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Original Research Article

Effect of antiretroviral therapy on progression of endothelial dysfunction in acquired immune deficiency syndrome patients

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ABSTRACT

Background: Though the natural history of Human Immunodeficiency Virus (HIV) infection in many patients has been dramatically altered through the use of antiretroviral therapy, this treatment paradigm of chronic chemotherapy may not be sustainable given the short and long-term toxicities of these medications. Currently, there is lack of data in the Indian literature regarding study of endothelial dysfunction in HIV patients. The purpose of our research was to study the effect of anti-retroviral therapy on the progression of endothelial dysfunction in HIV/AIDS patients.
Methods: The study comprises a total number of 30 adult HIV positive patients of both sex with confirmed HIV seropositivity and CD 4+T cell count < 200/µl. None of these patients had ever received anti retroviral therapy (ART). These patients were subjected to detailed clinical examination and markers of endothelial dysfunction - Flow mediated vasodilatation (FMD) of brachial artery, S. Nitrite and C-reactive protein (CRP) were performed before starting ART. Study group patients were started on ART, they received triple drug ART (Lamivudine 150 mg BD, Stavudine 30 mg BD, Nevirapine 200 mg BD). In patients on ART after a period of 6 months, markers of endothelial dysfunction - FMD, S. Nitrite and CRP tests were reassessed.
Results: FMD (4.08±3.58) and S. Nitrite (20.83±13.75) were also depressed after six months of anti retroviral therapy. Also, patients showed more CRP positivity and higher titres after ART. CD 4+T cell count before ART (124.16±84.46) and after ART (186.63±70.96). This rise in count was statistically significant.
Conclusions: HIV patients who are receiving ART demonstrate a number of metabolic abnormalities with more severe depression in markers of endothelial function.

Keywords: AIDS, Antiretroviral therapy, Endothelial dysfunction

INTRODUCTION

Since AIDS was first diagnosed in 1981, HIV/AIDS has become a global pandemic. As on 2017, approximately 36.9 million people are suffering with HIV globally, with 43% being women. There were about 940000 deaths from AIDS alone in 2018. Since the start of the epidemic, an estimated 77.3 million people have become infected with HIV and 35.4 million people have died of AIDS related illnesses.[1] Though the natural history of HIV infection in many patients has been dramatically altered through the use of antiretroviral therapy, this treatment paradigm of chronic chemotherapy may not be sustainable given the short and long-term toxicities of these medications. Among the more concerning are the development of atherogenic lipoprotein abnormalities and a state of relative insulin resistance. Logically, there has been concern that such metabolic disturbances may

predispose towards cardiovascular morbidity and mortality.[2] Although these therapies have only been in widespread use for a short period of time, a growing body of evidence from case reports and cohort studies has suggested that HIV-infected patients on ART my experience premature coronary artery disease.[3] The explanation of this phenomenon is most likely multifactorial such as 1) Direct effect of HIV-1 on the endothelium, 2) The virus might have some capacity to effect the production of nitric oxide as a pathogenic mechanism. 3) Drug-induced abnormalities in lipid and/or glucose metabolism.[4] HIV-infected individual with a CD4 + T lymphocyte of <200/µl had AIDS by definition, regardless of the presence of symptoms or opportunistic diseases.[5]

Endothelial dysfunction is defined as impairment of normal function of endothelium caused by injurious stimuli resulting in an imbalance between vasoconstrictors and vasodilators, pro and anti-coagulants, growth promoters and growth inhibitors. HIV patients have regulatory mechanisms that exist to maintain normal functioning of the endothelium disturbed. In vitro studies have also shown that interleukin 2 secretion, which is important for stimulation of T-cell responses, is diminished in HIV-exposed endothelium. Increased adherence of leucocytes, mostly of the monocyte/macrophage lineage, were observed in the endothelium. Endothelial dysfunction is prevalent in HIV patients as evidenced by decreased serum nitrite, impaired FMD and raised CRP levels.[6]

The syndrome of lipodystrophy has also been observed with Protease Inhibitors-sparing regimens.[7] The exact role of Nucleoside analog reverse transcriptase inhibitors (NRTIs) in the pathogenesis of lipodystrophy syndrome, however, remains unclear. Mitochondrial toxicity seems to play an important part in the development of this syndrome.[8]

Mitochondrial DNA cannot be repaired if mitochondrial polymerase gamma is inhibited. The NRTIs have been shown to inhibit this enzyme resulting in mitochondrial damage, which may eventually lead to the disruption of lipid storage, transport and glucose uptake by adipocytes, which might lead to lactic acidosis. A long-term follow up of various combinations of NRTIs revealed an overall prevalence of lipodystrophy of 35% at 2.5 years, but twice of this among those taking stavudine and didanosine together.[9]

Aim of the present endeavor was to study the effect of anti-retroviral therapy [NRTI-NNRTI (Nonnucleoside reverse transcriptase inhibitor) regimen] on the progression of endothelial dysfunction in AIDS patients.

METHODS

The study was conducted in the Department of Medicine, Delhi. A written informed consent was taken from all the patients. Appropriate approval of the Ethics Committee was also taken. 30 adult patients of either sex, with confirmed HIV seropositivity, registered in the ART clinic, Lok Nayak Hospital, were screened for entry into the study using the following inclusion and exclusion criteria.

Inclusion criteria

Adults with confirmed HIV infection by two different ELISA/Rapid/Simple (ERS) with different antigen preparations and CD4+T cell count <200/µl.

Exclusion criteria

- Children and adolescents,
- Heart failure,
- Coronary artery disease (CAD),
- Smokers,
- Hypertension and diabetes mellitus patients

None of these patients had ever received anti retroviral therapy.

The patients were evaluated by detailed history and clinical examination. Before ART and After ART, the following investigations were performed on all the Patients - CD 4 cell counts, Tests for endothelial dysfunction: Flow Mediated Dilatation (FMD) of the brachial artery, Serum Nitrite and CRP.

The study group patients with CD4+T cell count <200/µl were started on triple drug ART for a period of six months. This consisted of the NRTIs - Lamivudine 150 mg BD and Stavudine 30 mg BD, NNRTI - Nevirapine 200 mg BD.

CD 4 cell count was measured by flow cytometry using the FACS count instrument in analysed whole blood collected under aseptic precautions, Manufactured by Becton Dickinson Immunocytometry Systems, California.

FMD is a noninvasive method to evaluate endothelial function that uses postischemic (forearm) vasodilatation, causing enhanced flow in the brachial artery and consequently a shear stress-induced vasodilatation. Endothelial function was evaluated in all patients by measuring flow-mediated vasodilatation (FMD) of the brachial artery. Vasodilatation responses of the brachial arteries were measured by ultrasound technique with the help of skillful radiologist.

Serum nitrite was assayed using Griess reagent and microplate reader absorbance.

Serum CRP was assayed using the Rhelax CRP kit (manufactured by Tulip Diagnostic Pvt Ltd) based on the principle of agglutination. If CRP concentration is greater

than 0.6 mg/dl a visible agglutination reaction is observed.

Statistical analysis

The data was statistically analysed by t-test. P <0.05 was considered significant.

RESULTS

Study group patients with CD 4+ T cell count <200/µl were followed-up and after a period of six months, CD 4 counts and markers of endothelial function were repeated. During this period, patients were on anti retroviral therapy consisting of two nucleoside analogue reverse transcriptase inhibitors and one nonnucleoside reverse transcriptase inhibitor.

Table 1: Effect of ART (anti retroviral therapy) on CD 4+T cell count.

Test	Before ART (Mean±SD)	After ART (Mean± SD)	P Value
CD4+T cell count (/µl)	124.16 ±84.46	186.63 ±70.96	0.001

As given in the Table 1, there was significant difference in the CD 4+T cell count, six months after anti retroviral therapy. This rise in CD 4+T cell count was statistically significant (p=0.001).

FMD after anti retroviral therapy

FMD was performed in patients after six months. As shown in Table 2, there was no significant difference in the brachial artery diameter before and after anti retroviral therapy.

It was also noted that the brachial artery response to sublingual nitroglycerine (NID-Nitrate Induced Vasodilatation) was not significant. However, flow mediated dilatation was impaired after ART which was statistically significant (p=0.001).

Table 2: Effect of ART (anti retroviral therapy) on brachial artery diameter.

Flow-mediated dilatation	Before ART (Mean±SD)	After ART (Mean±SD)	P Value
Baseline Brachial A. diameter (mm)	3.45 ±0.472	3.37 ±0.355	0.557
FMD (% of increase over baseline)	7.07 ±2.89	4.08 ±3.58	0.001*
NID (% of increase over baseline)	16.37 ±6.74	15.89 ±6.35	0.869

* = significant

Serum nitrite after anti retroviral therapy

As given in the Table 3 there was significant statistical difference in levels of Serum Nitrite before and 6 months after anti retroviral therapy (p=0.001). Patients showed decrease in levels of S. nitrite after ART (with mean± SD of 20.83±13.75 - after ART compared to 26.43±15.38- before ART).

Table 3: Effect of ART (anti retroviral therapy) on serum nitrite.

Test	Before ART (Mean±SD)	After ART (Mean± SD)	P Value
S. Nitrite (µmol/L)	26.43±15.38	20.83±13.75	0.001

C-reactive protein after anti retroviral therapy

After six months of antiretro viral therapy, sera of the patients were again subjected to detection and later quantification for the acute phase reactant, C-reactive protein, using latex agglutination method.

Table 4: Effect of ART (anti retroviral therapy) on serum C-reactive protein.

Study group	Qualitative assay	Quantitative assay (serum dilutions)				
		1:2	1:4	1:8	1:16	1:32
	0.6 mg/dl	1.2 mg/dl	2.4 mg/dl	4.8 mg/dl	9.6 mg/dl	19.2 mg/dl
Pre-ART	19/30	4	3	6	3	0
Post-ART	20/30	4	6	5	3	1

Table 4 depicts the CRP profiles of the patients with CD 4 counts <200 µl, before and after initiation of anti retroviral therapy. As given in the table below, 19 of the 30 patients (63.3%) showed positive CRP levels before initiation of therapy. Six months after starting anti

retroviral therapy, 20 of the 30 patients (66.6%) had then demonstrated CRP positivity.

DISCUSSION

Anti retroviral therapy has dramatically improved the life expectancy of HIV patients, prompting increasing concerns related to chronic disease issues. Regimens containing combination of drugs are associated with unexpected metabolic complications such as lipodystrophy, dyslipidemia and few reports of premature coronary artery disease. Much of the western literature regarding the effects of ART on endothelial function has been concerned with the use of regimens containing protease inhibitors (PIs).[10] However, these complications are not exclusively associated with PI administration. Therapy with NRTI and NNRTIs has also been associated with the development of endothelial dysfunction, although abnormalities of plasma lipid levels, insulin resistance and lipodystrophy are more prevalent among patients receiving a PI-based regimen. After six months of ART, authors found that patients showed progression in endothelial dysfunction as measured by various endothelial markers. This is significant, considering the fact that the study population was free from cardiovascular disease and its risk factors like smoking, hypertension and diabetes mellitus.

Before ART CD4+T cell count was 124.16±84.46, after 6 months of ART the CD4+T cell count was 186.63±70.96. This rise in CD4+T cell count was statistically significant (Table 1). FMD of brachial artery was measured six months after initiation of ART in patients with CD 4 count <200/µl. Flow mediated dilatation was impaired after ART which was statistically significant. There was a fall in FMD from a mean of 7.07% of baseline brachial diameter before therapy to 4.08% after anti retroviral therapy indicating progression of endothelial dysfunction (Table 2). Bonnet et al, demonstrated that HIV-infected children had a vascular dysfunction as shown by impaired FMD of brachial artery. Endothelium-dependent dilatation was lower in HIV-infected children but non-endothelium dependent dilatation was similar to that observed in controls. They did not find differences for any of the vascular variables between HIV-infected children receiving ART and those never treated. All arterial variables were similar in children with and without dyslipidemia.[11]

Serum nitrite was again measured in patients with CD 4 count <200/µl at a period of six months from starting ART. A statistically significant difference in levels of S. Nitrite was observed. The mean S. Nitrite declined to 20.83 µmol/L after six months representing impaired endothelial function (Table 3). Sarman et al, studying endothelial dysfunction have found significantly lower plasma total nitrite + nitrate level in hypertensive type 2 diabetic patients compared to normotensive diabetic patients.[12] CRP was also measured after six months in the study group patients. 20 of the 30 patients (66.6%) had demonstrated CRP positivity after this interval, as compared to 19 patients (63.3%), before initiation of ART. Six months after starting treatment, patients also demonstrated higher CRP levels with titres ranging from 1.2 mg/dl to 19.2 mg/dl. 50% of the patients (15 out of 30) shower CRP titres >2.3 mg/dl (Table 4) depicting progression of endothelial dysfunction. Present study findings are consistent with those of the studies mentioned later, in which HIV infected patients shower higher levels of CRP. The highest category of CRP (>2.3 mg/dl) was associated with a faster progression of HIV to AIDS, independent of CD4 lymphocyte count and HIV RNA level. It was also found that CRP levels increased significantly over time in both individuals who did and did not progress to AIDS. This study indicates that CRP may provide additional prognostic information when used with CD4 lymphocyte counts and HIV RNA levels and could have potential cardiovascular implications in the association of CRP and cardiovascular disease remains in the presence of a chronic infection like HIV.[13] Hadigan et al, also reported that concentration of surrogate markers like CRP, tissue plasminogen activator and tissue plasminogen activator inhibitor-1 are increased in patients with HIV and metabolic abnormalities.[14]

CONCLUSION

Treatment with NRTI-NNRTI is associated with endothelial dysfunction. FMD was decreased in patients with CD4+T cell count <200 before and after ART, suggesting that there is progression of endothelial dysfunction. HIV patients who are receiving ART demonstrate a number of metabolic abnormalities with more severe depression in markers of endothelial function. Excess inflammation as denoted by raised CRP levels may contribute to increased cardiovascular risk in these patients.

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