Journal of
AIDS & Clinical Research
ISSN: 2155-6113

Krol et al., J AIDS Clinic Res 2012, 3:9
DOI: 10.4172/2155-6113.1000183

Short Communication Open Access

Increased Exhaled Hydrogen Peroxide in Human Immunodeficiency Virus-Infected Patients without Clinical Signs and Symptoms of Opportunistic Lung Disease

Maciej Krol[1]*, Arkadiusz Balcerowski[2], Maria Luczynska[3], Urszula Szkudlarek[4] and Dariusz Nowak[5]

[1]*Department of Sleep Medicine and Metabolic Disorders, Medical University of Lodz, Poland*
[2]*Acquired Immune Disorders Clinic, the Dr. W. Bieganski Memorial Regional Hospital, Lodz, Poland*
[3]*Department of Cell-to-Cell Communication, Medical University of Lodz, Poland*
[4]*Department of Experimental Physiology, Medical University of Lodz, Poland*
[5]*Department of Clinical Physiology, Medical University of Lodz, Poland*

Abstract

Background: HIV-infected subjects present with decreased antioxidant defense and increased activation of inflammatory cells which may lead to overproduction of oxidants. This study determined whether HIV-infected patients without clinical signs and symptoms of opportunistic lung disease (OLD-negative) exhaled more H_2O_2 than healthy controls and whether there was association between the exhalation of H_2O_2 and whole blood chemiluminescence (CL) and clinical variables.

Methods: A cross-sectional study was conducted. H_2O_2 in exhaled breath condensate and CL, resting and agonist-induced with N-formyl-methionyl-leucyl-phenylalanine (fMLP) were measured in 36 OLD-negative patients and 14 healthy controls. Univariate linear regression was used to summarize the average relationship and quantile regression analyzed the relationship at different points of the exhaled H_2O_2 distribution. Multivariate analyses were carried out using multiple linear regressions.

Results: The fold increase of the geometric mean exhaled H_2O_2 against healthy controls was 3.76-times higher in OLD-negative patients than in controls (95% CI: 2.65-5.33, $p<0.001$), whereas that of either resting or fMLP-induced CL was 1.46 or 1.63, respectively (95%: 1.17-1.83 and 1.27-2.08, $p<0.01$). Exhaled H_2O_2 was not associated with CL, either resting or fMLP-induced. Linear regression detected positive relationship between the exhalation of H_2O_2 and viral load (R-squared 0.23, $p<0.05$). The effects of viral load were best revealed at a higher exhalation of H_2O_2 (quantiles 0.6 and 0.7; both Pseudo R-squared 0.21, $p<0.05$). In a multivariate model, the main independent contributors to the exhalation of H_2O_2 were viral load and highly active antiretroviral therapy (HAART), which together accounted for 35% of the variance in exhaled H_2O_2. If the analysis was limited exclusively to HAART-treated, a better model fit was obtained (R-squared 0.79), confirming that viral load is the main contributor to the exhaled H_2O_2.

Conclusion: Inordinate increase in exhaled H_2O_2 may reflect airway oxidative stress in HIV-1 infection which may be related to viral load.

Keywords: Exhaled hydrogen peroxide; HIV-1; Oxidative stress; Whole blood chemiluminescence

Introduction

Since oxidative stress is implicated in both human immunodeficiency virus, type 1 (HIV-1) expression [1,2] and the pathogenesis of AIDS [3], this pro-oxidant and antioxidant imbalance has been widely described among HIV-infected patients [4-7]. Overproduction of Reactive Oxygen Species (ROS) is thought to be the result of various cell activation and altered redox status, mostly phagocytic [5], inappropriately compensated by antioxidants or antioxidant enzymes [8] and is also augmented by highly active antiretroviral therapy (HAART) [7,9]. Exhaled H_2O_2 belongs to non-invasive markers of ROS production in the airways [10,11]. Since H_2O_2 is a volatile compound, it is easily determined by breath analysis. Exhalation of H_2O_2 is elevated in respiratory tract disorders accompanied by an influx of activated inflammatory cells e.g. bronchial asthma [12,13], COPD [14,15] and pneumonia [16]. To date, this has not been described in HIV-infected patients. They typically show a marked decrease in the concentration of reduced glutathione (GSH) [17,18] and their alveolar macrophages spontaneously produce more superoxide anion [17] which undergo dismutation to H_2O_2. Moreover, progression of HIV-1 infection results in a decreased erythrocyte glutathione peroxidase (GSH-Px) activity and suppression of GSH plasma levels [19]. Since the GSH-GSH-Px system is involved in the decomposition of H_2O_2 one may suspect that HIV-infected subjects have increased H_2O_2 levels in the airways resulting in increased exhalation of H_2O_2. Nevertheless, any concomitant Opportunistic Lung Disease (OLD) can influence such measurements [20].

The mechanisms underlying changes in exhalation of H_2O_2 are likely multifactorial in nature. Exhaled H_2O_2 represents a pool of ROS derived from the NADPH-oxidase system and the mitochondrial chain that avoids decomposition by antioxidant systems, subsequently diffusing into the airway surface which is then blown out as vapor and or aerosolized respiratory fluid droplets released from the respiratory epithelial lining fluid finally collected as exhaled breathe condensate (EBC) [11]. Therefore,

***Corresponding author:** Maciej Krol, Medical University of Lodz, Aleja Tadeusza Kosciuszki 4, 90-419 Lodz, Poland, NIP: 725 18 43 739; Tel: +48-42-272-5656; Fax: +48-42-272-5652; E-mail: mkrol@sleeplab.pl

Received September 04, 2012; **Accepted** November 17, 2012; **Published** November 23, 2012

Citation: Krol M, Balcerowski A, Luczynska M, Szkudlarek U, Nowak D (2012) Increased Exhaled Hydrogen Peroxide in Human Immunodeficiency Virus-Infected Patients without Clinical Signs and Symptoms of Opportunistic Lung Disease. J AIDS Clinic Res 3:183. doi:10.4172/2155-6113.1000183

changes in antioxidant defense in the airway or modifications in the number and activity of pulmonary inflammatory cells may alter the concentration of H_2O_2 in EBC. In addition, possibly there is a relationship between exhaled H_2O_2 and the blood oxidant and antioxidant status as measured by luminol enhanced whole blood chemiluminescence. Szkudlarek et al. [21] found an association between increased exhalation of H_2O_2 and a greater light emission of whole blood in a cross-sectional study of 41 healthy subjects. This associative finding in HIV-infected patients would provide evidence of a shared mechanism in the oxidative response in the blood and the airways. Furthermore, since nucleoside reverse transcriptase inhibitors (NRTIs) have been shown to increase intracellular H_2O_2, treatment with NRTIs may also contribute to exhalation of H_2O_2 [22].

We conducted a cross-sectional study of HIV-infected men and women without clinical signs and symptoms of concomitant OLD (OLD-negative), accompanied by clinical signs and symptoms of concomitant respiratory tract infection (RTI) without a definite diagnosis of OLD (RTI-positive) and healthy control subjects to determine: 1) the respective amount of exhaled H_2O_2, 2) luminol enhanced whole blood chemiluminescence, either resting or agonist-induced, 3) whether exhaled H_2O_2 is associated with whole blood chemiluminescence and finally 4) whether exhaled H_2O_2 is associated with selected clinical variables, including HIV-infection duration, detectable viral load, treatment with HAART and HAART duration or a history of AIDS.

Materials and Methods

Study population

A total of 310 HIV-infected patients from the Acquired Immune Disorders Outpatient Clinic in Lodz, Poland were screened. The study included 36 OLD-negative subjects, 28 RTI-positive patients and 14 healthy controls. Each patient enrolled had to meet an inclusion criteria: age ≥ 18 and ≤ 60 years, HIV-1 seropositivity, a chest X-ray performed within 10 days prior to the enrollment and a written informed consent. The exclusion criteria included: any episode of alcohol or illicit drug abuse within the last 2 to 6 months before the study, respectively, any history of bronchial asthma, COPD, bronchiectasis, cystic fibrosis, tuberculosis, malignancies, renal or liver damage, pregnancy or breast feeding, pharmacological treatment other than HAART within the last 2 months and regular ingestion of supplements with known antioxidant properties (e.g. vitamin C) within the last 3 weeks before the study. At admission, patients were screened for clinical signs and symptoms of OLD, including: cough, purulent sputum, dyspnea, chest X-ray findings and a history of past respiratory tract infections within the last 3 months. Ongoing HAART was administered to 23 of OLD-negative patients and 7 of RTI-positive subjects. Patients were treated with either lopinavir 600 mg+ritonavir 150 mg twice-daily associated with stavudine 40 mg twice-daily and didanosine 400 mg once daily or lamivudine 150 mg+zidovudine 300 mg twice-daily in combination with efavirenz 600 mg once daily. Except HIV-1 seropositivity, healthy control subjects also had to meet all inclusion and exclusion criteria. The study was approved by the Ethics Committee of the Medical University of Lodz, Poland (RNN/216/03/KE).

Study protocol

All subjects enrolled (HIV-1 infected patients and healthy controls) were asked to come to the laboratory between the hours of 8 am to 10 pm for EBC collection. Subsequently, 9 ml blood samples were drawn into EDTAK3 Vacuette tubes (Greiner Labor Technik, Austria) for whole blood chemiluminescence assay, blood cell count and HIV-1 RNA copy number.

Exhaled breathe condensate sampling

2-3 mL of exhaled breath condensate was sampled during 15 min of spontaneous tidal volume breathing (respiratory rate ranged 14-20 bpm), using EcoScreen-1 (Erich Jaeger GmbH, Hoechberg, Germany), with saliva trap. Subjects wore a noseclip and rinsed their mouth with distilled water just before and after 7 min of collection [23]. Immediately after the procedure, EBC specimens were stored at -80°C [23,24], no longer than 7 days until H_2O_2 measurement. No amylase activity was detected in EBC specimens (control of salivary contamination) [16]. Subjects who were current smokers refrained from cigarette smoking 12 hrs preceding EBC collection.

Measurement of H_2O_2

HVA method was used to assess the concentration of H_2O_2 in EBC [25], as previously described [23,26]. The detection limit of the H_2O_2 assay was 0.05 μmol/L. The intra-assay variability did not exceed 2.5% for the standard 1 μmol/L of the H_2O_2 solution. The addition of catalase (30 U) to the EBC specimens of HIV-infected patients (n=4) and healthy subjects (n=3), which previously revealed that detectable exhaled H_2O_2 levels completely abolished HVA oxidation, demonstrates that the H_2O_2 assay is specific and other reactive compounds or oxygen species did not contribute to H_2O_2 readings. Individual results were means from duplicate measurements.

Whole blood chemiluminescence assay

The resting and fMLP-induced luminol enhanced whole blood chemiluminescence (CL) were measured as previously described [21,27]. Two CL parameters were assessed: resting CL prior to the addition of fMLP and peak light emission after the addition of an agonist to a final concentration of 20 μmol/L (fMLP-induced peak CL). Resting CL and fMLP-induced peak CL were expressed as mV per 10^4 phagocytes in the assayed blood sample. Individual results were obtained as a mean from triplicate measurements.

Other techniques

Blood cell count was performed using the 5-DIFF LH 750 Hematology Analyzer (Beckman-Coulter, Inc. USA). Blood CD_4 count was determined with anti-CD_4 monoclonal antibodies (Becton Dickinson, NJ, USA), following flow cytometry (Beckman Coulter Epics XL, USA). Serum anti-HIV-1 antibodies were detected with enzyme linked immunosorbent assay (Bio-Rad, USA). HIV-1 seropositivity was confirmed by Western blot analysis (Calypte Biomedical, USA). HIV-1 RNA copies (viral load) were determined by COBAS Amplicor HIV-1 monitor test (Roche, Branchburg, NJ, USA), with detection limit of 50 copies/mL and expressed as a common logarithm of RNA copies per mL of plasma.

Statistical analysis

Statistical analysis was carried out using the Stata 12 (Stata Corp., College Station, TX, USA). Normally distributed continuous variables and variables of log 10-transformed toward normality were compared between groups using one-way ANOVA with post-hoc Bonferroni adjustment and unpaired Student's t-test for equal variances; non-normally distributed data were compared using the Kruskall-Wallis rank test and the Wilcoxon rank-sum test. Categorical variables were compared between groups using the Pearson's chi-squared test and the Fisher's exact test. The one-way analysis of covariance (ANCOVA) was used for comparison of variables adjusted for covariate. Correlations between continuous variables were determined nonparametrically using Spearman's rho. Univariate linear regression analyses were carried out with nonparametric bootstrap and 10 000 replications (to avoid transformation of the dependent variables

where appropriate). Linear regression was used to summarize the average relationship while simultaneous quantile regression (*sqreg* function) was applied to analyze the relationship at different points of the distribution [28]. Multivariate analyses were performed using multiple linear regression with bootstrap estimates of coefficient standard errors and 10 000 replications. Independent variables were manually implemented based on clinical judgment where appropriate. The stepwise estimation technique was used with p to enter and p to leave both equal to 0.15, and unitless standardized coefficients were presented. Statistical significance was set at $p<0.05$. No formal adjustments for multiple comparisons were made.

Results

A total of 36 OLD-negative adults and 14 healthy control subjects matched for gender, age and smoking habits were studied. Moreover, a group of 28 RTI-positive patients, of whom 15 had a cough, 19 had purulent sputum, 6 were dyspneic, 11 had the chest X-ray findings and 12 with a history of past RTI within the last 3 months, were included in the analysis (Table 1). EBC of H_2O_2 as well as resting and fMLP-induced whole blood chemiluminescence measurements was observed in all patients. Viral load was determined in 28 of OLD-negative patients and 10 of RTI-positive subjects. Viraemia measurement was solely dependent on test availability. Table 1 shows the clinical and demographic findings of HIV-infected subjects and healthy controls. Evaluation of white blood cell (WBC) count and polymorphonuclear leukocytes (PMNs) count revealed significantly lower values in OLD-negative patients as compared to healthy controls (Table 1). Moreover, WBC and lymphocyte counts were higher, estimated duration of HIV-infection was shorter and the number of HAART-treated was lower in RTI-positive subjects as compared to OLD-negative patients.

Exhaled H_2O_2 and whole blood chemiluminescence

Old-negative vs. RTI-positive subjects: Increased oxidative status defined as elevated exhalation of H_2O_2 and enhanced whole blood chemiluminescence in comparison to healthy controls was a consistent feature of HIV infection, regardless of concomitant RTI (Table 2, Figures 1A and 1B). The highest significant difference was seen in exhaled H_2O_2 (Table 2, Figure 1B). Fold increase of the geometric mean exhaled H_2O_2

Variable	(1) Control (n=14)	(2) HIV-infected OLD-negative (n=36)	(3) HIV-infected RTI-positive (n=28)	p-value	*post-hoc* p-value	Difference	95% CI
Male gender, n (%)	8 (57%)	20 (55%)	19 (68%)	0.59[1]			
Age [yrs], median (95% CI, range)	31 (25.6 to 36.4, 20-52)	28.5 (25.5 to 31.5, 23-54)	29.5 (25.9 to 33.1, 20-56)	0.62[6]			
Smokers, n (%)	7 (50%)	23 (64%)	23 (82%)	0.09[1]			
Cough/Sputum/Dyspnea/Chest X-ray/Past RTI, n	0 / 0 / 0 / 0 / 0	0 / 0 / 0 / 0 / 0	15 / 19 / 6 / 11 / 12				
Hemoglobin [g/dL], mean (95% CI, range)	14.5 (13.8 to 15.3, 12.3-16.5)	14.3 (13.9 to 14.7, 11.4-16.7)	14.9 (14.4 to 15.5, 11.0-17.2)	0.18[3]			
White blood cells count [×10³ cells/μL], geometric mean (95% CI, range)	6.61 (5.82 to 7.50, 5.10-11.0)	4.75 (4.25 to 5.30, 2.01-8.72)	5.83 (5.24 to 6.49, 4.03-11.40)	<0.001[3]	0.002[4] 1 vs. 2 0.020[4] 2 vs. 3	0.72 1.23	(0.58 to 0.89) (1.03 to 1.47)
PMNs count [×10³ cells/μL], median (95% CI, range)	4.30 (3.65 to 4.95, 3.80-8.90)	3.14 (2.53 to 3.75, 1.06-7.66)	3.58 (2.76 to 4.40, 1.91-8.98)	0.003[6]	0.001[7] 1 vs. 2	-1.45	(-2.24 to -0.59)
Lymphocyte count [×10³ cells/μL], mean (95% CI, range)	1.79 (1.55 to 2.04, 1.30-2.30)	1.49 (1.30 to 1.68, 0.31-2.46)	1.86 (1.60 to 2.12, 0.26-3.30)	0.039[3]	0.043[4] 2 vs. 3	0.37	(0.02 to 0.72)
CD4 count [cells/μL], mean (95% CI, range)	N/A	361.6 (287.1 to 436.0, 6-1063)	391.7 (320.8 to 462.6, 90-822)	0.56[5]			
HIV-infection duration [yrs], median (95% CI, range)	N/A	4.2 (1.9 to 6.5, 0.2-12.0)	2.1 (0.7 to 3.6, 0.1-9.2)	0.003[8]		-1.8	(-3.3 to -0.7)
Viral load assays, n (%)	N/A	28 (78%)	10 (36%)	0.001[2]			
Detectable viral load[a], n (%)	N/A	19 (68%)	6 (60%)	0.71[2]			
Viral load [×10³ RNA copies/mL], geometric mean (95% CI, range)	N/A	1.08 (0.26 to 4.42, 0-935)	1.33 (0.07 to 23.71, 0-41.9)	0.88[5]			
Treatment with HAART, n (%)	N/A	23 (64%)	7 (25%)	0.003[2]		-39%	
Treatment duration [mths], geometric mean (95% CI, range)	N/A	17.4 (10.9 to 28.0, 1-84)	7.9 (1.7 to 36.0, 1-62.5)	0.15[5]			
History of AIDS[b], n (%)	N/A	14 (39%)	5 (18%)	0.10[2]			

p-values: [1]Pearson's chi-squared test; [2]Fisher's exact test; [3]one-way ANOVA; [4]*post hoc* ANOVA test with Bonferroni adjustment; [5]unpaired Student's *t*-test (equal variances); [6]Kruskal-Wallis rank test; [7]Wilcoxon rank-sum test with Bonferroni adjustment; [8]Wilcoxon rank-sum test.
HAART, highly active anti-retroviral therapy; PMNs, polymorphonuclear leukocytes.
[a]Detectable viral load defined as ≥50 copies of HIV-1 RNA/mL. [b]AIDS defined as a history of CD_4 count <200 cells/μL or AIDS-defining illness.

Table 1: Comparison of demographic and clinical variables between healthy controls vs. HIV-infected patients without clinical signs and symptoms of opportunistic lung disease (OLD-negative) or with clinical signs and symptoms of concomitant respiratory tract infection without a definite diagnosis of OLD (RTI-positive).

against healthy controls was about 2 to 3-times higher than that of either resting or fMLP-induced CL, respectively (*post hoc* ANOVA against healthy controls; all p<0.05) (Table 2, Figure 1B). Moreover, there were no significant differences between OLD-negative patients and RTI-positive subjects (post hoc ANOVA against OLD-negative; all p>0.05) (Table 1, Figure 1A). Adjustment of CL variables for the significant difference on PMNs count with ANCOVA changed the conclusion concerning the significant difference in resting and fMLP-induced CL (Table 2, Figure 1B). While controlling for the effect of PMNs count in OLD-negative patients, there were no significant differences in the PMNs-adjusted means of CL variables (ANCOVA testing against healthy controls with PMNs as covariate; all p>0.05 in OLD-negative patients). For the exhaled H_2O_2 the assumptions for ANCOVA with PMNs as a covariate were not met. Regardless of that, the strong increase in exhaled H_2O_2 was also highly significant in terms of 95% CI (the entire 95% confidence interval for the ratio of geometric means was well over 2-times that of HIV-infected to control geometric means ratio) (Figure 1B).

To recapitulate, there exist a highly significant difference in the exhalation of H_2O_2 between HIV-infected patients and healthy controls; what is more, exhaled H_2O_2 was the most prominent marker of oxidative stress in HIV-infected individuals, regardless of concomitant RTI.

HAART-naive vs. HAART-treated subjects: A total of 23 OLD-negative patients commenced aggressive antiretroviral treatment regimens with HAART and 13 OLD-negative subjects remained off therapy until clinically indicated (Table 3). Viral load assays confirmed a significant decrease in the number of HIV-1 RNA copies associated with antiretroviral treatment (p<0.05) (Table 3). Along with suppression of viral load no further differences occurred between HAART-naive and HAART-treated arms (*post hoc* ANOVA against HAART-naive; all p>0.05) (Table 3, Figure 2A). The analysis confirmed an elevated exhalation of H_2O_2 in comparison to healthy controls, regardless of HAART and no significant difference in resting and fMLP-induced CL after adjustment of CL variables for a difference in PMNs with ANCOVA (Table 3, Figures 2A and 2B).

Relationship between exhaled H_2O_2 and whole blood chemiluminescence: Spearman's rank correlations were calculated between exhaled H_2O_2 and CL variables. Exhaled H_2O_2 did not significantly correlate with resting CL and fMLP-induced peak CL in OLD-negative patients (all Spearman's rho p>0.05; detailed data not shown). This was in agreement with no significant associations between exhaled H_2O_2 and either resting CL or fMLP-induced peak CL in healthy controls (all Spearman's rho p>0.05; detailed data not shown).

Factors determining exhaled H_2O_2 in OLD-negative subjects

Univariate analyses: When exhaled H_2O_2 was established as dependent variable linear regression by nonparametric bootstrap did not find any evidence of significant association with any demographic or clinical variables except a detectable viral load being revealed as a significant and positive predictor for exhaled H_2O_2 (R-squared=0.23, p=0.014) (Table 4, Figure 3A). On the contrary, the linear regression by nonparametric bootstrap did not show any significant relations between CL variables, either resting CL or fMLP-induced peak CL and viral load (all p>0.05, detailed data not shown).

Moreover, quantile regression was employed to estimate the relationships between exhaled H_2O_2 and viral load for a large part of the exhaled H_2O_2 distribution. We present results by simultaneous bootstrap analysis narrowed to a range from 0.2 to 0.8 quantiles, as justified by the small sample (n=19) and large sampling variation for upper quantiles (Figure 3B). Quantile regression estimates indicated some significant and positive relations between exhaled H_2O_2 and viral load. The effects of viral load on exhaled H_2O_2 were best revealed at higher H_2O_2 exhalation as shown for quantiles from 0.6 to 0.7 (Figure 3B). There was a significant increase in exhaled H_2O_2 in response to viral load at quantile 0.6 (Pseudo R-squared=0.21, p=0.043) and at quantile 0.7 (Pseudo R-squared=0.21, p=0.042).

Insofar as it can be ascertained, the estimated effects of viral load were well represented by changes in exhaled H_2O_2.

Multivariate analysis: In order to determine the factors contributing to exhaled H_2O_2 (to generate hypotheses regarding the causes in variation of the exhalation of H_2O_2), a multivariate analysis was carried out with exhaled H_2O_2 as the dependent variable together with smoking habits, duration of HIV-infection (in years), a detectable viral load (in log10 of RNA copies/

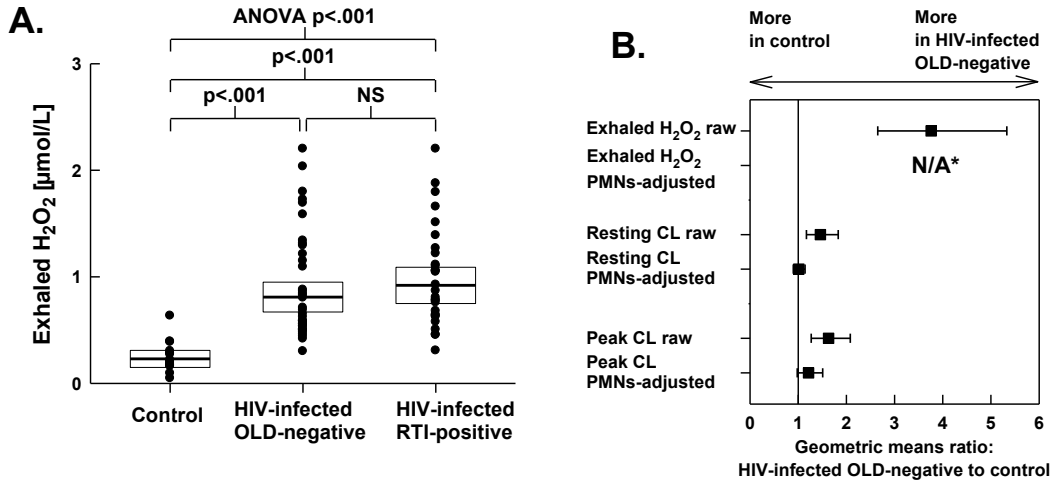

Figure 1: Exhaled H_2O_2 as marker of oxidative stress in HIV-infected patients without signs and symptoms of opportunistic lung disease (OLD-negative) and with signs and symptoms of concomitant respiratory tract infection without definite diagnosis of OLD (RTI-positive). (A - top) Dot plot of individual results in healthy controls and HIV-1 infected patients. Box plot shows the geometric mean as a solid line and 95% confidence intervals as a rectangle. (B - bottom) Fold increase of geometric mean exhaled H_2O_2, resting and fMLP-induced peak whole blood chemiluminescence (CL) in OLD-negative patients against healthy controls. Raw data vs. adjusted for differences in polymorphonuclear leukocytes count (PMNs) among groups with ANCOVA (not applicable for exhaled H_2O_2 - assumptions not met).

Variable	(1) Control (n=14)	(2) HIV-infected OLD-negative (n=36)	(3) HIV-infected RTI-positive (n=28)	p-value	post-hoc p-value	Difference	95% CI
Exhaled breath H_2O_2 [µmol/L], geometric mean (95% CI, range)	0.21 (0.15 to 0.31, 0.05-0.63)	0.80 (0.67 to 0.95, 0.30-2.20)	0.90 (0.75 to 1.09, 0.31-2.20)	<0.001[1]	<0.001[2] 1 vs. 2 <0.001[2] 1 vs. 3 1.00[2] 2 vs. 3	3.76 4.24	(2.53 to 5.58) (2.81 to 6.40)
Resting CL [mV/10^4 cells], geometric mean (95% CI, range)	0.59 (0.50 to 0.70, 0.28-0.84)	0.84 (0.74 to 0.95, 0.48-1.35)	0.71 (0.61 to 0.83, 0.35-1.28)	0.008[1]	0.006[2] 1 vs. 2 0.042[2] 1 vs. 3 1.00[2] 2 vs. 3	1.46 1.36	(1.10 to 1.94) (1.02 to 1.83)
fMLP-induced peak CL [mV/10^4 cells], geometric mean (95% CI, range)	0.92 (0.74 to 1.13, 0.42-2.24)	1.49 (1.30 to 1.71, 0.62-2.97)	1.67 (1.34 to 2.09, 0.63-4.71)	<0.001[1]	0.004[2] 1 vs. 2 <0.001[2] 1 vs. 3 0.98[2] 2 vs. 3	1.63 1.83	(1.20 to 2.19) (1.27 to 2.63)
Adjusted variables for significant difference on PMNs count:							
Exhaled breath H_2O_2 [µmol/L], geometric mean (95% CI)	N/A	N/A	N/A	N/A[3]			
Resting CL [mV/10^4 cells], geometric mean (95% CI)	0.71 (0.63 to 0.81)	0.75 (0.70 to 0.81)	0.81 (0.75 to 0.88)	0.18[4]			
fMLP-induced peak CL [mV/10^4 cells], geometric mean (95% CI)	1.03 (0.82 to 1.31)	1.40 (1.21 to 1.62)	1.71 (1.45 to 2.00)	0.004[4]	0.003[5] 1 vs. 3	1.65	(1.17 to 2.33)

p-values: [1]one-way ANOVA; [2]post hoc ANOVA test with Bonferroni adjustment; [3]one-way ANCOVA (assumptions not met); [4]one-way ANCOVA (assumptions met); [5]*post hoc* ANCOVA test with Bonferroni adjustment.
CL, whole blood chemiluminescence; fMLP, N-formyl-methionyl-leucyl-phenylalanine; N/A, not applicable; PMNs, polymorphonuclear leukocytes.

Table 2: Comparison of exhaled H_2O_2 and whole blood chemiluminescence between healthy controls vs. HIV-infected patients without clinical signs and symptoms of opportunistic lung disease (OLD-negative) or with clinical signs and symptoms of concomitant respiratory tract infection without a definite diagnosis of OLD (RTI-positive).

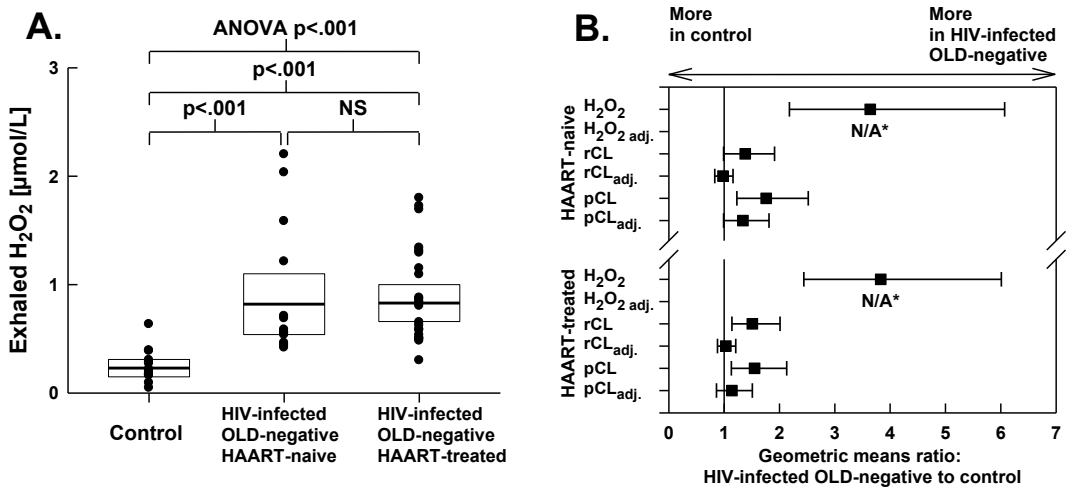

Figure 2: Exhaled H_2O_2 as marker of oxidative stress in HIV-infected patients without signs and symptoms of opportunistic lung disease (OLD-negative) and either HAART-naive or HAART-treated. (A - top) Dot plot of individual results in healthy controls and HIV-1 infected patients. Box plot shows the geometric mean as a solid line and 95% confidence intervals as a rectangle. (B - bottom) Fold increase of geometric mean exhaled H_2O_2 (H_2O_2), resting (rCL) and fMLP-induced peak whole blood chemiluminescence (pCL) in OLD-negative patients either HAART-naive or HAART-treated against healthy controls. Raw data vs. adjusted (adj.) for differences in polymorphonuclear leukocytes count (PMNs) among groups with ANCOVA (not applicable for exhaled H_2O_2 - assumptions not met).

mL), treatment with HAART and a history of AIDS as possible explanatory factors. In this model, the main contributors to exhaled H_2O_2 as described by a multiple linear regression equation were viral load and treatment with HAART. The standardized coefficients indicated that viral load contributed most considerably to the model, followed by treatment with HAART. Together, they accounted for 35% of the variance in exhaled H_2O_2 (Table 4). If the analysis was limited to HAART-treated only and CD_4 count (in cells/µL) was added as a possible predictor, then a better model fit was obtained (R-squared=0.79). The analysis confirmed that viral load is the main contributor to the exhaled H_2O_2; it also suggests that CD_4 count has slightly more influence than the duration of HIV-infection in the HAART-treated subgroup. Figure 3C shows a scatter graph with the predicted values of exhaled H_2O_2 on the X-axis from the multiple regression equation and the observed values of exhaled H_2O_2 on the Y-axis. Since the points fall close to the diagonal line, this illustrates the fit of the multiple regression model for prediction of H_2O_2 exhalation.

Discussion

Whereas systemic oxidative stress is a common feature of HIV-1 infection, the lungs are one of the major targets of HIV-1 attack. Accumulation of ROS induces airway inflammation that can be deteriorated by opportunistic lung diseases. Exhaled H_2O_2 is a known noninvasive inflammatory marker of the respiratory tract which has not been previously reported in HIV-1-infected patients. Moreover, the relationship between exhaled H_2O_2 and whole blood chemiluminescence has not been established. In this cross-sectional study, we found a high

level of exhaled H_2O_2 among HIV-infected patients as compared to healthy controls, regardless of concomitant respiratory tract infection and despite treatment with HAART. This was accompanied by greater luminol enhanced light emission of the whole blood, either resting or agonist-induced, even though an increase in the exhalation of H_2O_2 was more evident. Nevertheless, elevated exhalation of H_2O_2 and appreciable

Variable	(1) Control (n=14)	(2) HIV-infected OLD-negative HAART-naive (n=13)	(3) HIV-infected OLD-negative HAART-treated (n=23)	p-value	*post-hoc* p-value	Difference	95% CI
Viral load assays, n (%)	N/A	5 (39%)	23 (100%)	<0.001[1]			
Detectable viral load[a], n(%)	N/A	5 (100%)	14 (61%)	0.14[1]			
Viral load [x10^3 RNA copies/mL], geometric mean (95% CI, range)	N/A	13.65 (0.43 to 436.13, 0.84-935)	0.53 (0.11 to 2.47, 0-625)	0.047[2]		0.04	(0.002 to 0.95)
Exhaled breath H_2O_2 [µmol/L], geometric mean (95% CI, range)	0.21 (0.15 to 0.31, 0.05-0.63)	0.77 (0.54 to 1.10, 0.42-2.20)	0.81 (0.66 to 1.00, 0.30-1.80)	<0.001[3]	<0.001[4] 1 vs. 2 <0.001[4] 1 vs. 3 1.00[4] 2 vs. 3	3.64 3.83	(2.18 to 6.07) (2.44 to 6.01)
Resting CL [mV/10^4 cells], geometric mean (95% CI, range)	0.59 (0.50 to 0.70, 0.28-0.84)	0.79 (0.66 to 0.94, 0.47-1.22)	0.87 (0.73 to 1.04, 0.43-2.14)	0.004[3]	0.070[4] 1 vs. 2 0.004[4] 1 vs. 3 1.00[4] 2 vs. 3	1.51	(1.14 to 2.01)
fMLP-induced peak CL [mV/10^4 cells], geometric mean (95% CI, range)	0.92 (0.74 to 1.13, 0.42-2.24)	1.61 (1.29 to 2.02, 0.90-2.97)	1.42 (1.19 to 1.70, 0.62-2.87)	<0.001[3]	0.001[4] 1 vs. 2 0.005[4] 1 vs. 3 1.00[4] 2 vs. 3	1.76 1.55	(1.23 to 2.52) (1.13 to 2.13)
Adjusted variables for significant difference on PMNs count:							
Exhaled breath H_2O_2 [µmol/L] geometric mean (95% CI)	N/A	N/A	N/A	N/A[5]			
Resting CL [mV/10^4 cells], geometric mean (95% CI)	0.75 (0.68 to 0.83)	0.73 (0.67 to 0.80)	0.77 (0.72 to 0.83)	0.63[6]			
fMLP-induced peak CL [mV/10^4 cells], geometric mean (95% CI)	1.13 (0.95 to 1.35)	1.52 (1.28 to 1.79)	1.30 (1.14 to 1.47)	0.078[6]			

p-values: [1]Fisher's exact test; [2]unpaired Student's t-test (equal variances); [3]one-way ANOVA; [4]*post* hoc ANOVA test with Bonferroni adjustment; [5]one-way ANCOVA (assumptions not met); [6]one-way ANCOVA (assumptions met).
CL, whole blood chemiluminescence; fMLP, N-formyl-methionyl-leucyl-phenylalanine; HAART, highly active anti-retroviral therapy; N/A, not applicable; PMNs, polymorphonuclear leukocytes.

Table 3: Comparison of viral load, exhaled H_2O_2 and whole blood chemiluminescence between healthy controls vs. HIV-infected patients without clinical signs and symptoms of opportunistic lung disease (OLD-negative) and either HAART-naive or HAART-treated.

Type	Model	Dependent Variable	Independent Variable	Regression Type	R-squared	Coefficient (95% CI)	Standardized Coefficient	p-value
Univariate	1	Exhaled H_2O_2 [µmol/L] (n=19) (detectable viral load subgroup)	Log10 (detectable viral load) [RNA copies/mL]	Linear	0.23	0.180 (0.037 to 0.323)		0.014
Multivariate	2	Exhaled H_2O_2 [µmol/L] (n=19) (detectable viral load subgroup: HAART-naive and HAART-treated)	Log10 (detectable viral load) [RNA copies/mL] Treatment with HAART Constant	Linear, stepwise	0.35	0.229 (0.080 to 0.377) 0.401 (0.010 to 0.791) -0.189 (-0.818 to 0.440)	0.608 0.365	0.003 0.044 0.56
	3	Exhaled H_2O_2 [µmol/L] (n=14) (detectable viral load subgroup: HAART-treated only)	Log10 (detectable viral load) [RNA copies/mL] CD_4 count [cells/µL] HIV-infection duration [yrs] HAART duration [mths] Constant	Linear, stepwise	0.79	0.464 (0.270 to 0.659) 0.0017 (0.0003 to 0.0031) 0.155(0.011 to 0.298) -0.015 (-0.035 to 0.003) -1.50 (-2.56 to -0.44)	1.16 0.938 0.932 -0.944	<0.001 0.015 0.035 0.11 0.005

HAART, highly active anti-retroviral therapy

Table 4: Summary of regression models in HIV-infected patients without clinical signs and symptoms of opportunistic lung disease (OLD-negative).

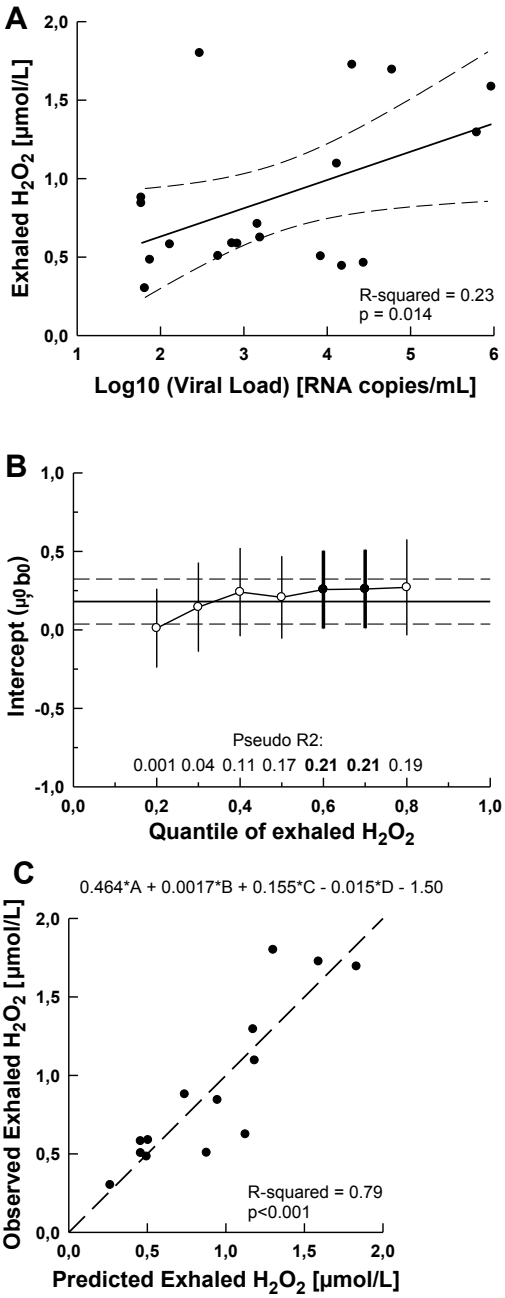

Figure 3: Regression models in HIV-infected patients without signs and symptoms of opportunistic lung disease (OLD-negative). Linear regression (A - top) to estimate changes in exhaled H_2O_2 (y) as a function of log10-transformed detectable HIV-1 viral load (x). Solid line: slope (β_1); long dashed lines: 95% confidence intervals. Quantile regression (B-middle) to estimate relationships between exhaled H_2O_2 and log10-transformed detectable HIV-1 viral load for the exhaled H_2O_2 distribution from 0.2 to 0.8 quantiles. Solid line: slopes for quantiles (b_1), connected with white circles (non-significant) or black circles (significant at $p<0.05$) and with error bars corresponding to 95% confidence intervals (bolded if $p<0.05$). Linear regression slope β_1 is shown as solid line with long dashed lines. corresponding to 95% confidence intervals. Multiple linear regression (C - bottom) in HAART-treated subgroup only, with exhaled H_2O_2 as the dependent variable and log10-transformed HIV-1 detectable viral load (A), CD_4 count (B), HIV-infection duration (C) and HAART duration (D) as the independent variables. Predicted values of exhaled H_2O_2 from the multiple regression equation are graphed on the X-axis and the observed values of exhaled H_2O_2 are plotted on the Y-axis.

chemiluminescence result from enhanced activity of phagocytes and may be a compensatory mechanism in response to the underlying immunodeficiency [29]. We found that the observed differences in CL variables were explained by an adjustment to a lower whole blood PMNs count. Elbim et al. confirmed that PMNs counts in HIV-infected patients were significantly decreased, though circulating PMNs were activated producing more H_2O_2 [30]. Interestingly, there was no association between exhaled H_2O_2 and whole blood chemiluminescence in HIV-infected patients. This associative finding had been previously reported in a study of 41 healthy subjects using the same methods to measure both the exhalation of H_2O_2 and light emission of blood phagocytes [21]. These results indicate that exhalation of H_2O_2 in HIV-infected patients does not dependent on ability of blood phagocytes to generate ROS, to a higher extent, this may involve phagocytes within the lungs.

In addition, increased exhalation of H_2O_2 in OLD-negative patients was associated with detectable viral load. The association was more evident at higher levels of exhaled H_2O_2 as revealed by quantile regression analysis. The mechanism underlying the observed associations aims to uncover direct casual pathway between viral load and exhaled H_2O_2. Numerous studies have shown that H_2O_2 strongly activates HIV long terminal repeat (LTR), containing sequences required for the initiation of HIV-1 transcription via a post-translational control of NF-kappaB [2,31-33]. Moreover, alveolar macrophages are susceptible to HIV-1 virus infection and can be recognized as latent viral reservoir [34]. These cells isolated from asymptomatic HIV-1 positive subjects exhibited a constitutive activation of phosphatidylinositol 3-kinase pathway [35]. The Nef (Negative Regulatory Factor) protein of the HIV-1 virus could be one of the activators in the signal transduction pathway leading to stimulation of the NADPH oxidase complex [36] and increased oxidants release from macrophages [37]. Additionally, Tat protein has been shown to induce the release of cytokines, thereby enhancing the production of H_2O_2 in a variety of cells, including macrophages [38,39]. In fact, a study by Buhl showed that alveolar macrophages isolated from the lungs of HIV-infected subjects presented with an increased spontaneous release of oxidants [40]. In all, these can favor conditions for increased H_2O_2 activity in the airways, rendering an augmentation in viral replication. This is in agreement with the findings of Elbim et al. who reported that basal production of H_2O_2 in whole blood monocytes is correlated with viral load [41]. These observations point towards the existence of a positive feedback interplay between the production of H_2O_2 in the airways and HIV-1 viral load. Possibly, this may be a leading mechanism responsible for increased exhaled H_2O_2 levels in HIV-infected patients.

Moreover, instead of an association with viral load there was a weaker association between increased exhaled H_2O_2 and treatment with HAART as revealed by a multiple regression analysis. This concurs with study by Mandas et al. showing oxidative imbalance in HIV-1 infected patients treated with antiretroviral therapy [7], and report by Ngondi et al. demonstrating enhancing (pro-oxidant) effect of HAART on systemic lipid peroxidation [9]. Although, in the latter study, the majority of HIV-1 infected patients were diagnosed relatively late and presented with an increased severity in clinical status along with active opportunistic infections [9].

When the analysis was narrowed exclusively to HAART-treated, we found other positive associations with CD_4 count and HIV-1-infection duration. Bucy et al. showed evidence that increase in CD_4 lymphocytes after HIV antiretroviral therapy reflects redistribution from lymphoid tissues [42]. The link between the increased exhalation of H_2O_2 and the duration of HIV-infection is likely complex, resulting from disease factors, such as decrease in the GSH concentration over time in the alveolar lining

fluid [18], decreased erythrocyte GSH-Px activity [43], interactions with HAART [9] and patient factors including genetically based susceptibility. For example, Delanghe et al. reported that HIV-seropositive patients with the antioxidant protein haptoglobin 2-2 phenotype, known to bind free hemoglobin more slowly, had a higher mortality and worse prognosis than patients with other phenotypes, suggesting enhanced hemoglobin-driven oxidative stress [44].

Our study has several limitations. Given our small sample size, which necessitates testing in larger groups, we were unable to fully explore all the hypotheses. Secondly, the cross-sectional study design makes the assessment of casual relationships difficult. Finally, we enrolled OLD-negative patients, which included normal chest X-rays, to avoid opportunistic infections, though asymptomatic presentations of OLD could not be excluded. Despite these limitations, the findings in our study should encourage an answer to the question of whether or not the increased exhalation of H_2O_2 in HIV-1 infected subjects evinces clinical significance. Implications for further studies are HIV associated pulmonary emphysema [45], since H_2O_2 is linked to breakdown of elastic fibers [46] and Kaposi's sarcoma [47], as H_2O_2 mediates herpesvirus reactivation from latency. Therefore, it is possible that determination of exhaled H_2O_2 can be helpful in the selection of patients with a higher risk of some HIV-1 associated diseases.

Acknowledgment

This work was supported by Medical University of Lodz Grants: 503/0-079-01/503-41 and 503/0-079-06/503-01.

References

1. Yao Y, Hoffer A, Chang CY, Puga A (1995) Dioxin activates HIV-1 gene expression by an oxidative stress pathway requiring a functional cytochrome P450 CYP1A1 enzyme. Environ Health Perspect 103: 366-371.
2. Pyo CW, Yang YL, Yoo NK, Choi SY (2008) Reactive oxygen species activate HIV long terminal repeat via post-translational control of NF-kappaB. Biochem Biophys Res Commun 376: 180-185.
3. Papadopulos-Eleopulos E (1988) Reappraisal of AIDS--is the oxidation induced by the risk factors the primary cause? Med Hypotheses 25: 151-162.
4. Walmsley SL, Winn LM, Harrison ML, Uetrecht JP, Wells PG (1997) Oxidative stress and thiol depletion in plasma and peripheral blood lymphocytes from HIV-infected patients: toxicological and pathological implications. AIDS 11: 1689-1697.
5. Elbim C, Pillet S, Prevost MH, Preira A, Girard PM, et al. (2001) The role of phagocytes in HIV-related oxidative stress. J Clin Virol 20: 99-109.
6. Deshmane SL, Mukerjee R, Fan S, Del Valle L, Michiels C, et al. (2009) Activation of the oxidative stress pathway by HIV-1 Vpr leads to induction of hypoxia-inducible factor 1alpha expression. J Biol Chem 284: 11364-11373.
7. 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.
8. Repetto M, Reides C, Gomez Carretero ML, Costa M, Griemberg G, et al. (1996) Oxidative stress in blood of HIV infected patients. Clin Chim Acta 255: 107-117.
9. Ngondi JL, Oben J, Forkah DM, Etame LH, Mbanya D (2006) The effect of different combination therapies on oxidative stress markers in HIV infected patients in Cameroon. AIDS Res Ther 3: 19.
10. Horváth I, Hunt J, Barnes PJ, Alving K, Antczak A, et al. (2005) Exhaled breath condensate: methodological recommendations and unresolved questions. Eur Respir J 26: 523-548.
11. Stolarek R, Bialasiewicz P, Krol M, Nowak D (2010) Breath analysis of hydrogen peroxide as a diagnostic tool. Clin Chim Acta 411: 1849-1861.
12. Jöbsis Q, Raatgeep HC, Hermans PW, de Jongste JC (1997) Hydrogen peroxide in exhaled air is increased in stable asthmatic children. Eur Respir J 10: 519-521.
13. Antczak A, Nowak D, Shariati B, Król M, Piasecka G, et al. (1997) Increased hydrogen peroxide and thiobarbituric acid-reactive products in expired breath condensate of asthmatic patients. Eur Respir J 10: 1235-1241.
14. Dekhuijzen PN, Aben KK, Dekker I, Aarts LP, Wielders PL, et al. (1996) Increased exhalation of hydrogen peroxide in patients with stable and unstable chronic obstructive pulmonary disease. Am J Respir Crit Care Med 154: 813-816.
15. Nowak D, Kasielski M, Antczak A, Pietras T, Bialasiewicz P (1999) Increased content of thiobarbituric acid-reactive substances and hydrogen peroxide in the expired breath condensate of patients with stable chronic obstructive pulmonary disease: no significant effect of cigarette smoking. Respir Med 93: 389-396.
16. Majewska E, Kasielski M, Luczynski R, Bartosz G, Bialasiewicz P, et al. (2004) Elevated exhalation of hydrogen peroxide and thiobarbituric acid reactive substances in patients with community acquired pneumonia. Respir Med 98: 669-676.
17. Buhl R (1994) Imbalance between oxidants and antioxidants in the lungs of HIV-seropositive individuals. Chem Biol Interact 91: 147-158.
18. Pacht ER, Diaz P, Clanton T, Hart J, Gadek JE (1997) Alveolar fluid glutathione decreases in asymptomatic HIV-seropositive subjects over time. Chest 112: 785-788.
19. Look MP, Rockstroh JK, Rao GS, Kreuzer KA, Barton S, et al. (1997) Serum selenium, plasma glutathione (GSH) and erythrocyte glutathione peroxidase (GSH-Px)-levels in asymptomatic versus symptomatic human immunodeficiency virus-1 (HIV-1)-infection. Eur J Clin Nutr 51: 266-272.
20. 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.
21. Szkudlarek U, Maria L, Kasielski M, Kaucka S, Nowak D (2003) Exhaled hydrogen peroxide correlates with the release of reactive oxygen species by blood phagocytes in healthy subjects. Respir Med 97: 718-725.
22. Ferraresi R, Troiano L, Roat E, Nemes E, Lugli E, et al. (2006) Protective effect of acetyl-L-carnitine against oxidative stress induced by antiretroviral drugs. FEBS Lett 580: 6612-6616.
23. Nowak D, Kalucka S, Białasiewicz P, Król M (2001) Exhalation of H2O2 and thiobarbituric acid reactive substances (TBARs) by healthy subjects. Free Radic Biol Med 30: 178-186.
24. Zappacosta B, Persichilli S, Mormile F, Minucci A, Russo A, et al. (2001) A fast chemiluminescent method for H(2)O(2) measurement in exhaled breath condensate. Clin Chim Acta 310: 187-191.
25. Ruch W, Cooper PH, Baggiolini M (1983) Assay of H2O2 production by macrophages and neutrophils with homovanillic acid and horse-radish peroxidase. J Immunol Methods 63: 347-357.
26. Łuczyñska M, Szkudlarek U, Dziankowska-Bartkowiak B, Waszczykowska E, Kasielski M, et al. (2003) Elevated exhalation of hydrogen peroxide in patients with systemic sclerosis. Eur J Clin Invest 33: 274-279.
27. Kukovetz EM, Bratschitsch G, Hofer HP, Egger G, Schaur RJ (1997) Influence of age on the release of reactive oxygen species by phagocytes as measured by a whole blood chemiluminescence assay. Free Radic Biol Med 22: 433-438.
28. Cade BS, Noon BR (2003) A gentle introduction to quantile regression for ecologists. Front Ecol Environ 1: 412-420.
29. Lee SS, Li PC, Yeung KT, Sitt WH (1993) Enhanced phagocyte chemiluminescence in asymptomatic HIV infection. Asian Pac J Allergy Immunol 11: 131-133.
30. Elbim C, Prevot MH, Bouscarat F, Franzini E, Chollet-Martin S, et al. (1994) Polymorphonuclear neutrophils from human immunodeficiency virus-infected patients show enhanced activation, diminished fMLP-induced L-selectin shedding, and an impaired oxidative burst after cytokine priming. Blood 84: 2759-2766.
31. Schreck R, Rieber P, Baeuerle PA (1991) Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. EMBO J 10: 2247-2258.
32. Kazazi F, Koehler JK, Klebanoff SJ (1996) Activation of the HIV long terminal repeat and viral production by H2O2-vanadate. Free Radic Biol Med 20: 813-820.

33. Klebanoff SJ, Headley CM (1999) Activation of the human immunodeficiency virus-1 long terminal repeat by respiratory burst oxidants of neutrophils. Blood 93: 350-356.

34. Reynoso R, Wieser M, Ojeda D, Bönisch M, Kühnel H, et al. (2012) HIV-1 induces telomerase activity in monocyte-derived macrophages, possibly safeguarding one of its reservoirs. J Virol 86: 10327-10337.

35. Tachado SD, Li X, Swan K, Patel N, Koziel H (2008) Constitutive activation of phosphatidylinositol 3-kinase signaling pathway down-regulates TLR4-mediated tumor necrosis factor-alpha release in alveolar macrophages from asymptomatic HIV-positive persons in vitro. J Biol Chem 283: 33191-33198.

36. Olivetta E, Mallozzi C, Ruggieri V, Pietraforte D, Federico M, et al. (2009) HIV-1 Nef induces p47(phox) phosphorylation leading to a rapid superoxide anion release from the U937 human monoblastic cell line. J Cell Biochem 106: 812-822.

37. Olivetta E, Pietraforte D, Schiavoni I, Minetti M, Federico M, et al. (2005) HIV-1 Nef regulates the release of superoxide anions from human macrophages. Biochem J 390: 591-602.

38. Westendorp MO, Shatrov VA, Schulze-Osthoff K, Frank R, Kraft M, et al. (1995) HIV-1 Tat potentiates TNF-induced NF-kappa B activation and cytotoxicity by altering the cellular redox state. EMBO J 14: 546-554.

39. Nath A, Conant K, Chen P, Scott C, Major EO (1999) Transient exposure to HIV-1 Tat protein results in cytokine production in macrophages and astrocytes. A hit and run phenomenon. J Biol Chem 274: 17098-17102.

40. Buhl R, Jaffe HA, Holroyd KJ, Borok Z, Roum JH, et al. (1993) Activation of alveolar macrophages in asymptomatic HIV-infected individuals. J Immunol 150: 1019-1028.

41. Elbim C, Pillet S, Prevost MH, Preira A, Girard PM, et al. (1999) Redox and activation status of monocytes from human immunodeficiency virus-infected patients: relationship with viral load. J Virol 73: 4561-4566.

42. Bucy RP, Hockett RD, Derdeyn CA, Saag MS, Squires K, et al. (1999) Initial increase in blood CD4(+) lymphocytes after HIV antiretroviral therapy reflects redistribution from lymphoid tissues. J Clin Invest 103: 1391-1398.

43. Ogunro PS, Ogungbamigbe TO, Elemie PO, Egbewale BE, Adewole TA (2006) Plasma selenium concentration and glutathione peroxidase activity in HIV-1/AIDS infected patients: a correlation with the disease progression. Niger Postgrad Med J 13: 1-5.

44. Delanghe JR, Langlois MR, Boelaert JR, Van Acker J, Van Wanzeele F, et al. (1998) Haptoglobin polymorphism, iron metabolism and mortality in HIV infection. AIDS 12: 1027-1032.

45. Petrache I, Diab K, Knox KS, Twigg HL 3rd, Stephens RS, et al. (2008) HIV associated pulmonary emphysema: a review of the literature and inquiry into its mechanism. Thorax 63: 463-469.

46. Cantor JO, Shteyngart B, Cerreta JM, Ma S, Turino GM (2006) Synergistic effect of hydrogen peroxide and elastase on elastic fiber injury *in vitro*. Exp Biol Med (Maywood) 231: 107-111.

47. Ye F, Zhou F, Bedolla RG, Jones T, Lei X, et al. (2011) Reactive oxygen species hydrogen peroxide mediates Kaposi's sarcoma-associated herpesvirus reactivation from latency. PLoS Pathog 7: e1002054.