ± 5.26	0.464
RBCs (cells/mL)[a]	4.05 x 10^6 ± 0.63 x 10^6	4.26 x 10^6 ± 0.49 x 10^6	0.31

[a]Values are expressed as mean ± standard deviation

[b]Differences between groups were assessed with 2-sided Wilcoxon-Mann-Whitney test

[*]Statistically significant difference

WBC: White Blood Cells; RBC: Red Blood Cells; Hb: Hemoglobin; HCT: Hematocrit; PLT: platelets

Table 2: Blood Parameters of HIV Positive Subjects in the Study.

Plasma pro-inflammatory cytokine levels correlate with depression symptoms in HIV-positive subjects

Depression affects not only the brain and behavior but also the rest of the body. However, the underlying mechanisms by which depression influences HIV/AIDS disease progression remain to be fully understood. Evidence suggests that a chronic inflammatory state plays a key role in regulating HIV infection and likely depression [23,24].

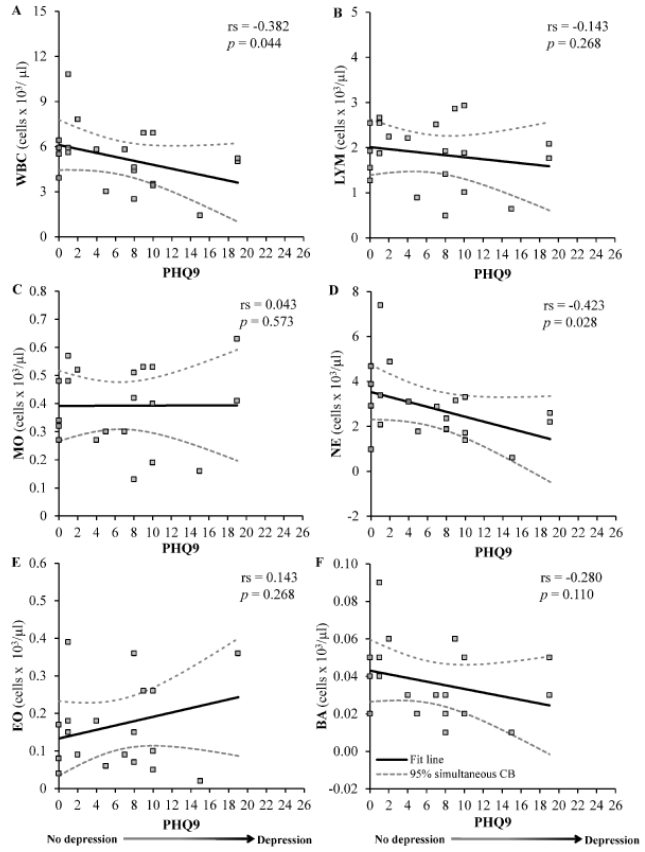

Figure 1: Correlations between blood cell number and depression symptoms. Two-sided Spearman correlation tests between the white blood cell numbers and levels of depression symptoms. Panels: A= total White Blood Cells (WBC); B= Lymphocytes (LYM); C= Monocytes (MO); D= Neutrophils (NE); E= Eosinophils (EO); F= Basophils (BA). CB: Confidence Band. The cut-off for significance was p = 0.05.

We assessed the levels of pro-inflammatory cytokines in plasma samples of HIV-infected individuals who were not experiencing depression symptoms (n=10) and compared them to the levels in HIV-infected individuals who were experiencing depression symptoms (n=13). The plasma pro-inflammatory cytokine levels of IL-15 (Figure 1A), IP-10 (Figure 1C), and IL-12 p40/p70 (Figure 1E) were significantly elevated in subjects with mild-moderate to severe depression symptoms compared with those of subjects having no depression symptoms (p = 0.044 and p = 0.025, respectively), indicating an enhanced inflammatory state in this group of participants. Moreover, the plasma levels of IL-15 (Figure 1B), IP-10 (Figure 1D), and IL-12 p40/p70 (Figure 1F), significantly correlated with the level of depression symptoms (p = 0.011; p = 0.050; p = 0.036, respectively), which correlation is indicative of a relationship between mental health status and the inflammatory response in HIV infection. We also analyzed the plasma levels of G-CSF (Figure 1G) and observed significantly elevated levels of this cytokine in subjects with depression symptoms compared to the levels observed in their non-depressed counterparts (p = 0.011). The levels of G-CSF also correlated with the level of depression symptoms (Figure 1H; p < 0.0001). Other cytokines (IL-1β, IL-1α, IFN-γ, MCP-1, MIP-1α, MIP-1β, among others) assessed within the MILLIPLEX HCYTMAG-60K-PX39 panel were detected at higher levels in individuals who were experiencing depression symptoms however, no significant differences were observed between the two groups (data not shown).

Table 2 shows the clinical information related to the blood parameters of the HIV-infected subjects enrolled in this study. According to the analyses, the depressed subjects have significantly decreased white blood cells (WBCs; p = 0.023) and platelets (p = 0.048) as compared to their non-depressed counterparts, and there was a slight inverse correlation of the former with depressive symptoms (rs = -0.382, p = 0.044). Within the WBCs, the neutrophils and the basophils were the subpopulations that were significantly decreased in depressed subjects. However, only the total WBCs (rs = -0.382, p = 0.044) and neutrophils (rs = -0.423, p = 0.0281) inversely correlated with depression symptoms (Figure 2, panels A and D). Other blood parameters, including RBCs, Hb, and HCT were not significant when comparing the two groups (Figures 2E and 2F).

HIV depressed and non-depressed patients have significant differences in lipid peroxidation

Overload of oxidative stress is commonly found in the development and progression of chronic diseases. It has been shown that lipids are the most susceptible biomolecules affected by oxidative stress [25]. Thus, to analyze the redox status between HIV-infected subjects who have/do not have depression symptoms we performed analysis for plasma levels of lipid peroxidation and antioxidant activity. We measured lipid peroxidation with two different assays.

Parameter	Non-depressed	Depressed(Mild-Moderate/Severe)	p value[b]
Catalase (nmol/min/mL)[a]	31.38 ± 20.12	41.01 ± 18.71	0.142
Superoxide dismutase (U/mL)[a]	3.14 ± 3.67	1.64 ± 0.87	0.261
Malondialdehyde (mM)[a]	11.41 ± 2.70	9.17 ± 3.34	0.033*
8-isoprostane (pg/mL)[a]	28.69 ± 17.66	14.21 ± 11.92	0.036*

[a]Values are expressed as mean ± standard deviation

[b]Differences between groups were assessed with Wilcoxon-Mann-Whitney test

*Statistically significant difference

Table 3: Oxidative stress parameters in depressed versus non-depressed HIV positive subjects.

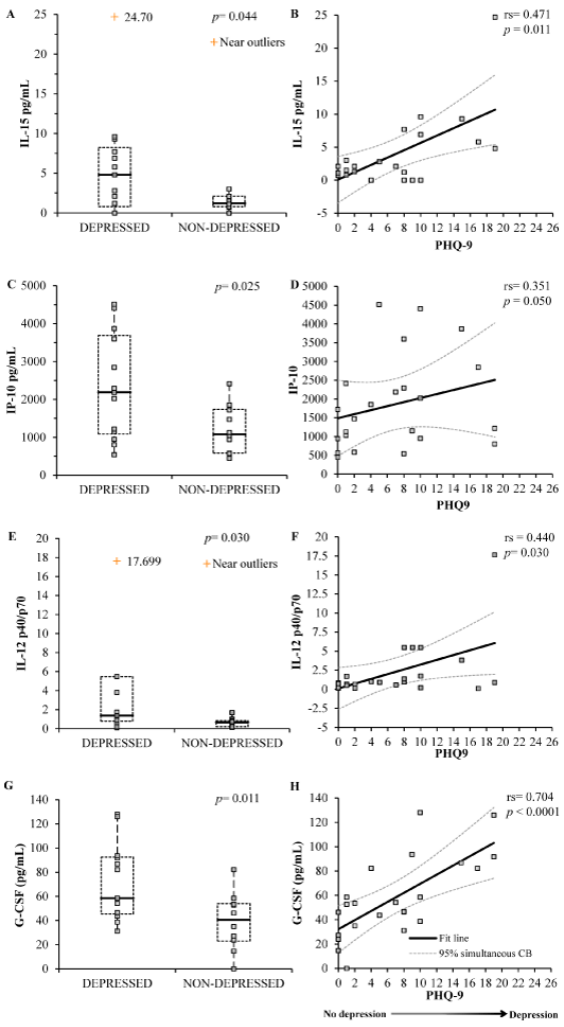

Figure 2: HIV-depressed patients have higher plasma IL-15, IP-10, IL-12 p40/p70 levels. Panels A, C, E, and G: Cytokines (panel A: IL-15; panel C, IP10; panel E: IL-12 p40/p70; panel F: G-CSF) were measured simultaneously using the Luminex's MAGPIX (with xPONENT 4.1 program) and a cytokine magnetic bead panel (MILLIPLEX HCYTMAG-60K-PX39). Significance was calculated using Wilcoxon-Mann-Whitney test. Solid black lines represent the median of each group and dashed boxes denote the first to third quartiles. Observed values greater than 1.5*IQR from the quartiles are highlighted as possible near outliers (+). Panels B, D, F and H: 2-sided Spearman correlation tests between the levels of IL-15 (panel B), IP-10 (panel D), IL-12 p40/p70 (panel F), or G-CSF (panel H) and the levels of depression symptoms. CB: Confidence Band. The cut-off for significance was p = 0.05.

The thiobarbituric acid reactive substances (TBARS) assay measures malondialdehyde (MDA), a naturally occurring decomposition product of lipid peroxidation. Alternatively, the 8-isoprostane enzyme immuno assay identifies a stable end-product of lipid peroxidation. When we analyzed oxidative stress parameters (Table 3), we did not see any significant differences in catalase and superoxide dismutase activities between the two groups. However, we observed significantly higher plasma levels of lipid peroxidation (malondialdehyde, p = 0.033; 8-isoprostane, p = 0.036) in HIV non-depressed subjects than in the HIV depressed subjects. The levels of 8-isoprostane in plasma inversely correlated with the levels of depression symptoms (rs = -0.560, p = 0.008), whereas there was a trend of inverse correlation between MDA and depression symptoms (rs = -0.302, p = 0.080) (Figure 3).

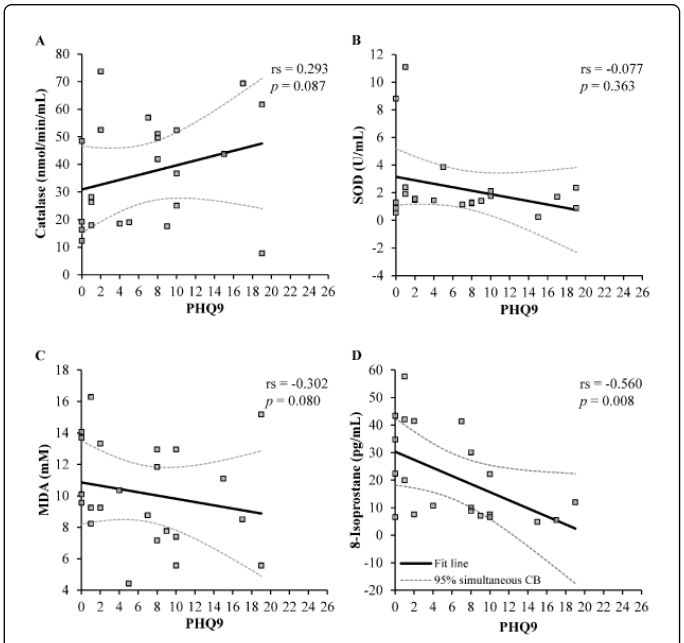

Figure 3: Lipid peroxidation levels inversely correlated with depression symptoms. Two-sided Spearman correlation tests between the levels of catalase (panel A), superoxide dismutase (panel B), malondealdehyde (panel C), or 8-isoprostane (panel D) and the levels of depression symptoms. CB: Confidence Band. The cut-off for significance was p = 0.05.

Discussion

Depression is the most common psychiatric diagnosis in people living with HIV/AIDS [9] and, according to a 2008 literature review by Leserman, almost 50% of HIV/AIDS patients suffering from depression are never diagnosed or treated for that co-morbid condition [26]. Poor management of depression is associated with reduced antiretroviral treatment adherence, which can lead to disease progression [27]. Recent studies have shown a correlation between pro-inflammatory cytokines, free radical species, and oxidants in the etiology of depression [13,14]. In addition, oxidative stress products can contribute to chronic inflammation and the development of pathologies [28]. The current study addressed the correlation of depressive symptoms on the inflammatory state and the oxidant/antioxidant balance in an HIV cohort.

We analyzed the plasma levels of the pro-inflammatory cytokines IL-15, IP-10, IL-12 p40/p70 and G-CSF. Our results demonstrate that participants with depression symptoms exhibit significantly higher levels of the pro-inflammatory cytokines IL-15, IP-10, IL-12 p40/p70 and G-CSF than do those participants without depression symptoms and the levels of these cytokines correlated with the levels of

depression symptoms. Our results are in agreement with a previous study that demonstrated significantly higher IP-10 levels in depressed patients when compared to controls [29]. The group also demonstrated that the levels of IP-10 decreased after antidepressant treatment [29]. In addition, there is significant evidence demonstrating that elevated peripheral cytokines (IL-1, IL-6, TNF-α and IFNγ) and other pro-inflammatory mediators (C-reactive protein) influence the development and progression of depression [30-34]. As well, gene expression profiling on post-mortem brain tissue from psychotropic drug-free individuals with a history of major depression revealed up-regulation of a variety of pro- and anti-inflammatory cytokines [35]. Additionally, studies on HIV patients co-infected with HCV revealed higher plasma levels of IL-15 compared to the levels in patients mono-infected with either HIV or HCV [36,37]. However, to our knowledge, this is the first report that correlates higher plasma levels of IL-15 to depression symptoms. IL-12 is a heterodimeric cytokine consisting of the p35 and p40 chains located in separate chromosomes [38]. The bioactive heterodimer (p70) plays an important role in the activities of natural killer cells (NK) and T lymphocytes. IL-12 p40 has been estimated to be secreted in excess as compared to IL-12 p70 by peripheral blood cells [39]. Our results showed that HIV subjects with depression symptoms had IL-12 p40 levels approximately 5 times higher than HIV subjects without depression, whereas the levels of IL-12 p70 were comparable between the two groups. This led to a significantly increased ratio of IL-12 p40/p70 in subjects with depression symptoms as compared to subjects who do not have depression symptoms. Previous studies have shown that overexpression of IL-12 p40 subunits forms more stable homodimers that bind to IL-12 β1 receptors, antagonizing IL-12 p70 subunit [40]. Thus, the increased IL-12 p40/p70 ratio could potentially lead to diminished NK and T cell functions in HIV subject with depression symptoms. We did not find previous studies about the specific role of IL-12 p40/p70 in depression, but interestingly, IL-12 is important for the establishment of a TH1 response and depression is characterized by shift from T naïve to TH1 responses [41]. Although the sample size was small, the levels of the pro-inflammatory cytokines reported in our study correlated with the levels of depression symptoms as we did not observe any significant differences in the percentage of HCV co-infection between the groups.

The disruption of hematological parameters is among the most common complications of HIV disease [42]. Several studies have shown the presence of anemia, leukopenia, and thrombocytopenia within the HIV-infected individuals, and these blood disorders are associated with rapid disease progression and increased mortality [43-48]. Though the participants in both groups were within the normal range of WBC (4-11 cells/μl) and PLT (150-400 cells/μl), we observed that depressed subjects had significantly decreased WBCs and PLTs compared to their non-depressed counterparts. Riviere and colleagues showed that precursors of platelets, also known as megakaryocytes, have high CXCR4 expression [49]. CXCR4 is one of the co-receptors for HIV-1 infection, suggesting that HIV-1 infects megakaryocytes directly and inhibits them to produce platelets [49]. Another study demonstrated that HIV-1 infection can also impair megakaryocyte maturation by reducing the formation of megakaryocytopoietic colony-forming units in infected subjects [50]. Also, a group from the Strategies for Management of Antiretroviral Therapy (SMART) study found that platelet counts increased in most of the HIV subjects after their re-introduction to treatment [51]. Although it may be likely that a combination of the mechanisms described above underlie the significantly lower platelet counts in HIV$^+$ depressed subjects in our study, we were not able to establish a correlation between treatment adherence and platelet counts. Self-reported treatment adherence in subjects experiencing depression symptoms ranged from 40% to 100%, and the subjects with lower treatment adherence did not necessarily have the lowest platelet count or the highest HIV viral load. Also, IFN-α based therapy of HCV infection has been reported to negatively affect peripheral platelet counts and induce depression in treated patients [52,53]. However, HIV/HCV co-infected subjects in our study were not under IFN-α based therapy at the time of sample collection. Thus it seems unlikely that the observed altered levels of IP-10, MDA, 8-isoprostane and platelet count be due to IFN-α based therapy of HCV infection.

The activation of the immune system and inflammatory responses occur as a result of the presence of stimuli, and infection with HIV is not an exception. HIV infection is characterized by a persistent chronic inflammatory state [54]. Moreover, it has been shown that during early HIV infection, patients are in oxidative imbalance, characterized by high levels of malondialdehyde (MDA), a product of lipid peroxidation, and reduced antioxidant capacity in serum [55]. We assessed the enzymatic activity and levels of oxidative stress markers in plasma samples from our HIV-infected cohort. We observed a trend of higher catalase and lower SOD levels in HIV subjects with depression symptoms. Catalase is induced by higher hydrogen peroxide (H_2O_2) levels and high H_2O_2 levels may in turn lead to increased peroxidation of lipids. The plasma levels of MDA and 8-isoprostane were significantly lower in the group of depressed subjects. The lower levels of lipid peroxidation may be partially explained by the higher levels of catalase enzyme activity in depressed subjects. In the presence of catalase, an in vitro study estimated that 18% of MDA disappeared [56]. Several other factors could also account for the discrepancy, ranging from differences in gender to differences in environment/pathogens or differences in the phases of neuroendocrine, counter-regulatory systems or severity and stage of HIV disease. In addition, since the non-depressed subjects had significantly higher levels of cART adherence, the antiretroviral therapy itself may be leading to the observed increased in lipid peroxidation. It is known that the antiretroviral treatment increases chemically reactive species in circulation, possibly by producing more oxidized metabolites deriving from the interaction between ROS and infected-cell biomolecules [57-59].

This study does have some limitations. The demographic information and results shown here are from a cross-sectional design. Thus, we need to implement strategies to continue monitoring the mental and physical health statuses of the HIV-infected subjects. In addition, the adherence to antiretroviral medications was self-reported. The actual percentage of adherence could be lower than that which was reported by some of the patients. Regardless, HIV infected subjects experiencing depressive symptoms have worse inflammatory markers demonstrating that it is important to deal with depression because of its physiological effects. Further studies with a larger sample size are needed to confirm whether stabilization of the depression symptoms is accompanied by a reduction in pro-inflammatory cytokines to support the role of cytokines in the pathophysiology of depression.

Acknowledgements

The authors would like to thank the Clinical Psychology Program, the AIDS Research Infrastructure Program at PSMHS, RCMI Publications Office, and Dr. Rafael Iván Iriarte from the Public Health

Program at PSMHS. We also want to acknowledge the efforts of the personnel from Special Health Clinic, Juana Díaz, Puerto Rico, who made a valuable contribution to our study. The project was supported by the following grants from the National Institutes of Health: NCRR-RCMI (RR003050) / NIMHD (G12-MD007579), NIGMS (R25GM084206), NCRR-INBRE (P20-RR016470), and MD007587. The work was also supported by institutional funds from PSMHS.

References

1. Barker E, Mackewicz CE, Levy JA (1995) Effects of TH1 and TH2 cytokines on CD8+ cell response against human immunodeficiency virus: implications for long-term survival. Proc Natl Acad Sci U S A 92: 11135-11139.
2. Stephensen CB, Marquis GS, Douglas SD, Kruzich LA., Wilson CM (2007) Glutathione, glutathione peroxidase, and selenium status in HIV-positive and HIV-negative adolescents and young adults. Am J Clin Nutr 85: 173-181.
3. Coaccioli S, Crapa G, Fantera M, Del Giorno R, Lavagna A, et al. (2010) Oxidant/antioxidant status in patients with chronic HIV infection. Clin Ter 161: 55-58.
4. Mandas A, Iorio EL, Congiu MG, Balestrieri C, Mereu A, et al. (2009) Oxidative imbalance in HIV-1 infected patients treated with antiretroviral therapy. J Biomed Biotechnol 2009: 749575.
5. Deeks SG, Lewin SR, Havlir DV (2013) The end of AIDS: HIV infection as a chronic disease. Lancet 382: 1525-1533.
6. Deribew A, Deribe K, Reda AA, Tesfaye M, Hailmichael Y, et al. (2013) Change in quality of life: a follow up study among patients with HIV infection with and without TB in Ethiopia. BMC public health 13: 408.
7. Mota-Miranda A, Gomes H, Serrão R, Araújo F (2004) Transmission of HIV-2: another perspective. Lancet Infect Dis 4: 265-266.
8. Benton TD (2008) Depression and HIV/AIDS. Curr Psychiatry Rep 10: 280-285.
9. Pence BW (2009) The impact of mental health and traumatic life experiences on antiretroviral treatment outcomes for people living with HIV/AIDS. J Antimicrob Chemother 63: 636-640.
10. Cook JA, Grey D, Burke J, Cohen MH, Gurtman AC, et al. (2004) Depressive symptoms and AIDS-related mortality among a multisite cohort of HIV-positive women. Am J Public Health 94: 1133-1140.
11. Lopresti AL, Maker GL, Hood SD, Drummond PD (2014) A review of peripheral biomarkers in major depression: the potential of inflammatory and oxidative stress biomarkers. Prog Neuropsychopharmacol Biol Psychiatry 48: 102-111.
12. Alciati A, Gallo L, Monforte AD, Brambilla F, Mellado C (2007) Major depression-related immunological changes and combination antiretroviral therapy in HIV-seropositive patients. Hum Psychopharmacol 22: 33-40.
13. Maes M, Galecki P, Chang YS, Berk M (2011) A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro)degenerative processes in that illness. Prog Neuropsychopharmacol Biol Psychiatry 35: 676-692.
14. Song C, Wang H (2011) Cytokines mediated inflammation and decreased neurogenesis in animal models of depression. Prog Neuropsychopharmacol Biol Psychiatry 35: 760-768.
15. Erlandson KM, Allshouse AA, Jankowski CM, Lee EJ, Rufner KM, et al. (2013) Association of functional impairment with inflammation and immune activation in HIV type 1-infected adults receiving effective antiretroviral therapy. J Infect Dis 208: 249-259.
16. Maes M, Kubera M, Leunis JC, Berk M, Geffard M (2013) In depression, bacterial translocation may drive inflammatory responses, oxidative and nitrosative stress (O&NS), and autoimmune responses directed against O&NS-damaged neoepitopes. Acta Psychiatr Scand 127: 344-354.
17. Morris D, Guerra C, Donohue C, Oh H, Khurasany M, et al. (2012) Unveiling the mechanisms for decreased glutathione in individuals with HIV infection. Clin Dev Immunol 2012: 734125.
18. Kroenke K, Spitzer RL, Williams JB (2001) The PHQ-9: validity of a brief depression severity measure. J Gen Intern Med 16: 606-613.
19. Löwe B, Unützer J, Callahan CM, Perkins AJ, Kroenke K (2004) Monitoring depression treatment outcomes with the patient health questionnaire-9. Med Care 42: 1194-1201.
20. Löwe B, Schenkel I, Carney-Doebbeling C, Göbel C (2006) Responsiveness of the PHQ-9 to Psychopharmacological Depression Treatment. Psychosomatics 47: 62-67.
21. Spitzer RL, Williams JB, Kroenke K, Hornyak R, McMurray J (2000) Validity and utility of the PRIME-MD patient health questionnaire in assessment of 3000 obstetric-gynecologic patients: the PRIME-MD Patient Health Questionnaire Obstetrics-Gynecology Study. Am J Obstet Gynecol 183: 759-769.
22. Löwe B, Kroenke K, Herzog W, Gräfe K (2004) Measuring depression outcome with a brief self-report instrument: sensitivity to change of the Patient Health Questionnaire (PHQ-9). J Affect Disord 81: 61-66.
23. Deeks SG, Phillips AN (2009) HIV infection, antiretroviral treatment, ageing, and non-AIDS related morbidity. BMJ 338: a3172.
24. McCombe JA, Auer RN, Maingat FG, Houston S, Gill MJ, et al. (2009) Neurologic immune reconstitution inflammatory syndrome in HIV/AIDS: outcome and epidemiology. Neurology 72: 835-841.
25. Rybka J, Kędziora-Kornatowska K, Banaś-Leżańska P, Majsterek I, Carvalho LA, et al. (2013) Interplay between the pro-oxidant and antioxidant systems and proinflammatory cytokine levels, in relation to iron metabolism and the erythron in depression. Free Radic Biol Med 63: 187-194.
26. Leserman J (2008) Role of depression, stress, and trauma in HIV disease progression. Psychosom Med 70: 539-545.
27. Ammassari A, Antinori A, Aloisi MS, Trotta MP, Murri R, et al. (2004) Depressive symptoms, neurocognitive impairment, and adherence to highly active antiretroviral therapy among HIV-infected persons. Psychosomatics 45: 394-402.
28. Krysko DV, Agostinis P, Krysko O, Garg AD, Bachert C, et al. (2011) Emerging role of damage-associated molecular patterns derived from mitochondria in inflammation. Trends Immunol 32: 157-164.
29. Wong ML, Dong C, Maestre-Mesa J, Licinio J (2008) Polymorphisms in inflammation-related genes are associated with susceptibility to major depression and antidepressant response. Mol Psychiatry 13: 800-812.
30. Raison CL, Capuron L, Miller AH (2006) Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends Immunol 27: 24-31.
31. Dantzer R (2006) Cytokine, sickness behavior, and depression. Neurol Clin 24: 441-460.
32. Dantzer R, Kelley KW (2007) Twenty years of research on cytokine-induced sickness behavior. Brain Behav Immun 21: 153-160.
33. García-Bueno B, Caso JR, Leza JC (2008) Stress as a neuroinflammatory condition in brain: damaging and protective mechanisms. Neurosci Biobehav Rev 32: 1136-1151.
34. Young JJ, Bruno D, Pomara N3 (2014) A review of the relationship between proinflammatory cytokines and major depressive disorder. J Affect Disord 169: 15-20.
35. Shelton RC, Claiborne J, Sidoryk-Wegrzynowicz M, Reddy R, Aschner M (2011) Altered expression of genes involved in inflammation and apoptosis in frontal cortex in major depression. Mol Psychiatry 16: 751-762.
36. Allison RD, Katsounas A, Koziol DE, Kleiner DE, Alter HJ, et al. (2009) Association of interleukin-15-induced peripheral immune activation with hepatic stellate cell activation in persons coinfected with hepatitis C virus and HIV. J Infect Dis 200: 619-623.
37. Rahman S, Connolly JE, Manuel SL, Chehimi J, Montaner LJ, et al. (2011) Unique Cytokine/Chemokine Signatures for HIV-1 and HCV Mono-infection versus Co-infection as Determined by the Luminex® Analyses. J Clin Cell Immunol 2.

38. Sieburth D, Jabs EW, Warrington JA, Li X, Lasota J, et al. (1992) Assignment of genes encoding a unique cytokine (IL12) composed of two unrelated subunits to chromosomes 3 and 5. Genomics 14: 59-62.
39. D'Andrea A, Rengaraju M, Valiante NM, Chehimi J, Kubin M, et al. (1992) Production of natural killer cell stimulatory factor (interleukin 12) by peripheral blood mononuclear cells. J Exp Med 176: 1387-1398.
40. Gillessen S, Carvajal D, Ling P, Podlaski FJ, Stremlo DL, et al. (1995) Mouse interleukin-12 (IL-12) p40 homodimer: a potent IL-12 antagonist. Eur J Immunol 25: 200-206.
41. Anderson G, Maes M, Berk M (2013) Schizophrenia is primed for an increased expression of depression through activation of immuno-inflammatory, oxidative and nitrosative stress, and tryptophan catabolite pathways. Prog Neuropsychopharmacol Biol Psychiatry 42: 101-114.
42. Awodele O, Olayemi SO, Nwite JA, Adeyemo TA (2012) Investigation of the levels of oxidative stress parameters in HIV and HIV-TB co-infected patients. J Infect Dev Ctries 6: 79-85.
43. Morfeldt-Månson L, Böttiger B, Nilsson B, von Stedingk LV (1991) Clinical signs and laboratory markers in predicting progression to AIDS in HIV-1 infected patients. Scand J Infect Dis 23: 443-449.
44. Moore RD, Keruly JC, Chaisson RE (1998) Anemia and survival in HIV infection. J Acquir Immune Defic Syndr Hum Retrovirol 19: 29-33.
45. Mocroft A, Kirk O, Barton SE, Dietrich M, Proenca R, et al. (1999) Anaemia is an independent predictive marker for clinical prognosis in HIV-infected patients from across Europe. EuroSIDA study group. AIDS 13: 943-950.
46. Sullivan PS, Hanson DL, Chu SY, Jones JL, Ciesielski CA (1997) Surveillance for thrombocytopenia in persons infected with HIV: results from the multistate Adult and Adolescent Spectrum of Disease Project. J Acquir Immune Defic Syndr Hum Retrovirol 14: 374-379.
47. Evans RH, Scadden DT (2000) Haematological aspects of HIV infection. Baillieres Best Pract Res Clin Haematol 13: 215-230.
48. De Santis GC, Brunetta DM, Vilar FC, Brandão RA, de Albernaz Muniz RZ, et al. (2011) Hematological abnormalities in HIV-infected patients. Int J Infect Dis 15: e808-e811.
49. Rivière C, Subra F, Cohen-Solal K, Cordette-Lagarde V, Letestu R, et al. (1999) Phenotypic and functional evidence for the expression of CXCR4 receptor during megakaryocytopoiesis. Blood 93: 1511-1523.
50. Costantini A, Giuliodoro S, Mancini S, Butini L, Regnery CM, et al. (2006) Impaired in-vitro growth of megakaryocytic colonies derived from CD34 cells of HIV-1-infected patients with active viral replication. AIDS 20: 1713-1720.
51. Zetterberg E, Neuhaus J, Baker JV, Somboonwit C, Llibre JM, et al. (2013) Platelet count kinetics following interruption of antiretroviral treatment. AIDS 27: 59-68.
52. Li L, Han DK, Lu J (2010) Interferon-alpha induced severe thrombocytopenia: a case report and review of the literature. World J Gastroenterol 16: 1414-1417.
53. Bonaccorso S, Marino V, Biondi M, Grimaldi F, Ippoliti F, et al. (2002) Depression induced by treatment with interferon-alpha in patients affected by hepatitis C virus. J Affect Disord 72: 237-241.
54. Cossarizza A, Pinti M, Nasi M, Gibellini L, Manzini S, et al. (2011) Increased plasma levels of extracellular mitochondrial DNA during HIV infection: a new role for mitochondrial damage-associated molecular patterns during inflammation. Mitochondrion 11: 750-755.
55. Suresh DR, Annam V, Pratibha K, Prasad BV (2009) Total antioxidant capacity--a novel early bio-chemical marker of oxidative stress in HIV infected individuals. J Biomed Sci 16: 61.
56. Bonnes-Taourel D, Guérin MC, Torreilles J (1992) Is malonaldehyde a valuable indicator of lipid peroxidation? Biochem Pharmacol 44: 985-988.
57. Akay C, Cooper M, Odeleye A, Jensen BK, White MG, et al. (2014) Antiretroviral drugs induce oxidative stress and neuronal damage in the central nervous system. J Neurovirol 20: 39-53.
58. Reyskens KM, Essop MF (2014) HIV protease inhibitors and onset of cardiovascular diseases: a central role for oxidative stress and dysregulation of the ubiquitin-proteasome system. Biochim Biophys Acta 1842: 256-268.
59. Caron M, Auclairt M, Vissian A, Vigouroux C, Capeau J (2008) Contribution of mitochondrial dysfunction and oxidative stress to cellular premature senescence induced by antiretroviral thymidine analogues. Antivir Ther 13: 27-38.