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Original Research Article

Prevalence of endothelial dysfunction in acquired immune deficiency syndrome patients and its correlation with degree of immunodeficiency

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ABSTRACT

Background: Number of factors play a role in endothelial dysfunction observed in AIDS patients, which can lead to atherosclerosis along with cardiovascular mortality and morbidity. Human immunodeficiency virus (HIV), the etiologic agent of AIDS causes several vascular disorders characterized by an evident activation and perturbation of endothelial cells. 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 prevalence of endothelial dysfunction in HIV/AIDS patients.
Methods: The study comprises a total number of 60 adult HIV positive patients of both sex (male and female) with confirmed HIV seropositivity. The patients were divided into two groups of 30 each, depending on the degree of immune dysfunction (CD 4 cell counts). Group I- patients with CD 4+T cell count>200/µl and group II-patients with CD 4+T cell count<200/µl. 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.
Results: The defect in endothelial function was most prevalent in patients with more severe immunosuppression. FMD of brachial artery was decreased in patients with CD 4+T cell count < 200/µl (7.07±2.89, p=0.00). S. nitrite was also significantly lower in group II patients (26.43±15.38), and these patients also showed more CRP positivity and higher CRP titres ranging from 1.2 mg/dl to 9.6 mg/dl.
Conclusions: The defect in endothelial function was most prevalent in patients with more severe immunosuppression. FMD of brachial artery was decreased in patients with CD 4+T cell count <200/µl (7.07±2.89, p=0.00). S. nitrite was also significantly lower in group II patients (26.43±15.38), and these patients also showed more CRP positivity and higher CRP titres ranging from 1.2 mg/dl to 9.6 mg/dl.

Keywords: AIDS, Endothelial dysfunction, Flow mediated vasodilatation, HIV

INTRODUCTION

HIV infection is characterized by an acquired and profound depression in cell mediated immunity resulting in a wide range of opportunistic infections and unusual neoplasm. The endothelium is a silent target of HIV-1, and infection of persons with HIV-1 results in a diffuse vascular process that is frequently not recognized. Endothelium directly or indirectly affected by HIV-1 may also contribute to the pathogenesis and expression of many common HIV-linked diseases such as cardiovascular disorders. The endothelium is the cell layer that lines blood vessels. For many years, it was believed to be a semipermeable barrier between the blood

and the interstitium, facilitating the exchange of water and small molecules. But recent experiments have shown that the endothelium also participates in metabolic, synthetic and regulatory pathways.[1] The main effect of stimulating the endothelium is vasodilatation in healthy people, and the local control of the vasculature depends on a balance between dilators and constrictors.[2,3] A healthy endothelium is also antiatherogenic, as it is able to inhibit platelet aggregation and adhesion, smooth muscle cell proliferation and leucocyte adhesion.[3]

Endothelial dysfunction is defined as an imbalance between vasoconstrictor and dilators, pro and anticoagulants, growth promoting and growth inhibitory factors, pro and antiatherogenic factors. Broadly it can be defined as an impairment in any of the normal functions of the endothelium caused by an injurious stimulus.

Widespread organ damage seen in HIV-infected patients appears to be in part, the result of transmission of HIV-infected cells across the endothelium, usually without actual infection of endothelial cells themselves, although whether endothelial cells can be infected by HIV is still open to debate. Under normal circumstances, the endothelial cell barrier plays a critical role in the protection of tissues, but in HIV-infected patients this barrier fails, permitting exposure to infections and other sources of damage. HIV patients have regulatory mechanisms that exist to maintain normal functioning of the endothelium disturbed. This leads to an increase in concentrations of various markers in the serum, such as Von willebrand factor (vWF), soluble thrombomodulin (sTM), angiotensin converting enzyme (ACE), E-selectin (ELAM-1) and endothelin.[4] A positive correlation between disease progression and vWF was shown in HIV positive patients, and this is believed to be a possible predisposing factor to thrombus formation.[5]

Brachial artery FMD is an extensively used noninvasive method to evaluate endothelial function first described by Celermajer.[6,7] Nitric oxide has a short biological half-life and is rapidly converted into stable metabolites, nitrite and nitrate.[8] Sarman B et al, studying endothelial dysfunction have found significantly lower plasma total nitrite+ nitrate level in hypertensives.[9] C-reactive protein (CRP) is an acute-phase protein produced by the liver in response to inflammation and infection. Lau B et al, reported in a study that elevated levels of CRP predict HIV disease progression, independent of CD4 T cell count and HIV RNA level.[10] It was 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.[11] Log (CRP) correlates with vWF and VCAM-1, the established markers of endothelial injury. Levels of CRP correlates with blunted vasodilator response to acetylcholine.[12]

The aim of the study was based on the study of the prevalence of endothelial dysfunction in patients with HIV/AIDS and its correlation with degree of immunodeficiency (CD 4 counts).

METHODS

The study was conducted in the department of medicine, Lok Nayak Hospital and department of radiodiagnosis, G.B. Pant hospital, Delhi, India. A written informed consent was taken from all the patients. Appropriate approval of the ethics committee was also taken. 60 adult patients of either sex, with confirmed HIV seropositivity, registered in the ART clinic, from June 2008 to May 2009, 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.

Exclusion criteria

Children and adolescents, heart failure, coronary artery disease (CAD), smokers, hypertension and diabetes mellitus patients were excluded.

A total number of 60 (sixty) patients with confirmed HIV serology were included in the study. These patients were divided into two groups, with 30 patients in each group, depending on the degree of immunodeficiency (CD 4 cell counts). None of these patients had ever received anti-retroviral therapy.

Group I: 30 patients with CD 4 >200 cells/µl and group II: 30 patients with CD 4<200 cells/µl (severe immune suppression).

The patients were evaluated by detailed history and clinical examination. 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.

CD 4 cell count was measured by flow cytometry using the FACS count instrument in unlysed 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. This technique is widely used, reliable and reproducible. 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.

The collected information was statistically analyzed by chi-square test using Statistica-99 software.

RESULTS

CD 4 cell counts in subjects

CD4 cell counts were estimated by flow cytometry in all patients and depicted in the (Table 1) below. CD4 cell counts were lower in group II (mean of 124.16±84.46) as compared to group I (mean 457.00±164.93) and the difference was statistically significant (p=0.000).

Table 1: CD 4 cell counts in subjects.

Study group	CD4+T cell count Mean±SD (cells/µl)
Total HIV patients (n=60)	290.58±208.50
Group I (patients with CD4 + T count > 200/µl) (n=30)	457.00±164.93
Group II (patients with CD4 + T count < 200/µl) (n=30)	124.16±84.46

Table 2: Flow-mediated dilatation as a marker of endothelial function.

	Group I CD4 + T count > 200/µl) (Mean±SD)	Group II CD4 + T count < 200/µl) (Mean±SD)	P value
Baseline brachial A. diameter (mm)	3.39±0.46	3.44±0.47	0.732
FMD (% of increase over baseline)	13.86±8.21	7.07±2.89	0.000
NID (% of increase over baseline)	16.52±9.23	16.37±6.75	0.944

Tests for endothelial function

Brachial artery FMD (endothelium dependent vasodilatation) was assessed in both the groups of patients by calculating the percentage change from the brachial artery diameter at the rest. Endothelium independent vasodilatation was also calculated by looking at the percentage change in diameter after giving sublingual nitroglycerine (nitrate induced vasodilatation-NID). There was no significant difference in the baseline brachial artery diameter in the subjects (p=0.732). It was also noted that the response of artery to sublingual nitroglycerine (NID) in the two groups demonstrated no significant difference (p=0.944). But flow mediated dilatation was significantly impaired in Group II (patients with CD 4+T cell count <200/µl) with FMD of 7.07±2.89 as compared to 13.86 ± 8.21 in group I. This was statistically significant (p=0.000) (Table 2).

Serum nitrite as a marker of endothelial function

As given in the (Table 3), there was significant statistical difference in levels of serum nitrite in the two groups of HIV patients (p=0.000), with patients in group II showing depressed S nitrite levels.

Table 3: Serum nitrite in both groups.

	Group I CD4 + T count >200µ/l) (Mean±SD)	Group II CD4 = T count <200/µl) (Mean±SD)	P value
S. Nitrite (µmol/L)	51.03±18.07	26.43±15.38	0.000

C-reactive protein as a marker of endothelial function

(Table 4) depicts the CRP profiles of the patients with different levels of CD 4 counts. Sera of the patients positive in the qualitative method were then quantified by subjecting them to serial dilutions. When serum was analyzed, 11 of the 30 (36.6%) patients with CD 4 count >200/µl were positive, while 19 of the 30 (63.3%) patients with CD4 <200 /µl demonstrated CRP positivity. The overall titres of the positive sera were higher in patients belonging to group II (CD4<200/µl) when

compared with patients belonging to group I (CD4+T cell count >200 cells/µl).

Table 4: Serum C-reactive protein in both groups.

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	19.2MG/DL
Group I CD4 >200/µL	11/30	3	2	1	0	0
Group II CD4<200 /µL	19/30	4	3	6	3	0

DISCUSSION

Since it was first diagnosed in 1981, HIV/AIDS has become a global pandemic, with cases reported from every country. In India HIV/AIDS epidemics are no longer localized within high risk groups and now the virus is spreading from high risk groups to general population and from urban to rural areas. More than 25 million people have died of AIDS since 1981. FMD can be very well used as a tool for noninvasive assessment of endothelial dysfunction and also as a predictor of future risk of atherosclerosis.[13-15] In present study we used FMD as a marker of endothelium dysfunction to show the early endothelium dysfunction in HIV-infected patients at varying stages of immune dysfunction (Table 2).

Various markers have been used in the study of endothelial dysfunction. One study has used fasting glucose, insulin and lipid levels and anthropometric measurements to determine the prevalence of endothelial dysfunction in HIV patients by comparing them with healthy controls.[11] Another study assessed vascular function by measuring endothelium-dependent and endothelium-independent dilation of brachial artery in HIV infected patients.[16] Other studies have also demonstrated raised plasma levels of endothelial markers such as von adhesion molecule-1 (VCAM-1) in HIV infected patients.[17] In present study, authors showed that HIV infected patients have an endothelial dysfunction which may be an early step in the process of atherosclerosis. This defect in endothelial function was most prevalent in patients with more severe immunosupression group II patients 7.07±2.89 (Table 2). Bonnet D et al, demonstrated that HIV-infected children had a vascular dysfunction as shown by impaired FMD of brachial artery.[16]

Patients in group II showed depressed levels of S. nitrite (26.43±15.38) as compared to those in group I (51.03±18.07). The nitrite levels in serum further supports the existence of endothelial dysfunction in HIV patients (Table 3). No much literature is available regarding measurement of S. nitrite as a marker of endothelial function in HIV/AIDS patients.

Group II patients showed higher levels of CRP with titres ranging from 1.2 mg/dl to 9.6 mg/dl and 12 of the patients (40%) showed CRP values >2.3 mg/dl. Only 10% of patients of group I had CRP values >2.3 mg/dl (Table 4). Our findings are consistent with those of the studies, in which HIV infected patients showed higher levels of CRP when compared with normal population. Dolan SE et al, reported that HIV-infected women demonstrated higher CRP.[18] Hadigan C et al, also reported that concentration of surrogate markers like C-reactive protein, tissue plasminogen activator and tissue plasminogen activator inhibitor-1 are increased in patients with HIV and metabolic abnormalities.[11]

CONCLUSION

The endothelial function showed a progressive decline as the levels of immune function decline. The patients, although at present free from cardiovascular events, had an impaired FMD implying already established preatherosclerotic changes. Endothelial dysfunction is prevalent in HIV patients as evidenced by decreased serum nitrite, impaired FMD and raised CRP levels.

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