Research Paper

Effects of Antiretroviral Therapy on Autonomic Function in Early HIV Infection: A Preliminary Report

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Abstract

Background: A prospective study was conducted in human immunodeficiency virus (HIV)-infected patients as they undergo alterations in their antiretroviral therapy (ART) to determine the effect of ART on autonomic function.

Methods: HIV-infected subjects who were either 1) naïve to ART and initiating ART, or 2) receiving ART and in HIV virologic failure for at least 4 months and were about to switch ART were enrolled in this study. Autonomic function assessment (cardiovagal, adrenergic, and sudomotor tests) was performed prior to and 4 months after initiating the new ART. Changes in clinical autonomic symptoms and virologic assessment were assessed.

Results: Twelve subjects completed the study: 92% male; median age (Q1, Q3) was 41.0 (28.0, 48.2) years; and 50% White/Non-Hispanic. Seventy-five percent were ART naïve while 25% were failing their ART regimen. The median CD4 count was 336.5 (245.3, 372.3) cells/mm³. All subjects achieved an undetectable HIV viral load by the 4-month follow-up visit. The majority of naïve subjects were started on an ART regimen of tenofovir / emtricitabine / efavirenz. There were no significant differences in autonomic function assessment, as measured by cardiovagal, adrenergic, and sudomotor tests, with regards to ART initiation.

Conclusion: This is the first study to examine the effects of initiating ART on autonomic function in early HIV infection. This study found no appreciable differences of ART on the autonomic nervous system when ART is initiated early in the course of HIV disease. ART may not contribute to short-term changes in autonomic function.

Key words: HIV, Antiretroviral Therapy, Autonomic Function.

Introduction

Autonomic symptoms such as orthostatic hypotension, syncope, impotence, urinary dysfunction, diminished sweating, and diarrhea are common among individuals infected with HIV [1, 2]. The prevalence of symptoms of clinical autonomic neuropathy in HIV has been variable depending on HIV disease status and treatment, ranging from 0 to 84% [1, 3, 4]. Compostella et al. found 19% of treated

HIV-infected subjects demonstrated severe autonomic neuropathy [5] while Correia found no significant difference in autonomic function between AIDS subjects on HAART and HIV-negative controls [6]. This increase in autonomic dysfunction is likely to be multi-factorial and involve the effects of HIV per se, as well as the direct and indirect effects of antiretroviral therapy [5].

The autonomic nervous system consists of two balanced subsystems: the parasympathetic and sympathetic nervous system, which are involved in the homeostasis of organs and physiological functions. Increased sympathetic activation is associated with metabolic syndrome, hypertension, ischemic heart disease, arrhythmia, and cardiomyopathy while decreased parasympathetic activation has been seen with aging and in baroreflex and chemoreflex impairment [7, 8].

The effects of stable antiretroviral therapy on autonomic function and CVD risk have yet to be fully investigated. A cross-sectional study found differences in autonomic function between HIV-infected individuals and HIV sero-negative controls, but not between non-virologically un-suppressed and virologically suppressed HIV infected individuals [9]. There were trends towards a decrease in parasympathetic modulation in the virologically non-suppressed group compared to the virologically suppressed group. The combination of heightened sympathetic and low parasympathetic activity commonly found in HIV-infected patients has been linked to increased cardiovascular morbidity and mortality [10].

Autonomic dysfunction may add to inflammatory changes leading to the development of a deleterious feedback loop. The end result may be a detrimental effect on endothelial function, which can increase CVD risk. Because of the potential benefits of treating HIV-infected patients early in its disease course and the concern of adverse effects from ART on the cardiovascular system, we conducted a prospective study involving patients as they undergo alterations in their ART. Therefore the purpose of this study is to evaluate the effects of the initiation of ART therapy on autonomic function with regards to the presence of plasma HIV RNA.

Methods

Study design. This is a prospective study that measured autonomic function in a cohort of HIV infected individuals undergoing changes in ART. All subjects signed informed consent, and the institutional review board (IRB) at the University of Hawaii approved the study (COOP IRB CHS # 16360).

Study Population. Subjects were obtained through a convenience sample of participants studied at the Hawaii Center for AIDS. HIV infected individuals over the age of 17 were eligible for this study. ART was defined as antiretroviral regimens outlined in the 2004 Department of Health and Human Services (DHHS) guidelines for at least 3 months prior to study entry [11]. Undetectable viral load was defined as HIV RNA by PCR by Roche Ultrasensitive Amplicor Version 1.5 < 50 copies/ml. As past or current severe immunosupression may have affected autonomic function, we chose only subjects who never had a CD4 nadir < 200 cells/mm³. Eligible subjects were:

- HIV-infected subjects naïve to ART who initiated ART and subsequently had an undetectable HIV RNA after 4 months of therapy
- HIV-infected subjects receiving ART who were in virologic failure (detectable HIV RNA viral load for at least 4 months) and changed ART, and are found to have an undetectable HIV RNA after 4 months of stable ART.

Exclusion criteria were as follows: known cardiovascular disease, arrhythmia, pregnancy, hypertension, and diabetes mellitus. Participants currently taking medication known to influence autonomic nerve function (such as antihypertensive medications and tricyclic antidepressants) were excluded.

Clinical Health Assessments

Several health characteristics were obtained, including demographics, clinical exam, past medical history, medication history, fasting lipid panel, fasting glucose and insulin, HIV RNA viral load, and CD4 count.

Autonomic Function Testing

The autonomic function tests performed can be grouped into three general categories of autonomic activity: cardiovagal, adrenergic, and sudomotor [12].

Cardiovagal testing entailed measuring heart rate variability (HRV) during a battery of maneuvers such as paced breathing, valsalva and head up tilt. Heart rate, blood pressure (BP), and chest expansion were monitored by a Colin Pilot 700 (Colin Medical Instruments Corp., San Antonio, TX), and an Atlas converter box (WR Testworks, Stillwater, MN). Data was recorded on a personal laptop using WR Testworks Suite 2.1 (WR Testworks, Stillwater, MN). HRV was analyzed as time and frequency domain characteristics. The time domain parameters measured the magnitude of variability and provided information about the vagal modulation of the heart. Frequency domain analysis yielded information about the amount of the overall variance (or power) in heart rate

resulting from periodic oscillations of heart rate at various frequencies, and thereby inferences about overall sympathovagal balance. High frequency (HF) power (0.14-0.30 Hz) inferred parasympathetic activity, while low frequency (LF) power (0.03-0.14 Hz) inferred sympathetic activity.

During the deep breathing (HR_{DB}) maneuver, the subjects were positioned supine and coached to breathe at a rate of six cycles per minute, in concert with an oscillating bar, for eight breathing cycles. By pacing the respirations, the respiratory-related vagal modulation of the heart rate were amplified. The mean heart rate variation was determined from the five largest consecutive variations between maximum and minimum heart rates. HR_{DB} provided an indicator of parasympathetic activity with higher variability generally reflecting greater parasympathetic modulation. Reduced heart rate variability has been shown to have prognostic implications for future cardiovascular disease events [13]. During the Valsalva maneuver, subjects were instructed to lie in the supine position and to blow through a bugle and maintain an expiratory pressure at 40 mm Hg mercury for 15 seconds. The procedure was repeated until two reproducible BP curves are obtained [14, 15]. From the Valsalva maneuver, inferences were made on sympathetic modulation.

Adrenergic testing evaluated sympathetic regulation of arterioles and to a lesser degree cardiac contractility by evaluating baroreflex-mediated reflex vasoconstriction at rest and in response to a head-up tilt maneuver. Continuous intra-arterial waveform recordings were acquired to make this assessment. Systolic, diastolic, and mean blood pressure (BP) were recorded at heart level using the Colin Pilot (Colin Medical Instruments Corp, San Antonio Texas) during rest, Valsalva maneuver, and head-up tilt. Adrenergic testing is a better measure of sympathetic activity than low frequency power in the frequency analysis of cardiovagal data, which can be contaminated by overlapping signals of the vagal nerve [12].

The head-up tilt test involved having the subject rest for a 10-minute rest period before the subject was tilted to an angle of 70 degrees for 10 minutes or until symptoms of impending syncope were experienced by the subject. If a subject experienced presyncopal symptoms, the patient was returned to the supine position and the heart rate and blood pressure measures were recorded. Symptoms of syncope or near syncope were recorded.

Spectral (frequency) analysis of systolic blood pressure variability was performed using methods standardized from the Autonomic Disorders Program Project [16, 17]. The recordings of blood pressure (BP), heart rate, and respiration during rest and head up tilt were subjected to spectral analysis. We utilized the Mayo Clinic algorithm for this function [18]. Bad point removal was automated and user-definable using statistical methods (percentiles), and subsequently provided a choice of pre-selected low and high frequency bandwidths that correlated with sympathetic and parasympathetic modulations. Data were linearly interpolated and resampled at 4 Hz before fast Fourier transformation was utilized to smooth the data. This method has been determined to be consistent with the established methods of analysis of autonomic function [19].

Quantitative sudomotor axon reflex test (QSART) evaluates post-ganglionic sympathetic sudomotor function, an assessment of distal small nerve fiber activity. QSART, which measures the autonomic nerves that control sweating, is useful in assessing the autonomic function in peripheral neuropathies and some types of pain disorders. This procedure is useful in HIV-infected patients given that many such individuals on ART suffer from peripheral neuropathy. The test required a mild electrical stimulation on the skin, called iontophoresis, which allowed an acetylcholine gel to stimulate the sweat glands. QSART measures the volume of sweat produced by this stimulation, which can be measured quantitatively. Recordings were obtained from four sites (the left forearm, the proximal and distal portions of the left leg, and the proximal portion of the left foot) to determine the extent of peripheral neuropathy involvement. Typically, there is an ascending pattern of abnormal QSART findings in HIV-associated neuropathy. The QSART has a high sensitivity, specificity, and reproducibility with a coefficient of variation of 8.0% [20].

Statistical analysis. The primary objective was to assess the relationship of change in autonomic function in subjects before and after initiation of ART. Categorical variables were compared using the chi square test. Continuous variables are presented as medians along with their interquartile range (Q1, Q3) and analyzed by Wilcoxon rank test. Although the Shapiro-Wilks normality test showed that the majority of autonomic function test results were normal in distribution, the requirement that the distribution of the differences in paired data be approximately normal could not be confirmed given the small sample size. Even after transformation of the data, we were not satisfied with these assumptions because of the wide variability of study results. We chose the paired Wilcoxon signed rank test as the nonparametric alternative to the paired t-tests. This nonparametric method is useful in dealing with unexpected, outlying

observations that might be problematic with a parametric approach [21]. A two-sided probability of p<0.05 was used to determine statistical significance. All statistical analyses were performed using the JMP statistical program (SAS Institute Inc, Cary, NC).

Results

Subject characteristics. Twenty-one subjects were enrolled in this study. Twelve subjects had completed both study visits, 3 subjects discontinued the study because they failed to achieve an undetectable HIV viral load by the end of study visit, 2 subjects were discontinued due to loss of follow-up, and 4 subjects are still enrolled in the study awaiting their end-of-study visit.

The preliminary study results of the 12 completed subjects are presented. Demographic and clinical characteristics of the subjects are summarized in Table 1. The majority of subjects were males. The median age (interquartile range) was 41.0 (28.0, 48.2) years. Seventy five percent were ART naïve while 25% were failing their ART regimen. The median duration of HIV duration was 0.8 years. The median CD4 count (interquartile range) was 336.5 (245.3, 372.3) cells/mm^3. Only one subject had a CD4 counts < 200 cells/mm^3 (subject's CD4 count of 197 cells/mm^3). All subjects achieved an undetectable HIV viral load by the second visit. The majority of naïve subjects were started on an ART regimen of tenofovir / emtricitabine / efavirenz. Failing subjects were initiated on a variety of ART regimens.

None of the subjects had impaired fasting glucose or diabetes. There were no subjects with a resting systolic blood pressure > 140 mm Hg or diastolic blood pressure > 90 mm Hg. Low high density lipoprotein (<40 mg/dl) was noted in 4 subjects. None of the subjects had elevated low density lipoprotein, with the highest measure of 158 mg/dl.

Primary Outcomes – Autonomic Function Test Results. The autonomic function results are displayed in Table 2. There were no differences in time domain results of heart rate deep breathing and Valsalva ratio in regards to the initiation of ART when assessed by paired Wilcoxon signed rank test. One subject scored below the 5th percentile of standardized norms (Mayo Clinic and WR Medical) for the heart rate deep breathing test at entry, while 2 subjects scored below the 5th percentile at the end of study. The opposite occurred for the Valsalva maneuver: two subjects scored below the 5th percentile of standardized norms at entry, while only 1 subject scored below the 5th percentile at the end of study. Only one subject had consistently scored below the 5th percentile before and after initiating ART during the heart rate deep breathing test. There were no patterns noted for the other subjects who had low scores.

The spectral analysis of heart rate variability (cardiovagal results) was not significant, although there were higher changes in both low and high frequency power during the tilt maneuver. This may suggest an increase in both parasympathetic and sympathetic modulation as a result of ART. The greatest increase in high and low frequency power was noted in subjects failing their current ART regimen, but limitations in the number of failing subjects (n=3) made it difficult to generalize these findings.

The differences in adrenergic function in respect to ART were not significant as measured by the paired Wilcoxon signed rank test. The non-significant findings in adrenergic measures did not lend support to the possible increased low and high frequency power results during tilt seen in the cardiovagal spectral analysis of heart rate variability. None of the subjects had syncope or near syncope symptoms.

The QSART results showed a significant increase in sweat response in the distal leg of subjects initiating ART. However, the non-significant QSART finding in the foot make it unlikely that the significant findings in the distal leg are real (versus chance finding). Only one of the subjects (failing ART) reported symptoms of peripheral neuropathy (numbness and tingling). None of the subjects had abnormal findings on the neurologic examination (clinical examination of vibration and deep tendon reflexes). The other QSART sites (proximal leg and arm) did not reveal any abnormalities. The QSART responses at entry in all the subjects were above the 5th percentile of the standardized norms provided by the QSART manufacturer (WR Medical Electronics Co, Rochester, MN).

Spearman's rank correlation showed no significant correlation between the autonomic function tests and baseline CD4 count or change in study CD4 count, with the exception of changes in high and low frequency responses during tilt. Similarly, no correlation was noted between the autonomic function tests and baseline HIV viral load or change in study HIV viral load. There was no correlation between age and the baseline autonomic function measures. Change in CD4 count and change in high and low frequency responses during tilt had a significant negative correlation (-0.664, p=0.026 and -0.618, p=0.043 respectively). The correlations between the change in both CD4 Viral load and autonomic function tests are shown in Table 3. Correlations of baseline data for both autonomic function and CD4 and viral load levels were not significant and are not shown.

Table 1. Baseline Characteristics.

	HIV + Subjects
N	12
Age, years	41.0 (28.0, 48.2)
Gender, male/female	11/1
Ethnicity, n (%)	
Caucasian	6 (50)
Hispanic	2 (17)
Asian	1 (8)
Pacific Islander	2 (17)
African American	1 (8)
Body Mass Index, kg/m²	23.6 (22.0, 26.3)
Tobacco Use	
Current Use, n (%)	5 (42)
Former Use, n (%)	6 (50)
History of dyslipidemia or elevated blood pressure	0
Duration of HIV infection, years	0.80 (0.33, 13.00)
History of co-morbidities	
History of substance abuse, n (%)	2 (17%)
History of alcohol abuse, n (%)	1 (8)
Hepatitis C infection, n (%)	1 (8)
Treatment naïve / Treatment failure	9/3
Duration of antiretroviral medication use in treatment failure subjects, years	3.00 (0.40, 12.00)
Antiretroviral Medication Initiated	
<u>**Naïve Subjects**</u>	
tenofovir / emtricitabine / efavirenz <u>verses</u> tenofovir / emtricitabine / elvitegravir / experimental boosting agent GS 9350 (Subject enrolled in research study)	1
tenofovir / emtricitabine / atazanavir / ritonavir <u>verses</u> tenofovir / emtricitabine / elvitegravir / experimental boosting agent GS 9350 (Subjects enrolled in research study)	2
tenofovir / emtricitabine / efavirenz	6
<u>**Failing Subjects**</u>	
tenofovir / emtricitabine / efavirenz	1
tenofovir /darunavir / ritonavir / raltegravir	1
abacavir/lamivudine/darunavir/ritonavir/raltegravir	1
Heart Rate, beats/min	73 (66.5, 80)
Systolic Blood Pressure, mmHg	117 (111, 121.5)
Diastolic Blood Pressure, mmHg	70 (62.5, 75.5)
CD4 count, cells/mm³	336.5 (245.3, 372.3)
HIV Viral Load, copies/mL	19550 (9303, 50700)
Fasting Glucose, mg/dL	86.5 (75.8, 87.8)
High Density Lipoprotein, mg/dL	43 (36.5, 45.5)
Low Density Lipoprotein, mg/dL	100.0 (86.5, 123.5)
Triglyceride, mg/dL	84.0 (47.8, 128.0)
Total cholesterol, mg/dL	165.0 (138.3, 183.3)

˚Continuous values are presented as median with interquartile range in brackets (Q_1, Q_3).

Table 2. Autonomic Function Measures. Measures of autonomic function performed: Heart Rate Deep Breathing (HRDB), Valsalva Ratio (VR), and quantitative sudomotor axon reflex test. Non-parametric Wilcoxon Sign-Rank test conducted on paired samples.

Autonomic Function	Entry (Before Initiation of ART)	End of Study (After Initiation of ART)	p	Power
Cardiovagal Tests				
Heart Rate during Deep Paced Breathing (HR$_{DB}$) Difference, beats/min	16.35 (13.83, 25.10)	19.01 (12.10, 26.42)	0.57	**0.05**
Valsalva Heart Rate ratio	2.22 (1.60, 2.62)	1.96 (1.89, 2.33)	0.30	**0.30**
Spectral Analysis of Heart Rate Variability (Cardiovagal)				
Resting Low Frequency Power, ms^2/Hz	255.22 (48.05, 468.48)	207.02 (89.91, 378.56)	0.68	**0.14**
Resting High Frequency Power, ms^2/Hz	167.90 (114.70, 457.2)	243.00 (121.95, 401.88)	0.62	**0.07**
Tilt Low Frequency Power, ms^2/Hz	155.77 (78.90, 238.68)	244.96 (59.99, 335.20)	0.07	**0.46**
Tilt High Frequency Power, ms^2/Hz	66.79 (40.14, 106.14)	118.00 (37.75, 167.18)	0.06	**0.33**
Adrenergic Tests				
Spectral Analysis of blood pressure variability (Adrenergic)				
Resting Low Frequency Power, mmHg^2/Hz	0.84 (0.30, 1.57)	0.27 (0.17, 1.16)	0.62	**0.06**
Resting High Frequency Power, mmHg^2/Hz	0.71 (0.47, 0.97)	0.63 (0.15, 1.79)	0.97	**0.10**
Tilt Low Frequency Power, mmHg^2/Hz	3.27 (1.02, 5.58)	3.07 (1.89, 6.62)	0.85	**0.05**
Tilt High Frequency Power, mmHg^2/Hz	1.21 (0.66, 3.33)	1.38 (0.80, 3.78)	0.77	**0.05**
Quantitative Sudomotor Axon Reflex Test (QSART)				
Forearm, nanoliters/10 minutes	0.96 (0.45, 1.80)	1.98 (0.40, 2.42)	0.15	**0.95**
Proximal Leg, nanoliters/10 minutes	0.70 (0.51, 1.15)	1.17 (0.48, 1.96)	0.64	**0.95**
Distal Leg, nanoliters/10 minutes	1.08 (0.28, 1.72)	2.16 (0.66, 2.26)	0.03	**0.95**
Foot, nanoliters/10 minutes	0.90 (0.07, 1.20)	0.67 (0.50, 0.97)	0.90	**0.95**

˚Continuous values are presented as median with interquartile range in brackets (Q_1, Q_3).

Table 3. Spearman's Correlation Coefficient Values. Correlation between change in autonomic function and change in CD4 and viral load levels.

Change in Autonomic Function	Change in CD4	Change in Viral Load	Change in Log of Viral Load
Quantitative Sudomotor Axon Reflex Test (QSART)			
Foot Volume	0.045 (0.89)	-0.036 (0.92)	0.082 (0.81)
Distal Leg Volume	0.427 (0.19)	-0.355 (0.29)	0.424 (0.19)
Proximal Leg Volume	-0.115 (0.75)	-0.552 (0.10)	0.553 (0.10)
Arm Volume	-0.309 (0.36)	-0.009 (0.98)	0.096 (0.78)
Total QSART Volume	0.079 (0.83)	-0.467 (0.17)	0.571 (0.08)
Cardiovagal Tests			
Heart Rate Deep Breathing Average Difference	0.436 (0.18)	-0.300 (0.37)	0.383 (0.25)
Valsalva Ratio	0.509 (0.11)	0.382 (0.25)	-0.419 (0.20)
Spectral Analysis of Heart Rate Variability (Cardiovagal)			
Resting Low Frequency Power, ms^2/Hz	-0.009 (0.98)	0.591 (0.06)	-0.629 (0.05)
Resting High Frequency Power, ms^2/Hz	-0.082 (0.81)	0.255 (0.45)	-0.214 (0.53)
Tilt Low Frequency Power, ms^2/Hz	-0.664 (0.03)*	0.482 (0.13)	-0.419 (0.20)
Tilt High Frequency Power, ms^2/Hz,	-0.618 (0.04)*	0.164 (0.63)	-0.077 (0.82)
Adrenergic Tests			
Spectral Analysis of blood pressure variability (Adrenergic)			
Resting Low Frequency Power, mmHg^2/Hz	0.300 (0.37)	0.527 (0.10)	-0.451 (0.16)
Resting High Frequency Power, mmHg^2/Hz	0.200 (0.56)	0.492 (0.13)	-0.433 (0.18)
Tilt Low Frequency Power, mmHg^2/Hz	0.027 (0.94)	0.164 (0.63)	-0.214 (0.53)
Tilt High Frequency Power, mmHg^2/Hz	0.591 (0.06)	-0.409 (0.21)	0.433 (0.18)

* indicates correlation with p<0.05.

Discussion

This prospective study is the first study to examine the effect of initiating ART on autonomic function in early HIV infection. All the subjects in our study achieved a virologic response of being undetectable 4 months after initiating ART. There were no significant differences in the cardiovagal, adrenergic, or sudomotor autonomic tests in subjects before and after initiating ART. There were no correlations between the autonomic function tests and baseline CD4 count and HIV viral load. The correlations between CD4 count and high and low power spectral analysis of the tilt test could potentially be chance findings since no other measures of autonomic function supported these findings.

HIV is thought to alter sympathovagal balance, resulting in increased sympathetic tone. The exact mechanism by which HIV modulates autonomic function remains unknown but may involve HIV induced changes in the brain responsible for ANS function. HIV has a predilection for the central nervous system and localizes in high concentration in the hippocampus, basal ganglia, and other regions involved in hypothalamic regulation [22]. Intraventricular injection of gp120 HIV envelope protein in rats impaired function of the suprachiasmatic nucleus within the hypothalamus. Injury to the suprachiasmatic nucleus has been associated with increased sympathetic modulation [23]. Autonomic dysfunction is associated with HIV-infection and has been correlated with the severity of HIV disease progression [4, 24, 25]. Antiretroviral therapy (ART) may improve autonomic function in individuals with acquired immunodeficiency syndrome (AIDS). Individuals with AIDS receiving ART have better measures of autonomic function compared to individuals with AIDS who are not taking ART [26]. Our findings do not reveal any autonomic dysfunction in HIV infected individuals who are asymptomatic and are in their early stages of their HIV infection. Our study cohort had few comorbidities and did not have advanced HIV/AIDS as compared to the other studies. The duration of their HIV infection was within 6 years, suggesting the impact of HIV infection on the cardiovascular system is limited in early infection. Additionally, the majority of subjects were ART naïve, which removed the influence of antiretroviral medications on the cardiovascular and neurologic systems. These study findings were different from our earlier study, which found autonomic dysfunction in older HIV infected individuals who received at least 4 years of ART.

The study results suggest that ART does not contribute to short-term changes in autonomic function. Additional longitudinal measurement of autonomic function will be needed to determine if the findings of autonomic dysfunction in prior studies is due to advanced disease, complications from older ART regimens or from a combination of both. The link between ART and the autonomic nervous system has been demonstrated in the scientific literature. Impaired response to ART has been reported in HIV infected individuals with high ANS activity [27]. Sympathetic neurons terminate in the parenchyma of all primary and secondary lymphoid organs and release norepinephrine into T cell rich compartments [28, 29]. Norepinephrine binds to β_2 adrenoreceptors and results in leukocyte activation, localization and cytokine production via $G_{\alpha s}$ protein-mediated induction of cAMP/protein kinase A (PKA) signaling [27, 30]. Norepinephrine stimulation thereby alters lymphocyte function by reducing cellular activation, suppressing HIV-modulating cytokines, and altering cell traffic and adhesion, and cytotoxic activity [27]. Neurotransmitters, particularly norepinephrine, can accelerate HIV replication in vitro [27, 30]. This suggests that neural activity may directly promote residual viral replication by chemokine receptor up regulation and enhanced viral gene expression [27, 30]. HIV may remodel systemic host function such as autonomic balance to promote its viral replication [22]. Conversely, products of inflammation such as TNF and interleukin-1 have been shown to have influence on autonomic nervous activity by central and/or peripheral mechanisms [31, 32]. Cardiac autonomic dysfunction and increased inflammatory activity potentially leads to a "vicious cycle" whereby both lead to endothelial dysfunction and atherosclerosis via positive feedback mechanism. Our preliminary study did not analyze markers of inflammation. As per our eligibility criteria, none of our subjects had a CD4 nadir less than 200 cell/mm^3. This may have excluded individuals with active inflammation from participating in our study and thus may have led to an attenuation of association between ART and autonomic dysfunction.

While our study was limited by small sample size, our point estimates did not indicate any trend towards sympathovagal imbalance in this relatively healthy HIV-infected population. Serum for immunologic and inflammatory markers has been stored and may provide a mechanism of autonomic dysfunction. The nonparametric Wilcoxon rank sum test used in the analysis may lack power as compared with more traditional parametric approaches. However, this nonparametric method was simple to carry out and required very limited assumptions to be

made about the format of the data, especially given the small sample size. Post-test power analysis showed that the lack of difference found in the QSART test was not due to a type II error and is not significant. The sample size for this study produces a power of .92 for the non-significant results found in the HR_{DB} average difference given the effect size shown in this study. For the results in the Valsalva ratio, the chosen sample size produces a power of .70 with a p value of .30. The lack of significant findings in these parameters could still be due to a lack of statistical power; however, smaller differences than those reported in this study would not be clinically significant. The correlations found between tilt test spectral analysis and CD4 count will further investigated in the final study.

This study found no appreciable differences of ART on the autonomic nervous system when ART is initiated early in the course of disease. The findings suggest that ART may not contribute to short-term changes in autonomic function in healthy subjects early in their disease course. The lack of significant differences before and after initiating ART may be due to excluding people who were more susceptible to autonomic dysfunction, limited exposure to ART, small sample size or a combination of all these factors. More studies are needed to increase our understanding of the impact of ART on susceptible individuals with comorbidities such as chronic obstructive pulmonary disease, asthma, diabetes and cardiovascular disease.

Acknowledgements

Competing Interests

The authors have declared that no competing interest exists.

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