

Research Article

Oxidative Imbalance in HIV-1 Infected Patients Treated with Antiretroviral Therapy

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Received 10 February 2009; Revised 19 July 2009; Accepted 23 September 2009

Recommended by George Perry

It is generally accepted that oxidative stress is involved in HIV infection. However, the role in oxidative balance of Highly Active Antiretroviral Therapy (HAART) is still debated. In our study we assessed serum oxidant and antioxidant levels in an HIV-1-infected population treated with HAART, and compared them with those of untreated HIV-1 patients and HIV-1-negative subjects. The study included 116 HIV-1-infected patients (86 HAART-treated and 30 untreated), and 46 HIV-negative controls. Serum oxidant levels were significantly higher in the HIV-1 treated group as compared to untreated and control groups. In addition, a decrease of serum total antioxidant status was observed in the HIV-1 treated group. To be noted is that patients who rigorously follow antiretroviral therapy (optimal HAART adherence) have significantly higher oxidative status than those who do not closely follow the therapy (poor HAART adherence). Analysis of variance revealed no significant further increase in oxidative status in HIV-1-infected patients taking antiretroviral and other drugs with the exception of psychiatric drugs (e.g. anxiolytics or antidepressants). Taken together, our results indicate that HAART may affect oxidative stress in HIV-1-infected patients and suggest that antiretroviral therapy plays an important role in the synergy of HIV infection and oxidative stress.

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1. Background

Highly Active Antiretroviral Therapy (HAART) is currently the therapy of choice for HIV-infected patients [1, 2]. However, despite remarkable viral replication suppression and immune response restoration, long-term HAART appears of limited use in many patients [3], because of additional adverse effects [4, 5] and/or regimen adherence difficulties [6]. Furthermore, poor adherence to HAART can have serious consequences, including loss of serum HIV suppression, development of drug-resistant HIV strains, and increased probability of illness progression [6]. It is widely reported that oxidative stress—an imbalance between production and elimination of chemically reactive species, such as Reactive Oxygen Species (ROS) [7]—is involved in HIV infection [8]. Viral Tat protein induces enhanced ROS production

in HIV-infected patients by mitochondrial generation of superoxide anion [9], which in turn may activate Nuclear Factor κ B (NF- κ B) [8], thus increasing HIV transcription. It has also been found that HIV infection is able to indirectly inhibit glutathione synthesis [10], which is the main endogenous antioxidant.

HAART may increase chemically reactive species in circulation, possibly by producing more oxidized metabolites deriving from the interaction between ROS and infected-cell biomolecules [11–16]. This is supported by several biochemical mechanisms, such as mitochondrial interference, following treatment with HAART-NRTI (Nucleoside Reverse Transcriptase Inhibitors) [14–16], and activation of the P450 hepatic system by HAART, when comprising Protease Inhibitors (PI) [13]. Insufficient intake or malabsorption of nutrients—common in HIV patients—may further worsen

these conditions [17, 18]. It has been suggested that immune system reactivation may, itself, be a direct cause of oxidative stress [11] in HAART, when comprising Nonnucleoside Reverse Transcriptase Inhibitors (NNRTI), such as efavirenz. Increased levels of serum isoprostane—a class of bioactive molecules derived from *in vivo* nonenzymatic oxidation of arachidonic acid—usually observed in smokers [19, 20], were also seen in patients taking efavirenz. On the basis of these findings, we aimed to assess the effect of HAART on oxidative stress in an HIV-1-infected population. To do this we used commercially available tests: (i) d-ROMs test to assess serum oxidative status and (ii) OXY-Adsorbent test to assess serum total antioxidant status.

2. Methods

2.1. Subjects. One hundred sixty-two subjects were enrolled by the *Department of Internal Medical Sciences, University of Cagliari, Italy*. Following written informed consent, they were divided into three groups: (1) HIV-1-negative subjects (controls), (2) HAART-treated HIV-1-infected patients and (3) untreated HIV-1-infected patients, as shown in Table 1. Controls comprised 46 subjects, apparently healthy, on adequate diets, mean age 39.5 ± 11.4 , of whom 21 females. No control took drugs or nutraceuticals. The second group comprised 86 HIV-1-infected patients, mean age 40.9 ± 8.1 , of whom 39 females, on adequate diets, all treated applying a therapy plan recommended by international guidelines [2]. One PI and two NRTIs were given to 49 patients: 15 received PI of nelfinavir, 8, lopinavir, 8, indinavir, 5, atazanavir, 5, saquinavir, 4, amprenavir, and 4, fosamprenavir; all PIs were boosted, with the exception of nelfinavir. One NNRTI and two NRTI were given to 33 patients: 19 received NNRTI of nevirapine and 14, efavirenz. Due to therapeutic regimen failure, the remaining four had to be treated with 1 NRTI + 1 NNRTI + 1 PI. As NRTI therapy plan, 51 patients received epivir, 35, stavudine, 30, didanosine, 28, zidovudine, 19, tenofovir, and 5, abacavir. The third group comprised 30 HIV-1-infected patients, mean age 35.9 ± 7.9 , of whom 14 females, on adequate diets. None of the group took drugs or nutraceuticals. The treated patients received HAART for an average of 6.3 ± 2.2 years. Patients taking at least 80% of their prescribed medication were arbitrarily considered as having optimal adherence, while those taking under 80% were considered as having poor adherence. Seventy-one of the 116 HIV-1-infected patients were smokers (22.5 ± 9.5 cigarettes daily), of whom 14 in the untreated and 57 in the treated group. There were 20 smokers in the control group (23.1 ± 9.1 cigarettes daily).

2.2. Oxidative Stress Assessment. Blood samples taken after a minimum of 12 hours' fasting were centrifuged and serum was divided into aliquots and stored at -80°C until used. To assess serum total oxidant and antioxidant levels we utilized the commercially available d-ROMs and OXY-Adsorbent tests (Diacron International, Grosseto, Italy), respectively [21, 22]. d-ROMs serum levels of 250–300 CARR U/mL were considered normal [21, 22], and OXY-Adsorbent serum levels higher than $350 \mu\text{moles HClO/mL}$ were considered

normal [22]. These tests were performed according to manufacturers' instructions.

2.3. Other Laboratory Assays and Clinical Data. Glycemia, cholesterolemia, triglyceridemia, alanine aminotransferase (ALT), HBsAg, anti-HBs, anti-HBc, and anti-HCV antibodies were assessed in all enrolled subjects, using the same blood samples used in the oxidative status and antioxidant reef tests. Quantitative serum viral load and blood CD4 T-cells were analyzed in HIV patients. Conventional and ultrasensitive Roche Amplicore HIV Assay (Roche Molecular System, Alameda, CA, USA) was used to assess serum HIV RNA. Viral loads equal or superior to 50 copies/mL serum can be detected by this test. CD4 T-cells were assessed by flux cytometry using the IMK multiset commercial kit (Becton Dickinson, Mountain View, CA, USA). Clinical data, including smoking habits, Body Mass Index (BMI), antiretroviral therapy regimen, and HAART adherence, were collected at the time of laboratory tests.

2.4. Statistical Analysis. Data are expressed as mean \pm SD. In preliminary analyses, we used χ^2 tests for nominal variables and one-way analysis of variance (ANOVA) (Statistic-StatSoft) for continuous variables, to compare data from the three groups analyzed. Statistical significance levels of at least $P < .05$ were used for all tests. To assess the influence of HIV-RNA on oxidative status, the mean d-ROMs levels of treated patients with ≤ 400 copies/mL HIV-RNA were compared to those of treated patients with > 400 copies/mL (ANOVA). The mean d-ROMs levels of treated patients with $\geq 350/\text{mL}$ CD4 T-cell count and those of treated patients with $< 350/\text{mL}$ CD4 T-cell count were compared (ANOVA), to assess the influence of immunological conditions on oxidative status. To evaluate the influence of normal and altered levels of glycemia, ALT, cholesterolemia, and triglyceridemia on oxidative status, we compared (ANOVA) mean d-ROMs levels in treated patients with glycemia ≤ 100 mg/dL versus > 100 mg/dL, ALT ≤ 41 U/L versus > 41 U/L, cholesterolemia ≤ 180 mg/dL versus > 180 mg/dL, and triglyceridemia ≤ 170 mg/dL versus > 170 mg/dL. In order to render results more evident, we also determined the Odds Ratio (OR) for the above parameters and multivariate logistic regression for the clinical and laboratory variables of treated patients.

3. Results

3.1. Clinical Data. The clinical data of the 116 HIV-1-infected patients (86 HAART-treated and 30 untreated) and of 46 controls are given in Table 1. As shown, the three groups differ significantly regarding age, number of smokers, BMI, cholesterolemia, triglyceridemia, and ALT and HIV-RNA levels.

3.2. Comparisons of Oxidative Balance Markers in HIV-1 Treated and Untreated Groups and Controls. Oxidative balance markers for the three experimental groups are given in Tables 2 and 3. The number of subjects with increased d-ROMs (> 300 CARR U/mL) was significantly higher in HIV-1 patients compared to controls ($P = .000$).

TABLE 1: Clinical data of HIV patients and controls.

	Controls	HIV-1-treated	HIV-1-untreated	<i>P</i>
Number	46	86	30	
Age (years)	39.5 ± 11.4	40.9 ± 8.1	35.9 ± 7.9	.038*
Age range	21–61	16–65	21–45	
Female/male	21/25	39/47	14/16	.992*
Current smokers	20	57	14	.021 [§]
Cigarettes daily	23.1 ± 9.1	25.7 ± 9.8	21.7 ± 9.4	.290*
BMI	21.7 ± 1.4	22.8 ± 2.7	23.0 ± 2.2	.017*
Cholesterolemia (mg/dL)	183.0 ± 27.4	195.4 ± 39.4	164.7 ± 25.4	.000*
Triglyceridemia (mg/dL)	84.7 ± 39.8	181.1 ± 179.8	90.5 ± 38.9	.000*
Glycemia (mg/dL)	88.4 ± 10.3	89.7 ± 19.2	90.5 ± 10.8	.836*
ALT (UI/L)	21.1 ± 8.2	46.5 ± 38.9	40.13 ± 36.5	.000*
HCV positive	0	39	13	
HBV positive	0	1	0	
HCV + HDV positive	0	4	1	
HAART-PI	—	49	—	
HAART-NNRTI	—	33	—	
HAART-PI + NNRTI	—	4	—	
CD4 T-cell count (cells/mL)	nd	554.4 ± 279.2	528.9 ± 119.5	.629*
CD4 T-cell count ≥ 500 cells/mL	—	46	16	.864 [§]
CD4 T-cell count 499–200 cells/mL	—	32	14	.693 [§]
CD4 T-cell count < 200 cells/mL	—	8	0	.220 [§]
HIV-RNA level (log ₁₀ cp/mL)	nd	2.2 ± 0.8	4.1 ± 0.4	.000*
HIV-RNA < 400 cp/mL	—	69	0	

Data are expressed as mean ± SD.

*Calculated by One-Way ANOVA.

[§]Calculated by χ^2 .

As expected, HIV-1-infected patients display higher serum oxidant levels than controls ($P = .000$) (Table 2). However, within the HIV-1-infected group, untreated patients had significantly lower oxidant levels than treated ones ($P = .001$, Table 3). Oxidative status was more pronounced in females compared to males in all three groups considered. However, in untreated patients, gender difference was not statistically significant ($P = .08$) (Table 3).

The number of subjects with decreased OXY-Adsorbent (< 350 $\mu\text{mol HClO/mL}$) was not statistically different among the three groups (Table 2). Treated patients had antioxidant reef levels significantly lower than controls and untreated patients ($P = .000$). No significant difference in antioxidant reef levels was observed in males and females treated with HAART ($P = .59$), while control and untreated males had antioxidant reef levels which were significantly lower than corresponding females ($P = .027$, $P = .018$, resp.).

3.3. Oxidative Balance and Smoking. Smoking is linked to an increase in oxidative stress [19, 20]. For this reason we next assessed the influence of smoking on oxidative balance in the three experimental groups.

As shown in Table 2, d-ROMs was significantly higher in treated patients—both smokers and nonsmokers—compared to controls and untreated patients—both smokers

and nonsmokers ($P = .000$). On the other hand, OXY-Adsorbent was lower in treated smokers compared to those in the control and untreated groups ($P = .000$), while no difference in OXY-Adsorbent was observed in nonsmokers in the three groups (Table 2). As expected, control smokers had higher d-ROMs levels than control nonsmokers ($P = .001$). However, smoking significantly affected d-ROMs in neither treated nor untreated patients ($P = .8$, $P = .2$, resp.).

OXY-Adsorbent levels did not differ in smoker and nonsmoker controls ($P = .299$) nor in smoker and nonsmoker-untreated patients ($P = .841$), while they were significantly higher in nonsmoker-treated patients compared to smoker ones ($P = .022$).

3.4. Oxidative Status and HAART. Given the high d-ROMs levels in treated patients, it seemed to us to be of interest to evaluate the relationship between oxidative status and HAART adherence. Patients with optimal adherence had significantly higher d-ROMs levels than those with poor adherence ($P = .008$; OR = 4.2, 95% C.I. of 1.1–15.5) (Table 3). Differences in the following variables CD4 T-cell count, HIV-RNA level, antiretroviral-drug class, HCV coinfection, ALT, glycemia, cholesterolemia, and triglyceridemia were not significantly linked to d-ROMs levels (Table 3). An additional increase in d-ROMs was observed in patients receiving HAART plus psychiatric drugs (Table 3).

TABLE 2: Comparison of d-ROMs and Oxy-Adsorbent tests between the three groups.

	Controls	HIV-1 treated	HIV-1 untreated	<i>P</i>
d-ROMs (CARR U/mL)	295.4 ± 58.9	366.1 ± 68.8	322.1 ± 41.2	.000*
d-ROMs (CARR U/mL) in females	319.7 ± 66.6	393.0 ± 78.4	336.3 ± 49.1	.000*
d-ROMs (CARR U/mL) in males	275.0 ± 43.1	343.8 ± 50.5	309.6 ± 29.0	.000*
d-ROMs increased: no. of subjects	18	72	18	.000 [§]
OXY-Adsorbent (μmol HClO/mL)	433.6 ± 73	411.6 ± 48.2	458.7 ± 50.6	.000*
OXY-Adsorbent (μmol HClO/mL) in females	461.1 ± 77.6	408.5 ± 44.3	477.2 ± 57.1	.000*
Oxy-Adsorbent (μmol HClO/mL) in males	410.6 ± 61.4	414.1 ± 51.4	430.0 ± 53.5	.228*
Oxy-Adsorbent reduced: no. of subjects	4	6	0	.276 [§]
Smokers				
d-ROMs (CARR U/mL)	325.9 ± 68.8	362.1 ± 60.4	312.1 ± 30.0	.000*
Oxy-Adsorbent (μmol HClO/mL)	420.7 ± 67.3	403.2 ± 45.0	457.6 ± 59.1	.000*
Nonsmokers				
d-ROMs (CARR U/mL)	272.0 ± 36.6	366.1 ± 56.4	331.4 ± 48.7	.000*
Oxy-Adsorbent (μmol HClO/mL)	443.5 ± 76.9	428.1 ± 50.6	453.1 ± 61.8	.113*

Data are expressed as mean ± SD.

*Calculated by One-Way ANOVA.

[§]Calculated by χ^2 .

Multivariate logistic regression confirmed significant oxidative-status increase in optimal-adherence-treated patients ($P = .032$) and in women ($P = .005$), while they did not reveal any link between HAART and psychiatric drugs ($P = .14$).

4. Discussion

The d-ROMs and OXY-Adsorbent tests, both available commercially, were used to determine oxidative status and antioxidant-reef status, respectively, in the serum of one treated and one untreated group of HIV-1-infected patients and of one control group. It has been widely reported that HIV infection causes oxidative stress [8, 23–25], which is an imbalance between oxidant and antioxidant status. Under our experimental conditions, significantly higher d-ROMs levels were found in HIV-1-infected patients—both treated and untreated—compared to controls, while OXY-Adsorbent levels were significantly reduced in HAART patients. However, as reported by other authors [26], we too found that treated HIV-1-infected patients had significantly higher d-ROMs levels than untreated ones or controls. Taken together, our results indicate that HAART may affect oxidative stress in HIV-1-infected patients and suggest that antiretroviral therapy plays an important role in the synergy of HIV infection and oxidative stress [26]. Accordingly, under our experimental conditions, when treated patients alone are considered, viral load does not seem to affect oxidative status. Very significantly, patients who rigorously follow antiretroviral therapy (optimal HAART adherence) have higher oxidative status than those who do not closely follow the therapy (poor HAART adherence). Different therapy regimens do not seem to affect oxidative status. Analysis of variance revealed no significant further increase in oxidative status in HIV-1-infected patients taking antiretroviral and other drugs (antibiotics,

antihypertensive drugs, painkillers, lipid-lowering agents), with the exception of psychiatric drugs (e.g., anxiolytics or antidepressants). However, multivariate logistic regression analysis confirmed the statistical significance of these findings for only two variables: optimal HAART adherence and female gender. CD4 T-cell count, HIV-HCV co-infection, glycemia, cholesterolemia, and triglyceridemia did not appear to correlate with oxidative status. However, triglyceridemia and cholesterolemia were significantly lower in controls and untreated patients compared to treated ones, highlighting the fact that HAART causes dislipidemia [27]. This condition, in turn, may increase the risk of cardiovascular disorders. The antioxidant component, assessed (with the OXY-Adsorbent test) as serum total antioxidant capacity, was within normal levels ($> 350 \mu\text{mol HClO/mL}$) in most HIV-1 patients enrolled. This finding is only apparently in contrast with literature [17], which reports reduced antioxidant capacity in HIV-infected patients, predominantly those at an advanced stage of the illness. In fact, the pathology status of HIV-1-infected patients under study, except for 8 treated patients (6.9%) with less than 200 CD4 T-cell/mL, was not yet advanced. It is of interest that the 30 HIV-1-infected patients who, given their adequate immunological and virological states, were untreated, had a higher antioxidant reef than HAART-treated patients. The lowest antioxidant-reef levels were found in treated patients, while the highest ones were found in untreated patients. These findings are not readily explainable; however, the lowest antioxidant-reef levels—which were found in treated patients—might be not only due to the therapy itself but also due to an increased utilization of endogenous antioxidants, following increased ROS production in these patients. The remaining important aspects of our study concern gender and smoking. Oxidative status in female-treated patients and female controls was significantly higher than in males. A possible explanation is

TABLE 3: Comparison of d-ROMs concentration in the three groups, dichotomously classified by clinical and laboratory variables.

Variables	d-ROMs		P*	OR (CI 95%)
Controls				
Gender	Female	Male		
	319.7 ± 66.6	275.0 ± 43.1	.009	6.5 (1.7–24.3)
Smoking	Yes	No		
	325.9 ± 68.8	272.0 ± 36.6	.001	6.3 (1.7–23.7)
HIV-1 infection				
HAART	Yes	No		
	366.1 ± 68.6	322.4 ± 41.5	.001	3.4 (1.3–8.7)
HIV-1 treated				
Gender	Female	Male		
	393.0 ± 78.4	343.8 ± 50.5	.000	3.7 (0.9–14.2)
Smoking	Yes	No		
	362.1 ± 60.4	366.1 ± 56.4	.8	0.7 (0.2–2.6)
CD4 T-cells	≥ 350 cells/mL	< 350 cells/mL		
	364.0 ± 69.1	373.1 ± 69.0	.6	0.9 (0.2–3.5)
HIV-RNA	≤ 400 cp/mL	>400 cp/mL		
	364.6 ± 70.1	372.3 ± 64.8	.7	1.1 (0.3–4.6)
Drug class	PI	NNRTI		
	372.5 ± 75.1	359.7 ± 61.6	.4	1.3 (0.4–4.4)
Nelfinavir	Yes	No		
	398.2 ± 95.6	359.9 ± 61.2	.056	∞
Nevirapine	Yes	No		
	372.3 ± 63.4	364.4 ± 70.6	.7	1.8 (0.4–9.1)
Efavirenz	Yes	No		
	348.1 ± 54.2	370.6 ± 71.6	.2	0.4 (0.1–1.5)
HAART adherence	Optimal	Poor		
	378.5 ± 71.7	336.0 ± 50.7	.008	4.2 (1.1–15.5)
Other drugs + HAART	Yes	No		
	371.8 ± 61.7	363.1 ± 72.7	.6	2.2 (0.6–8.6)
Psychiatric drugs + HAART	Yes	No		
	399.4 ± 51.5	359.7 ± 70.1	.047	∞
HIV-HCV co-infected	Yes	No		
	359.7 ± 57.9	371.3 ± 79.1	.5	1.1 80.3–3.3
ALT	≤ 41 UI/L	< 40 UI/L		
	374.2 ± 80.8	355.0 ± 46.3	.2	1.0 (0.33–3.3)
Glycemia	≤ 100 mg/dL	< 100 mg/dL		
	372.3 ± 71.1	342.9 ± 54.9	.1	2.3 (0.7–8.1)
Cholesterolemia	≤ 180 mg/dL	> 180 mg/dL		
	355.0 ± 61.6	372.7 ± 72.5	.2	0.7 (0.2–2.4)
Triglyceridemia	≤ 170 mg/dL	> 170 mg/dL		
	371.2 ± 79.4	358.4 ± 48.5	.4	0.8 (0.2–2.8)
HIV-1 untreated				
Gender	Female	Male		
	336.3 ± 49.1	309.6 ± 28.9	.076	0.8 (0.2–3.5)
Smoking	Yes	No		
	312.1 ± 30.0	331.4 ± 48.7	.2	0.8 (0.2–3.5)
HIV-HCV co-infected	Yes	No		
	330.4 ± 38.9	314.7 ± 43.0	.3	2.5 (0.5–11.4)
ALT	≤ 41 UI/L	< 40 UI/L		
	316.9 ± 40.2	332.4 ± 43.3	.3	0.2 (0.04–1.4)

Data are expressed as mean ± SD.

*Calculated by One-Way ANOVA.

that in these two groups there were several women of perimenopausal or menopausal age, whose conditions, as is known, increase ROS production [28, 29]. By contrast, the majority of untreated female patients under study were of fertile age and did not significantly differ in oxidative status from males in the same group. Our study, as did previous ones [19, 20], revealed (as expected) that smoker controls had a significant increase in oxidant levels compared to nonsmoker controls. Surprisingly, no difference in oxidative status between treated and untreated HIV-1-infected groups was seen when, within these groups, smokers and nonsmokers were compared, even though the number of smokers in the HIV-1 group was significantly higher than in the control group. This result suggests that increased oxidative stress in HIV-1 patients mainly depends on the infection and/or antiretroviral therapy.

5. Conclusion

In summary, our results show that HIV-1 infection increases oxidative status, and that it is further increased by HAART. Consequently they suggest that HAART plays an important role in determining oxidative stress in these patients. Supporting this, patients with optimal adherence show higher oxidative stress than those with poor adherence. In addition, they suggest that antiretroviral therapy also affects metabolic status. Viral load and CD4 do not appear to be involved in oxidative stress occurring in HIV-1 patients.

Competing Interests

The authors declare that they have no competing and/or financial interests.

Authors' Contributions

AM was primarily responsible for the design of the study, for developing the analysis concept, for analysis of data, and for writing the manuscript. ELI provided help with oxidative balance determination. MGC and CB were responsible for data management. AM and DC were responsible for analysis. SD oversaw all aspects of this study. NC was responsible for the biochemical parts and participated in the writing of the study. All authors have read and approved the final manuscript.

Acknowledgment

The authors wish to thank volunteers and HIV-1 patients, all of whom enthusiastically agreed to participate in this study. They would also like to thank Professor Angelo Balestrieri for his invaluable general advice and Professor Edward John Steeden for his excellent help in translating this paper. The technical assistance given by Noemi Ruggeri is much appreciated.

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