Oxidative stress, microparticles, and E-selectin do not depend on HIV suppression

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Background. Oxidative stress and inflammation are considered predictors of diseases associated with aging. Markers of oxidative stress, inflammation, and endothelial activation were investigated in people with HIV on antiretroviral treatment to determine whether they had an immunosenescent phenotype that might predispose to the development of premature age-related diseases.

Patients and Methods. This study was conducted on 213 subjects with HIV. The control groups consisted of healthy HIV-negative adults. The level of oxidative stress was measured by assessing the production of malondialdehyde levels, which were detected by thiobarbituric acid reactive substance (TBARS) assay. The level of microparticles indicated the presence of inflammation and endothelial activation was measured by E-selectin levels. Significant differences were determined by appropriate statistical tests, depending on the distribution of variables. Relationships between continuous variables were quantified using Spearman's rank correlation coefficient.

Results. TBARS, and microparticle and E-selectin levels were significantly higher in untreated and treated subjects with HIV compared with HIV-negative controls (P<0.001). The levels of the investigated markers were not significantly different between untreated and treated patients and no significant correlation of these markers was found with $CD4^+$ count, $CD4^+/CD8^+$ ratio, and the number of HIV-1 RNA copies.

Conclusions. Elevated markers of oxidative stress, inflammatory and endothelial activation were independent of the virologic and immunologic status of people with HIV. These results support the hypothesis that residual viremia in cellular reservoirs of various tissues is a key factor related to the premature aging of the immune system and predisposition to the premature development of diseases associated with aging.

Key words: oxidative stress, microparticles, E-selectin, HIV suppression

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INTRODUCTION

Oxidative stress (OS) can be understood as an imbalance between antioxidant mechanisms and the production of reactive oxygen species (ROS). The pathogenesis of age-dependent endothelial dysfunction is causally related to the production of ROS and OS, as well as to vascular inflammation and vascular inflammation, and impaired nitric oxide signaling[1-3].

The phenomenon of "vascular aging" encompasses all age-associated changes in vessels. Aged vessels are more prone to atherosclerotic lesions, vascular injury, impaired angiogenesis, and calcifications and therefore, aging endothelium is increasingly unable to regulate all its functions, manifesting as significantly impaired endothelium-dependent relaxation (endothelial dysfunction) in elderly people[4].

Endothelial (vascular) dysfunction and low-grade inflammation are associated with cardiovascular disease as well as other disorders related to aging, including type 2 diabetes, neurodegenerative processes, end-stage renal, and liver disease. It is now clear that people living with HIV (PLWH) and those treated with antiretroviral treatment (ART) have enhanced susceptibility to these complications associated with aging, termed "non-AIDS diseases". Results from several studies have shown that this population might have a higher prevalence and earlier age of onset for many non-AIDS diseases compared with age-matched uninfected individuals[5].

In this context, ROS is a general term used to describe oxygen intermediates with high reactive capacity towards various biological molecules. Different types of ROS are characterized by their varying abilities to react with biological molecules[6]. The primary and major sources of

endogenous ROS production are mitochondria[6-11], which are involved in energy production and have evolved as principal intracellular signaling platforms that regulate innate immunity[11] and initiate a process called "sterile inflammation" (ref.[4]). ROS have a very short life span and therefore are difficult to detect.

The level of OS can be measured by assessing malondialdehyde (MDA) levels[12]. MDA is a low molecular-weight end product formed via the decomposition of primary and secondary lipid peroxidation products. However, only certain lipid peroxidation products generate MDA; it is not the sole end product of fatty peroxide formation or decomposition, nor is it a substance generated exclusively through lipid peroxidation[13]. However, excessive MDA production is associated with different pathological states and is a potential indicator of health-related acute and chronic problems[9,14,15], including carcinoma[12].

Microparticles (MPs) are a nanosized diverse mixture of cell membrane fragments that mirror the phenotypic features of the originating cell surface[16,17] and typically carry source-cell-specific signatures[17-19]. In optimal conditions, cells consistently shed minimal amounts of MPs that facilitate cell-to-cell communication[20,21].

MPs with their cargo can modify the phenotype, activate signaling cascades and influence cell function after cellular uptake by the recipient cells[17,21]. MPs emerge as possible biomarkers identifying diverse diseases linked with inflammation and procoagulant activity[22]. Research in clinical settings has demonstrated the key involvement of MPs in the pathophysiology of conditions associated with inflammation, earning them the occasional designation as the "holy grail of inflammation"[23].

The endothelium is a highly specialized organ responsible for the control of inflammation and coagulation. In response to proinflammatory stimuli or trauma, the endothelium upregulates cellular adhesion molecules and procoagulant factors[24]. E-selectin is specifically synthesized by activated endothelial cells and its expression by endothelium is a hallmark of vascular inflammation and endothelial activation[7,25-28]. E-selectin expression is almost absent on normal endothelial cells, but its expression is induced rapidly in response to proinflammatory cytokines[6,25] or during periods of various stresses[28].

The objective of this study was to investigate the levels of MDA, MPs, and E-selectin in naïve PLWH and those on ART. Differences in these parameters were compared between these groups. We investigated whether markers of OS, and inflammation and endothelial activation were related to the immunological status and viral suppression of this population.

PATIENTS AND METHODS

Research participants

Following approval from the local ethics committee and obtaining written informed consent, we recruited adult individuals (aged ≥ 18 years) living with HIV from the outpatient clinic of the Department of Infectious Diseases at the Faculty Hospital Brno and the Faculty of Medicine of Masaryk University in Brno. A total of 213 HIV patients participated in the study, with 172 of them undergoing ART, receiving a standard triple combination therapy. Some patients had previously been on at least one other antiretroviral regimen. Additionally, there was a group of forty-one treatment-naïve individuals with HIV before initiating ART. Exclusion criteria comprised individuals with active opportunistic infections, other acute illnesses, or significant chronic conditions (such as hepatitis B or hepatitis C). For comparative purposes, three control groups comprising healthy HIV-negative adult blood donors of Caucasian origin were included. The body mass index (BMI) was computed as the weight in kilograms divided by the square of the height in meters.

Blood sampling, plasma preparation, and conservation of markers of oxidative stress

Blood plasma was obtained via a direct venipuncture using a 0.109 mol/L citrate anticoagulant (9+1) solution. Two hours after collection, the plasma supernatant was promptly transferred to Eppendorf tubes and subjected to a 15-minute centrifugation at 1,500 ×g at room temperature. The resulting plasma was rapidly frozen and stored at −80 °C. Prior to utilization, the plasma was defrosted for 15 min at 37 °C.

Thiobarbituric Acid Reactive Substances (TBARS) assay of blood plasma

MDA levels were measured by TBARS assay. In the first step, 67 mg of thiobarbituric acid was dissolved in 1 mL of dimethylsulfoxide and then added to 9 mL of deionized water. In a separate tube, 200 µL of blood plasma sample was mixed with 400 µL of 10% trichloroacetic acid and incubated in an ice bath for 15 min. After incubation, the mixture was centrifuged at 3,000 ×g for 15 mins and 400 µL of supernatant (or 400 µL of saline solution for blank purposes) was mixed with 400 µL of the previously prepared thiobarbituric acid solution. The mixture was heated at 100 °C for 10 min and then cooled. Finally, the absorbance of samples and blanks at 532 nm was measured in standard disposable polystyrene cuvettes. Concentration of MDA in mol/L was calculated using the extinction coefficient for complex thiobarbituric acid – MDA, which was equal to 156,000 $M^{-1}cm^{-1}$.

Blood sampling, plasma preparation, and conservation of MP and E-selectin

Blood plasma was acquired through a direct venipuncture, adhering to recommended procedures, using a 0.109 mol/L citrate anticoagulant (9+1) solution. Within a 2-hour timeframe after collection, the plasma supernatant was promptly transferred to Eppendorf tubes. Subsequently, it underwent a 15-minute centrifugation at 1,500 ×g, followed by a rapid centrifugation lasting 2 min at 13,000 ×g at room temperature for MPs, and a 15-minute centrifugation at 2,500 ×g at room temperature for E-selectin. The resulting plasma was swiftly frozen and stored at −80 °C. Before application, the plasma underwent a 15-minute thawing process at 37 °C.

Microparticle measurement

The assessment of microparticle procoagulant activity in plasma, irrespective of their origin, employed a photometric technique with ZYMUPHEN MP-Activity (Hyphen BioMed, Neuville-sur-Oise, France). This was accomplished using an automated enzyme-linked immunosorbent assay (ELISA) instrument, DS2 (Dynex Technologies, Inc., Chantilly, VA, USA). The assay protocol involved diluting the plasma sample and enriching it with calcium, Factor Xa, and thrombin inhibitors. The prepared mixture was then introduced into a well microtiter plate with streptavidin and biotinylated annexin V, followed by an incubation period. After a washing step, the Factor Xa–Va mixture, containing calcium and purified prothrombin, was added. In instances where MPs were present in the tested sample, they adhered to annexin V, revealing their phospholipid surface. This enabled FXa–FVa to activate prothrombin into thrombin in the presence of calcium. The phospholipid concentration demonstrated a direct correlation with the amount of thrombin generation, as determined by its specific activity on the thrombin substrate. The reaction was concluded with 2% citric acid, and the absorbance was measured at 405 nM. The reference limit, established following the manufacturer's instructions, was set at 0–5.0 nM.

E-selectin measurement

Human E-selectin Platinum ELISA (Affymetrix, eBioscience, Santa Clara, CA95051, USA) was used for the quantitative detection of human E-selectin in plasma using an automatic ELISA instrument, DS2 (Dynex Technologies, Inc.). The assay principle was as follows: an anti-human E-selectin coating antibody was adsorbed onto microwells. Human E-selectin present in the sample or standard binds to antibodies adsorbed on the microwells. An HRP-conjugated anti-human E-selectin antibody was added, which binds to human E-selectin captured by the first antibody. Following incubation, unbound HRP-conjugated anti-human E-selectin is removed during a wash step, and substrate solution reactive with HRP is added to the wells. A colored product is formed in proportion to the amount of human E-selectin present in the sample or standard. The reaction was terminated by the addition of acid and the absorbance was measured at 450 nm. A standard curve prepared from six human E-selectin standard dilutions was used to determine the human E-selectin concentration. The reference limit was set in accordance with the manufacturer's instructions at 17.5–88.1 ng/mL.

Statistical analysis

Descriptive features were presented through absolute and relative frequencies for categorical factors and through the median in conjunction with the 5th and 95th percentiles for continuous variables. Both parametric methods (t-test, ANOVA) and non-parametric techniques (Mann-Whitney U test, Kolmogorov-Smirnov, Kruskal-Wallis) were employed to contrast disparities in the distribution of each continuous variable among diverse groups. The selection of each test hinged on multiple criteria, including the quantity of groups under comparison and the normality of the distribution. Post-hoc tests for comparing multiple groups were employed to evaluate differences among them. In instances of ANOVA and Kruskal-Wallis tests, Tukey's test and the Benjamini-Hochberg method (FDR), respectively, were applied. Associations between categorical factors were explored using Pearson's χ^2 test, and associations between continuous variables were assessed using Spearman's rank correlation coefficient. The statistical significance of the correlation coefficient was determined through the asymptotic t approximation. R software, version 3.5.1, was used for all statistical analyses, with significance considered for P-values less than 0.05.

RESULTS

Patient characteristics

The patient characteristics indicated subjects were matched for age and BMI between the groups. The median age was 37.7 years (minimum 25.3; maximum 58.6) with a BMI of 22.0 (minimum 18.5; maximum 28.4) in untreated PLWH. This group consisted of 92.7% male patients and 7.3% female patients. Blood samples from untreated PLWH were collected before the initiation of ART.

At the time of collection, no patient was being treated for any other disease or for diabetes, dyslipidemia, or hypertension

In the treatment group, 81.4% of PLWH were men and 18.6% were women, and the median age was 42.1 years (minimum 27.2; maximum 65.2) with a BMI of 24.6 (minimum 19.1; maximum 32.8). Thirteen patients were treated for hypertension, five were treated for diabetes, and eighteen were treated for dyslipidemia. One patient had a heart attack previously.

The three separate control groups for markers of OS, MP, and E-selectin levels consisted of healthy active blood donors. The active blood donors did not have hypertension, diabetes, or dyslipidemia, had not been treated for any disease, and had no risk factors (Table 1). They were tested for various diseases according to the prescribed protocol before the scheduled blood collection.

TBARS, MP, and E-selectin levels in untreated and treated PLWH

Table 1, and Fig. 1 and 2 show that TBARS levels were significantly higher in 30 PLWH without ART compared with 50 HIV-negative controls (4.54 μmol/L; 1.79 μmol/L; $P<0.001$). TBARS levels were significantly higher in 172 PLWH on ART compared with 50 HIV-negative controls (4.63 μmol/L; 1.79 μmol/L; $P<0.001$) and were not statistically significant compared with PLWH without ART.

Table 1, and Fig. 3 and 4 show that MP levels were significantly higher in 30 PLWH without ART compared with 40 HIV-negative controls (12.60 nmol/L; 3.16 nmol/L; $P<0.001$). MP levels were significantly higher in 139 PLWH on ART compared with 40 HIV-negative con-

Table 1. Patient characteristics in controls, untreated and treated subjects with HIV.

	HIV–	HIV+	HIV+ART	*P*
Number of patients	60[a]	41[b]	172[c]	
Sex				
Female	–	3 (7.3%)	32 18.6%)	0.129
Male	–	38 (92.7%)	140 (81.4%)	
Median (5^{th};95^{th})				
Age, years	–	37.7 (25.3; 58.6) n = 41	42.1 (27.2; 65.2) n = 172	0.112
BMI	–	22.0 (18.5; 28.4) n = 41	24.6 (19.1; 32.8) n = 163	0.001
TBARS, μmol/L	1.79[b,c] (0.73; 6.38) n = 50	4.54[a] (1.38; 12.42) n = 30	4.63[a] (1.85; 14.88) n = 172	< 0.001
MP, nM	3.16[b,c] (0.35; 6.24) n = 40	12.60[a] (5.19; 52.80) n = 30	10.94[a] (4.50; 45.40) n = 139	< 0.001
E-selectin, ng/mL	31.35[b,c] (9.40; 65.80) n = 60	41.90[a] (21.00; 94.60) n = 14	43.12[a] (17.10; 85.60) n = 114	< 0.001
$CD4^+$ cell count/μL	–	351 (11; 686) n = 39	667 (187; 1212) n = 172	< 0.001
$CD4^+/CD8^+$ ratio	–	0.43 (0.03; 1.98) n = 38	0.81 (0.21; 1.88) n = 167	< 0.001
Number of HIV-1 RNA copies/mL; viral load	–	44,300 (317; 4,850,000) n = 38	0 (0; 4410) n = 172	< 0.001
Treatment duration, years	–	–	6.59 (0.56–19.05) n = 172	
Number of patients with				
Hypertension	0	0	13	
Diabetes	0	0	5	
Dyslipidemia	0	0	18	
History of CV events	0	0	1	

[a-c]Indicate statistically significant differences between the two categories of a-c.
ART, antiretroviral therapy; CV, cardiovascular; MP, microparticle; TBARS, thiobarbituric acid reactive substance; BMI, body mass index.

Table 2. Correlation coefficients in untreated subjects with HIV.

	TBARS	MP	E-selectin	$CD4^+$	$CD4^+/CD8^+$
MP	−0.03	×			
E-selectin	0.49	0.04	×		
$CD4^+$	−0.01	0.28	−0.33	×	
$CD4^+/CD8^+$	−0.02	0.12	−0.31	0.70	×
VL	−0.22	−0.35	0.16	−0.52	−0.48

MP, microparticle; VL, viral load; TBARS, thiobarbituric acid reactive substance.

Table 3. Correlation coefficients in treated subjects with HIV on ART.

	TBARS	MP	E-selectin	$CD4^+$	$CD4^+/CD8^+$
MP	−0.08	×			
E-selectin	−0.04	−0.18	×		
$CD4^+$	0.06	−0.06	0.21	×	
$CD4^+/CD8^+$	0.02	−0.09	0.01	0.61	×
VL	0.08	−0.04	0.01	−0.24	−0.34

MP, microparticle; VL, viral load; TBARS, thiobarbituric acid reactive substance.

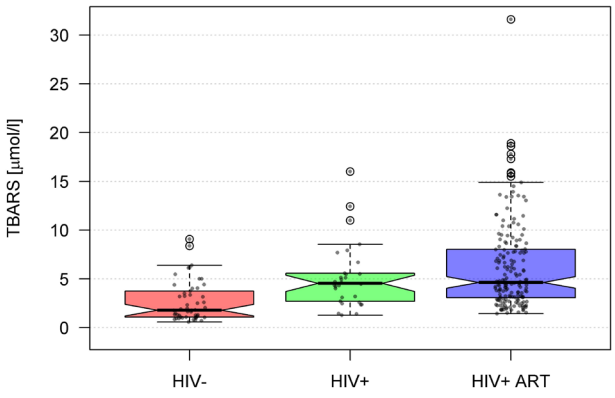

Fig. 1. TBARS (µmol/L) levels in controls, and untreated and treated PLWH.
HIV, control group; HIV+, people living with HIV without ART; HIV+ART, people living with HIV on ART.

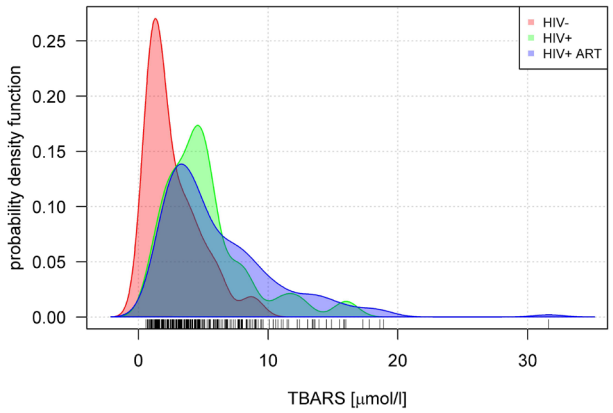

Fig. 2. Probability density function of TBARS levels (µmol/L).
HIV, control group; HIV+, people living with HIV without ART; HIV+ART, people living with HIV on ART.

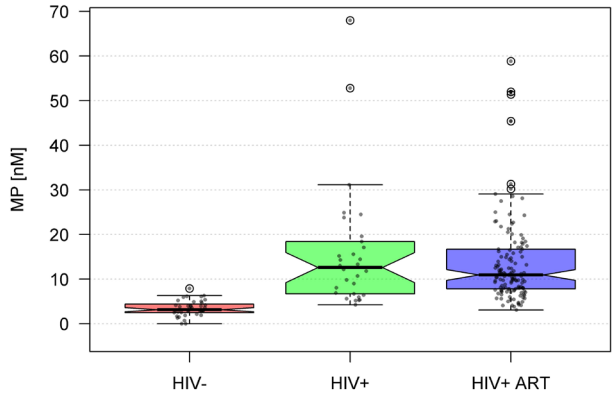

Fig. 3. Microparticle levels (nM) in controls, and untreated and treated PLWH.
HIV, control group; HIV+, people living with HIV without ART; HIV+ART, people living with HIV on ART.

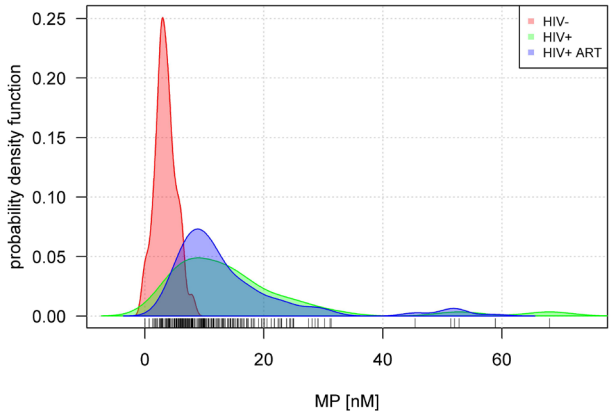

Fig. 4. Probability density function of MP levels (nM).
HIV, control group; HIV+, people living with HIV without ART; HIV+ART, people living with HIV on ART.

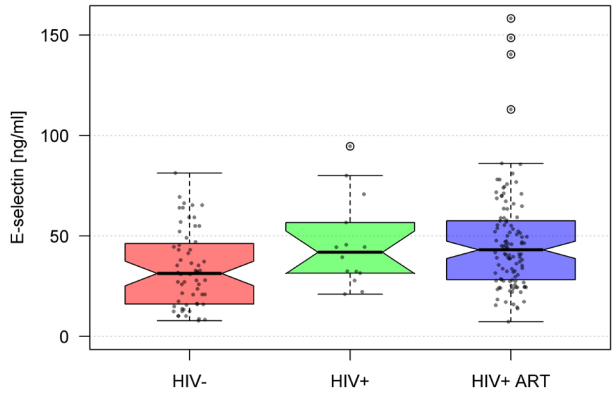

Fig. 5. E-selectin levels (ng/mL) in controls, and untreated and treated PLWH.
HIV, control group; HIV+, people living with HIV without ART; HIV+ART, people living with HIV on ART.

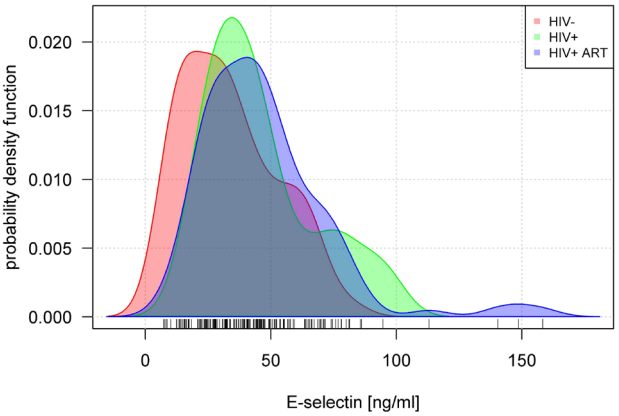

Fig. 6. Probability density function of E-sectin levels (ng/mL).
HIV, control group; HIV+, people living with HIV without ART; HIV+ART, people living with HIV on ART.

trols (10.94 nmol/L; 3.16 nmol/L; P<0.001). MP levels in PLWH on ART were not statistically significant compared with PLWH without ART.

Table 1, and Fig. 5 and 6 show that E-selectin levels were significantly higher in 14 PLWH without ART compared with 60 HIV-negative controls (41.90 ng/mL; 31.35 ng/mL; P<0.001). E-selectin levels were significantly higher in 114 PLWH on ART compared with 60 HIV-negative controls (43.12 ng/mL; 31.35 ng/mL; P<0.001). E-selectin levels in PLWH on ART were not statistically significant compared with subjects without ART.

TBARS, MP, E-selectin levels, CD4$^+$ cell count, CD4$^+$/CD8$^+$ ratio, and the number of HIV-1 RNA copies/mL

The median CD4$^+$ cell count was 351 cells/μL in PLWH without treatment and 667 cells/μL in treated patients (Table 1), which indicated immunological recovery. The median CD4$^+$/CD8$^+$ ratio was 0.43 in patients without treatment and 0.81 in treated patients. The median of both parameters was significantly higher in PLWH on ART compared with untreated patients (P<0.001).

The median number of HIV-1 RNA copies/ml in plasma was 44,300 copies/mL in PLWH without treatment, indicating intense viral replication. The median number of HIV-1 RNA copies/ml in plasma was undetectable in patients on treatment, indicating viral suppression (Table 1). There was a statistically significant difference in viral load between the untreated and treated groups (P<0.001).

TBARS, E-selectin, and MP levels were not significantly different between groups without treatment and with treatment (Table 1) regardless of immunologic status and viral suppression.

Correlation between TBARS, MPs, E-selectin, CD4$^+$ count, CD4$^+$/CD8$^+$ ratio, and the number of HIV-1 RNA copies

No significant correlations were detected between TBARS, and MP and E-selectin levels with CD4$^+$ count, CD4$^+$/CD8$^+$ ratio, and number of HIV-1 RNA copies in naïve PLWH. We observed a positive correlation between the CD4$^+$ count and CD4$^+$/CD8$^+$ ratio and a negative correlation between the CD4$^+$ count and number of HIV-1 copies/mL (Table 2).

No significant correlations were found between TBARS, and MP and E-selectin levels with CD4$^+$ count, CD4$^+$/CD8$^+$ ratio, and number of HIV-1 RNA copies in PLWH on ART. We observed a positive correlation between the CD4$^+$ count and CD4$^+$/CD8$^+$ ratio (Table 3).

DISCUSSION

Untreated HIV infection was associated with significantly higher levels of TBARS that did not change, even in PLWH on ART. In simple or highly purified systems, the TBARS for MDA provides an appropriate estimate of lipid peroxidation. In more complex biological systems, many compounds (including simple and complex carbohydrates, protein oxidation products, and nucleic acid oxidation products) react with thiobarbituric acid to produce colored adducts[29]. The TBARS method is nonspecific for MDA. However, MDA determination and TBARS offer an empirical view of the complex process of peroxidation in the human body[12] and are associated with various acute and chronic pathological states[9,14,15].

Clinical studies of HIV-infected individuals receiving ART have been inconclusive[1,2,6,30-34]. Musisi et al. found similar conclusions to us[1]. However, Mandas et al. observed that serum ROS levels were significantly higher in PLWH treated with ART compared with untreated individuals[2,32]. In contrast to our findings, Awodele et al. reported that PLWH without ART had higher lipid peroxidation compared with subjects on ART (ref.[30,31,34]). However, these studies did not have large numbers of participants and a comparison between the studies was limited.

A significantly higher level of MPs in antiretroviral naïve PLWH and subjects on ART than in HIV-negative controls demonstrated increased procoagulant activity in both groups of PLWH. MPs are increased in a wide range of inflammatory disorders[20,21,35] and are likely to contribute to disease complications because they promote thrombotic activity as part of a cascade of deleterious responses[18,36-38]. ROS might mediate the release of MPs from parent cells[39,40] but the detailed molecular processes that lead to their formation and release are not yet clear[22]. In our earlier published study, we observed that the concentration of microparticles (MPs) remains consistently elevated during antiretroviral therapy and does not vary with its duration[17].

Previous studies that have examined levels of E-selectin in HIV infection have been controversial. Some studies found that E-selectin levels were significantly increased in ART-naive PLWH compared with a group on ART and with an HIV-negative control group. Other studies found that E-selectin levels were decreased by ART and that E-selectin levels were increased during the acute phase of HIV infection but not during the chronic phase[28]. In our study, E-selectin levels indicated that vascular inflammation was significantly augmented, even in patients with complete viral suppression that was detected in peripheral blood. We found significant differences in E-selectin levels between PLWH naïve and treated PLWH on ART compared with non-infected individuals.

These results suggest HIV-1 infection itself is implicated in the acceleration and enhancement of OS, MP levels, and endothelial activation and dysfunction. All indications are that ART is not capable of normalizing the inflammatory response that is induced by HIV itself. As a result, some degree of immune activation persists. This residual immune activity is also indicated by a CD4$^+$/CD8$^+$ ratio < 1, which is an indirect marker of immune activation, immune senescence, and inflammation[41]. The significant role of HIV-1 in inducing an inflammatory response is supported by the conclusion of our previous study, where it was found that the level of MPs remains significantly elevated in treated HIV patients. Importantly, this elevation was found to be independent of the duration of ART (ref.[17]).

Several mechanisms have been suggested to explain how HIV infection can induce these processes, including the direct HIV infection of cells, via other markers of inflammation, via the effect of HIV proteins, and other mechanisms. The experimental evidence supports a functional role for HIV viral proteins in the disruption of endothelial cell biology. For example, among these viral proteins, gp120, Tat, and Nef have a major role in the pathogenesis of endothelial dysfunction[5,20,24,28] and, in recent years, there has been increased attention toward the consequences of HIV infection itself[5].

However, the potential effect of antiretroviral drugs cannot be ruled out. A study by Hijmans et al. demonstrated that MPs from ART-treated PLWH induced significantly more functional endothelial cell alterations compared with untreated PLWH (ref.[20]). Moreover, there is evidence that key antioxidant enzymes may be inhibited by antiretrovirals or their metabolites[1,2,31,42], indicating they might further potentiate the functional activity of ROS.

This hypothesis was also supported by the result of our study, which was published by our research team earlier. The level of reduced glutathione, which represents an important antioxidant mechanism of the human body, was investigated. The lowest level of reduced glutathione was detected in PLWH who were taking effective antiretroviral therapy and had achieved viral suppression. Paradoxically, the most effectively treated PLWH had the lowest serum antioxidant capacity[3].

The course of the whole cascade is probably very complex and at multiple levels is influenced by other numerous mechanisms and factors including various inflammatory biomarkers, decreased antioxidant capacity, microbial translocation, coinfections, telomere length shortening, and reduction of naïve T cells[24,41]. The effects of these and other additional factors may explain the statistically insignificant direct correlation between TBARS, MPs and E-selectin observed in our study. However, as Fig. 2,4 and 6 show, the probability density function of individual parameters was very similar. This may indicate a pathophysiological link between these parameters.

The exact mechanisms that lead to derivative MPs from parent cells and the activation of the endothelium are unclear[11,18,43]. However, experimental data suggest that ROS production from cells is the primary trigger of a cascade that leads to the expression of an immunosenescent phenotype[8,44,45] and to aberrant innate immune responses, which are considered a common factor that drives sterile inflammatory pathology in many conditions associated with aging[7,36,46,47].

The present study shows that the abnormal production of ROS, chronic inflammation, and endothelial dysfunction persisted in PLWH, as well as a certain degree of immune activation, despite effective ART, very good $CD4^+$ cell counts, and viral suppression. This leads to the disease pathophysiology and increased risk of developing diseases that are observed in older categories of the HIV-negative general population and are characteristic of an aging organism and immunosenescence[27,28,48,49].

This study had several limitations. First, this study had a low number of subjects in the untreated group with a low number of samples for the measurement of E-selectin, which may have limited the strength of the analysis. Second, aging affects most aspects of cell biology. In our study, patients were not stratified by age, duration of treatment, duration of viral suppression, ART composition, blood lipid levels, or other factors that might affect these parameters. In addition, different populations may have different levels of OS related to differences in their lifestyle, environmental pollutants, genetic variability, and other predispositions. Assembling homogeneous groups is therefore relatively difficult.

CONCLUSION

The present study presents global information on a representative number of ART treated PLWH and supports the hypothesis that residual viremia in cellular reservoirs of various tissue, rather than exposure to ART, is presumably a key factor related to the premature aging of the immune system[27,50]. However, ART may affect and accelerate functional changes. It is obvious that new therapeutic strategies are necessary. Primarily, it is most important to develop drugs that eliminate persistent chronic inflammation, which may also be of great benefit for the treatment of age-related diseases in the general HIV-negative population. Of note, circulating MPs represent novel mediators and viable targets for future therapeutic intervention. However, the highest therapeutic goal is the definitive elimination of the virus from the body of PLWH.

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