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Integrase inhibitor-based antiretroviral treatments decrease oxidative stress caused by HIV infection

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Abstract. – OBJECTIVE: Several chronic illnesses, including HIV infection are associated with oxidative stress. In addition to HIV itself, some antiretrovirals also increase oxidative stress while decreasing viral replication. To investigate the alterations in oxidative stress parameters and thiol-disulphide homeostasis in people living with HIV who were receiving integrase inhibitor-based antiretroviral therapy.

PATIENTS AND METHODS: Thirty treatment-naive adult people living with HIV were prospectively enrolled in the study. Sera were collected from patients twice: at the beginning of antiretroviral therapy (group 1) and 6 months later (group 2). Thirty age-matched healthy volunteers were enrolled in the study as the control group (group 3). Serum levels of total antioxidant status (TAS) and total oxidative status (TOS) were determined using an automated measurement method. Serum malondialdehyde (MDA) and protein carbonyl (PC) levels were measured spectrophotometrically. CD4+ T-cells were counted flow cytometrically. A mathematical equation was used to calculate the oxidative stress index (OSI) and determine disulfide levels (DIS).

RESULTS: TOS, OSI, MDA, and PC levels were significantly increased in treatment-naive people living with HIV than in those receiving ART (p<0.001). Total and native thiol were significantly lower in both HIV-infected groups than in the control group (p<0.001). PC and MDA levels were significantly higher in both HIV-infected groups than in the control group (p<0.001). In correlation analysis, MDA and age were negatively correlated, whereas TAS was positively correlated with CD4+ T-cell count in treatment-naive people living with HIV. Age was positively correlated with TOS (r:0.421, p:0.023) in healthy controls.

CONCLUSIONS: Integrase inhibitor-based antiretroviral treatments decrease the oxidative stress caused by HIV infection and may be a good therapeutic option in people living with HIV.

Key Words:
Oxidative stress, HIV infection, Integrase inhibitor-based antiretroviral treatment.

Introduction

Reactive oxygen species (ROS) are produced by living organisms as a result of cellular metabolism. There is a balance between oxidants and antioxidants in the body ordinarily. A shift in the balance between oxidant/antioxidant status in favor of oxidants is termed "oxidative stress" (OS). OS can injure cells and tissues by lipid peroxidation, protein oxidation (PO), and DNA damage[1-4]. Human cells have several enzymatic and non-enzymatic antioxidants that can limit ROS associated damage[5].

Thiols, also known as native thiols, are important antioxidants that contain sulfhydryl groups (SH) and play a key role in the eradication of ROS. Thiol proteins account for 52.9% of serum total antioxidant status (TAS) in healthy individuals[6]. Under OS, thiols release hydrogen into the environment and form a disulfide bond (S-S). The released hydrogen binds excess oxygen, leading to deactivation of ROS and thereby protect tissues from oxidative damage. Normally, there is a balance between thiols and the disulphide (DIS) called dynamic thiol/disulphide homeostasis. OS occurs when this balance shifts towards the DIS[2].

Several chronic illnesses are associated with OS-related events, including HIV infection[6-10]. OS plays an important role both in the pathogenesis of HIV disease, and in the progression from the asymptomatic stage to the development of AIDS[11,12]. Several studies have reported that OS increases with HIV infection[1,2,11-13]. Furthermore, the HIV-1 transactivator of transcription (Tat)

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protein induces increased ROS production *via* mitochondrial generation of superoxide anion, that activates nuclear factor ƙB (NF-ƙB). NF-ƙB is involved in transcription[2]. Due to the activated transcription factors caused by the increased exposure to ROS, adaptive responses are triggered, apoptosis is initiated, and cells become more resistant to insults. Increased ROS results in more mitochondrial damage, and the cell cycle halts to enable DNA repair[14]. Ongoing OS was also found to be a predictor of all-cause mortality in people living with HIV. It is independent of the established HIV-associated predictors such as $CD4^+$ T-cell count, HIV viral load and subclinical inflammation[11].

Antiretroviral drugs are classified according to steps they block in the HIV life cycle. Integrase inhibitor-based antiretroviral treatments are preferred regimens in recent guidelines[15]. Uncontrolled viral replication was not associated with increased OS, however different classes of antiretroviral treatment (ART) were shown to induce OS *via* several biochemical reactions. For example, mitochondrial toxicity following nucleoside reverse transcriptase inhibitor (NRTI) use and activation of the cytochrome P450 hepatic enzyme system by protease inhibitors (PIs)[2,5,8]. Despite this, OS induced by ART is thought to be less than OS induced by HIV infection[1]. No increase in OS with integrase inhibitor use was detected in cell culture investigations[16].

Besides HIV, several disease states, especially age-related ones that cause chronic inflammation, such as atherosclerosis, cardiovascular diseases, cancer, neurodegenerative disorders are associated with oxidation and modification of thiols[17]. Age-dependent plasma oxidation can occur even in healthy individuals[18,19]. With recent improvements in ART, people living with HIV can have a life expectancy approximately in line with that of the general population[20]. People living with HIV develop age-related complications such as atherosclerosis, diabetes, and neurodegenerative disorders earlier than those without HIV[2]. Aging people living with HIV are exposed to more ROS produced by HIV and ART in addition to ongoing age-dependent oxidation. Thus, the leading cause of mortality in people living with HIV who are receiving ART is cardiovascular diseases, possibly owing to underlying chronic inflammation triggered by ROS[21].

This study aimed to determine the direct effect of integrase inhibitor-based ART on thiol/disulfide homeostasis and OS parameters. We assessed a group of ART-naive people living with HIV before and 6 months after ART initiation compared with a group of matched healthy controls.

Patients and Methods

Study Population

This observational study was approved by the Local Ethical Committee of Bezmialem Vakif University, Istanbul, Turkey (No: 71306642/050-01-04/119). We prospectively enrolled 30 adult people living with HIV (≥18 years) who volunteered to participate in the study between 2017-2018, independent of ART status. We followed-up them for 6 months. All participants provided written informed consent before the study. Exclusion criteria included co-infections such as hepatitis and other opportunistic infections that could affect OS parameters. Sera were collected from patients twice: at the beginning of ART (group 1) and 6 months after ART initiation (group 2). ART regimens were chosen according to recent HIV guidelines for each patient independent of the study. Thirty age-matched healthy volunteers were enrolled as the control group (group 3).

Biochemical Analyses

Serum levels of total antioxidant status (TAS), total oxidative status (TOS), total thiol (TT), and native thiol (NT) were determined using automated photometric methods (Erel O, Rel Assay®, Turkey). Serum malondialdehyde (MDA) and protein carbonyl (PC) contents were measured spectrophotometrically. $CD4^+$ T-cells were counted using a flow cytometry (BD, FACS-CANTO II, Biosciences, Franklin Lakes, NJ, USA). OS index (OSI) was calculated according to the following formula: OSI (Arbitrary Unit)=TOS (μmol H_2O_2 Eq/L)/ TAS (mmol Trolox Eq/L). Disulfide (DIS) concentrations were calculated as half of the difference between levels of the TT and NT.

Statistical Analysis

Data distribution was assessed using the Shapiro-Wilk test. Normally distributed paired comparisons were performed using paired *t*-tests, and non-normally distributed paired comparisons were performed using the Wilcoxon test. Student's *t*-tests and the Mann Whitney U tests were used to compare two independent groups. Spearman's rank correlation coefficients were

Table I. Characteristics of study groups.

Parameters	Group 1 ART-Naive No. 30	Group 2 on ART No. 30	Controls No. 30
Age, mean (± STD)	38.7 (± 10.3)	38.7 (± 10.3)	38.7 (± 10.3)
Gender, men, n (%)	26 (86.7)	26 (86.7)	26 (86.7)
HIV-RNA (IU/mL) mean (± STD)	2,973,036 (± 9,040,487)	136 (± 389)	NA†
CD4+ T-cell count (cells/mm³) mean (± STD)	370 (± 334)	581 ± 358	NA†
Antiretroviral treatment n (%)			
• TDF/FTC/EVGc	10 (33.3)	10 (33.3)	NA†
• TAF/FTC/EVGc	7 (23.3)	7 (23.3)	
• TDF/FTC+DTG	11 (36.6)	11 (36.6)	
• ABC/3TC/DTG	2 (6.6)	2 (6.6)	

†NA: not applicable. HIV: human immunodeficiency virus, STD: standard deviation, TDF: Tenofovir Disoproxil Fumarate, FTC: Emtricitabine, EVGc: Elvitegravir-cobicistat, TAF: Tenofovir Alafenamide, DTG: Dolutegravir, ABC: Abacavir.

calculated. Statistical analysis of all data was performed using SPSS version 22.0 (SPSS, IBM; Armonk, NY, USA), and $p \leq 0.05$ was considered statistically significant; 95% confidence intervals were also reported.

Results

Characteristics of patients and healthy controls are summarized in Table I. Differences in all OS parameters except TAS and DIS were statistically significant on comparing groups 1 and 3 (Table II).

TT and NT levels were significantly lower in both people living with HIV groups than in the control group ($p<0.001$). Additionally, TT and NT levels were significantly lower in treatment naive people living with HIV, compared with levels at 6 months after ART initiation ($p<0.001$). There were significant differences in DIS levels when comparing groups 1 and 3 (p:0.049) and comparing groups 2 and 3 (p:0.048). But no significant difference was detected in DIS levels between groups 1 and 2 (p:0.49). PC and MDA levels were significantly higher in people living with HIV than in healthy controls ($p<0.001$). PC and MDA levels were also significantly higher in treatment naive people living with HIV compared with levels at 6 months after ART initiation ($p<0.001$).

Correlation analyses showed that MDA and age were negatively correlated, whereas TAS was positively correlated with $CD4^+$ T-cell count in group 1 (Table III). Age was negatively correlated with $CD4^+$ T-cells in group 2. Age was positively correlated with TOS (r:0.421, p:0.023) and TT (r:0.461, p:0.01) but negatively correlated with PC (r: -0.416, p:0.022) in healthy controls.

Table II. Comparison of mean values of oxidative stress parameters of study groups.

Parameters	Mean (± SD) Group 1-Naive	Mean (± SD) Group 2-on ART	Mean (± SD) Group 3-Control
TAS (mmol Trolox Eq/L)	1.87 (± 2.56)‡	2.03 (± 2.45)‡	2.09 (± 2.46)
TOS (µmol H_2O_2 Eq/L)	20.49 (± 5.65)§‡	12.05 (± 4.07)‡	11.14 (± 3.41)
OSI (Arbitrary Units)	24.83 (± 18.02)§‡	10.25 (± 5.89)‡	7.38 (± 4.13)
DIS (µmol/L)	27.4 (± 107.74)‡	45.1 (± 108.15)‡	23.3 (± 91.86)
MDA (nmol/mL)	18.3 (± 6.76§‡	10.9 (± 6.66)‡	7.6 (± 5.96)
PC (nmol/mg protein)	1339.9 (± 486.75)§‡	959.1 (± 365.48)‡	911 (± 313.15)
TT (µmol/L)	351.7 (± 146)§‡	473.6 (±1 78.11)‡	586.7 (± 13.22)
NT (µmol/L)	296.9 (± 137.97)§‡	383.5 (± 161.05)‡	540.1 (± 136.79)
CD4+ T-cell (mm³)	371 (± 335)§	581 (± 358)	NA†

†NA: not applicable; §: $p<0.01$ comparison with group 2; ‡: $p<0.05$ comparison with group 3.

Table III. Correlation of CD4+ T cell with other parameters in group 1.

Parameters	The correlation coefficient (r)	*p*-value
TOS	-0.007	0.97
TAS	**0.381**	**0.038***
OSI	-0.186	0.325
TT	0.066	0.729
NT	0.188	0.319
DIS	0.002	0.993
MDA	**-0.582**	**0.001***
PC	-0.031	0.869
Age	**-0.371**	**0.048***

*: Statistically significant at $p<0.05$.

Discussion

We found increased OS, OSI, MDA, and PC levels but decreased TAS, TT, and NT levels in treatment-naive people living with HIV compared with those in healthy controls. These findings are consistent with those of most previous studies[11-13]. However, OS was not increased as expected, but rather decreased with the use of integrase inhibitor-based ART for 6 months. This is in contrast with previous researches of patients using non-integrase inhibitor-based ART. Wanchu et al[22] reported increased OS in people living with HIV based on lipid peroxidation, whereas no significant difference in OS parameters was reported with ART consisting of two NRTI and one non-nucleoside reverse transcriptase inhibitor (NNRTI). In a study from Cameroon, using a combination of NRTIs and NNRTIs, both HIV infection and ART were found to increase OS parameters, whereas ART compounded this effect by lipid oxidation[23]. Despite these studies, Hulgan et al[8] suggest that therapeutic control of HIV replication by ART including PI, NRTI, and NNRTI, rather than HIV infection, was associated with increased OS. NRTIs and NNRTIs are associated with mitochondrial dysfunction, whereas PIs are associated with the activation of the P450 hepatic enzyme system[24]. In another study that evaluated OS, inflammation, and endothelial function**,** OS was not found to differ between NRTIs and NNRTIs, whereas significant differences were detected between the control[25].

Furthermore, OS, OSI, MDA, PC, and DIS levels were significantly increased on comparing people living with HIV receiving ART with healthy controls in our study. Although most of our patients were virologically suppressed at 6 months after ART initiation, OS parameters remained elevated when compared with those in healthy controls. However, it is unclear, if these levels would decline further after more than 6 months of ART.

In our report, increased OS due to HIV infection was associated with significant thiol depletion in ART-naive people living with HIV, which is consistent with findings of previous researches[26]. However, both OS and thiol depletion were lower in the ART group than in the ART-naive people living with the HIV group, although they remained higher in the ART group than in the control group. TAS was also significantly decreased in ART-naive patients. Because thiols are the largest component of the antioxidant system, this was an expected result. There was a trend towards an increase in TAS with ART. This suggests that HIV infection directly affects TAS levels regardless of ART. This antioxidant deficiency in HIV-1 seropositive population is probably due to depletion of antioxidant molecules. They are consumed in the process of protecting cells against ROS induced oxidative damage which is related to the advancement of the disease to AIDS. A weakened antioxidant defense system, in turn, could lead to further enhancement in lipid peroxidation[22,27]. In a study from Nigeria, antioxidant levels were significantly reduced in HIV-infected patients but were also improved in people living with HIV receiving ART[28].

$CD4^+$ T-cell count is negatively correlated with MDA concentration (r: -0.515, *p*: 0.001) in ART-naive people living with HIV. This is similar to the results found in HIV-infected children in a previous study[29]. This finding is further confirmed by another study showing a negative correlation between Center for Disease Control (CDC) illness classifications and MDA levels[30]. Age and $CD4^+$ T-cell count (r: -0.371, *p*: 0.048)

were also negatively correlated in ART-naive people living with HIV. With age-related perspective, both an unavoidable reduction in T-cell production occurs, and existing naive T cells may also become intrinsically dysfunctional in all populations[31]. These may be the causes of the negative correlation between the $CD4^+$ T cell count and age. Age was also negatively correlated with the $CD4^+$ T-cell count in group 2. The $CD4^+$ T-cell response after ART initiation may be delayed and weakened in older patients because of decreased T-cell production. Moreover, age was positively correlated with TOS in healthy controls, which is in keeping with findings from previous studies[18,19]. Aging has been described as a low-grade inflammatory state, termed as "inflammaging", which may guide us in ART selection that will not further increase OS in aging people living with HIV, although we could not show a correlation between age and OS in people living with HIV in our study[32].

The limitation of our paper is the small size of the study population, which did not allow us to compare elvitegravir-based regimens with dolutegravir-based ones. Further clinical researches are needed to investigate the long-term effects of integrase inhibitor-based ART.

This is the first study showing the decrease in OS parameters with ART in people living with HIV. OS plays critical role in most chronic diseases such as HIV. So clinicians may prefer to follow up the OS levels as well, for better clinical outcomes in these chronic diseases.

Conclusions

We suggest that integrase inhibitor-based ART decreases the OS caused by HIV infection. People living with HIV need a lifelong treatment, integrase inhibitor-based ART may be a good therapeutic option.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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References

1) Sharma B. Oxidative stress in HIV patients receiving antiretroviral therapy. Curr HIV Res 2014; 12: 13-21.
2) Popoola TD, Awodele O. Interplay between antiretroviral therapy and oxidative stress in HIV seropositive patients. Afr J Med Med Sci 2016; 45: 5-21.
3) Galleze A, Kocyigit A, Cherif N, Attal N, Touil-Boukoffa C, Raache R. Assessment of oxidative/anti-oxidative markers and DNA damage profile induced by chemotherapy in algerian children with lymphoma. Drug Chem Toxicol 2019: 1-5.
4) Teto G, Kanmogne GD, Torimiro JN, Alemnji G, Nguemaim FN, Takou D, Nanfack A, Tazoacha A. Lipid peroxidation and total cholesterol in HAART-naïve patients infected with circulating recombinant forms of human immunodeficiency virus type-1 in Cameroon. PLoS One 2013; 8: e65126.
5) Sundaram M, Saghayam S, Priya B, Venkatesh KK, Balakrishnan P, Shankar EM, Murugavel KG, Solomon S, Kumarasamy N. Changes in antioxidant profile among HIV-infected individuals on generic highly active antiretroviral therapy in southern India. Int J Infect Dis 2008; 12: e61-66.
6) Erturan I, Kumbul DoĞuç D, Korkmaz S, Büyükbayram HI, Yildirim M, Kocabey Uzun S. Evaluation of oxidative stress in patients with recalcitrant warts. J Eur Acad Dermatol Venereol 2019; 33: 1952-1957.
7) Colado Simao AN, Victorino VJ, Morimoto HK, Reiche EM, Panis C. Redox-driven events in the human immunodeficiency virus type 1 (HIV-1) infection and their clinical implications. Curr HIV Res 2015; 13: 143-150.
8) Hulgan T, Morrow J, D'Aquila RT, Raffanti S, Morgan M, Rebeiro P, Haas DW. Oxidant stress is increased during treatment of human immunodeficiency virus infection. Clin Infect Dis 2003; 37: 1711-1717.
9) Williams AA, Sitole LJ, Meyer D. HIV/HAART-associated oxidative stress is detectable by metabonomics. Mol Biosyst 2017; 13: 2202-2217.
10) Falay Gur T, Erdemir AV, Gurel MS, Kocyigit A, Guler EM, Erdil D. The investigation of the relationships of demodex density with inflammatory response and oxidative stress in rosacea. Arch Dermatol Res 2018; 310: 759-767.
11) Masiá M, Padilla S, Fernández M, Rodríguez C, Moreno A, Oteo JA, Antela A, Moreno S, Del Amo J, Gutiérrez F. Oxidative stress predicts all-cause mortality in HIV-infected patients. PLoS One 2016; 11: e0153456.
12) Ivanov AV, Valuev-Elliston VT, Ivanova ON, Kochetkov SN, Starodubova ES, Bartosch B, Isaguliants MG. Oxidative Stress during HIV Infection: Mechanisms and Consequences. Oxid Med Cell Longev 2016; 2016: 8910396.
13) Awodele O, Olayemi SO, Nwite JA, Adeyemo TA. Investigation of the levels of oxidative stress param-

eters in HIV and HIV-TB co-infected patients. J Infect Dev Ctries 2012; 6: 79-85.

14) Ikekpeazu JE, Orji OC, Uchendu IK, Ezeanyika LUS. Mitochondrial and oxidative impacts of short and long-term administration of HAART on HIV patients. Curr Clin Pharmacol 2019. Epub 2019/09/06. doi: 10.2174/1574884714666190905162237.

15) EACS Guidelines Version 10.0 - November 2019 2020 [cited 2020 15.6.2020]. Available from: https://www.eacsociety.org/guidelines/eacs-guidelines/eacs-guidelines.html.

16) Afonso P, Auclair M, Caron-Debarle M, Capeau J. Impact of CCR5, integrase and protease inhibitors on human endothelial cell function, stress, inflammation and senescence. Antivir Ther 2017; 22: 645-657.

17) Oliveira PVS, Laurindo FRM. Implications of plasma thiol redox in disease. Clin Sci (Lond) 2018; 132: 1257-1280.

18) Blanco RA, Ziegler TR, Carlson BA, Cheng PY, Park Y, Cotsonis GA, Accardi CJ, Jones DP. Diurnal variation in glutathione and cysteine redox states in human plasma. Am J Clin Nutr 2007; 86: 1016-1023.

19) Rossi R, Giustarini D, Milzani A, Dalle-Donne I. Cysteinylation and homocysteinylation of plasma protein thiols during ageing of healthy human beings. J Cell Mol Med 2009; 13: 3131-3140.

20) Burki T. People ageing with HIV face an uncertain future. Lancet HIV 2019; 6: e816-e817.

21) Laurence J, Elhadad S, Ahamed J. HIV-associated cardiovascular disease: importance of platelet activation and cardiac fibrosis in the setting of specific antiretroviral therapies. Open Heart 2018; 5: e000823.

22) Wanchu A, Rana SV, Pallikkuth S, Sachdeva RK. Short communication: oxidative stress in HIV-infected individuals: a cross-sectional study. AIDS Res Hum Retroviruses 2009; 25: 1307-1311.

23) Ngondi JL, Oben J, Forkah DM, Etame LH, Mbanya D. The effect of different combination therapies on oxidative stress markers in HIV infected patients in Cameroon. AIDS Res Ther 2006; 3: 19.

24) Mandas A, Iorio EL, Congiu MG, Balestrieri C, Mereu A, Cau D, Dessì S, Curreli N. Oxidative imbalance in HIV-1 infected patients treated with antiretroviral therapy. J Biomed Biotechnol 2009; 2009: 749575.

25) Dysangco A, Liu Z, Stein JH, Dube MP, Gupta SK. HIV infection, antiretroviral therapy, and measures of endothelial function, inflammation, metabolism, and oxidative stress. PLoS One 2017; 12: e0183511..

26) Walmsley SL, Winn LM, Harrison ML, Uetrecht JP, Wells PG. Oxidative stress and thiol depletion in plasma and peripheral blood lymphocytes from HIV-infected patients: toxicological and pathological implications. AIDS 1997; 11: 1689-1697.

27) Suresh DR, Annam V, Pratibha K, Prasad BV. Total antioxidant capacity--a novel early bio-chemical marker of oxidative stress in HIV infected individuals. J Biomed Sci 2009; 16: 61.

28) Coco-Bassey SB, Asemota EA, Okoroiwu HU, Etura JE, Efiong EE, Inyang IJ, Uko EK. Glutathione, glutathione peroxidase and some hematological parameters of HIV-seropositive subjects attending clinic in University of Calabar teaching hospital, Calabar, Nigeria. BMC Infect Dis 2019; 19: 944.

29) Jareño EJ, Bosch-Morell F, Fernández-Delgado R, Donat J, Romero FJ. Serum malondialdehyde in HIV-seropositive children negatively correlates with CD4+ lymphocytes count. Biofactors 1998; 8: 129-132.

30) Jareño EJ, Romá J, Romero B, Marín N, Muriach M, Johnsen S, Bosch-Morell F, Marselou L, Romero FJ. areño EJ, Romá J, Romero B, Marín N, Muriach M, Johnsen S, Bosch-Morell F, Marselou L, Romero FJ. Serum malondialdehyde correlates with therapeutic efficiency of high activity antiretroviral therapies (HAART) in HIV-1 infected children. Free Radic Res 2002; 36: 341-344.

31) Minato N, Hattori M, Hamazaki Y. Physiology and pathology of T-cell aging. Int Immunol 2020; 32: 223-231.

32) Bauer ME, Fuente Mde L. The role of oxidative and inflammatory stress and persistent viral infections in immunosenescence. Mech Ageing Dev 2016; 158: 27-37.