

Renal Function among HIV Infected Patients on Combination Antiretroviral Therapy: A Longitudinal Cohort Study

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ABSTRACT

Introduction: A large number of Human Immunodeficiency Virus (HIV) infected patients are taking combination Antiretroviral Therapy (cART) worldwide as it has led to dramatic improvements in them with a decreased viral load as well as an increase in Cluster of Differentiation (CD4+) T cell count. Though the incidence of HIV associated Chronic Kidney Disease (CKD) has decreased with the use of effective cART, the prevalence of End Stage Renal Disease (ESRD) in HIV positive patients has increased due to the risen longevity owing to them.

Aim: To study the renal function abnormalities in HIV infected patients and to compare the change in renal function of treatment naïve patients with patients on triple drug regimen (cART).

Materials and Methods: This prospective longitudinal cohort study was conducted on 54 Enzyme Linked Immunosorbent Assay (ELISA) positive HIV patients belonging to the age group of 18-70 years of both the genders over a period of two years from August 2017 to September 2019 in MKCG Medical College and Hospital, Berhampur, Odisha, India. Forty nine HIV infected patients naïve to cART and five patients on cART for a minimum period of three months were included in this study. All patients were treated with triple therapy regimens of either ZLN (Zidovudine 300 mg+Lamivudine 150 mg+Nevirapine 200 mg) or TLE (Tenofovir 300 mg+Lamivudine 150 mg+Efavirenz 600 mg) daily; in a single dose at bed time. Renal function parameters like serum urea, serum creatinine, Creatinine Clearance (CrCl), estimated Glomerular Filtration Rate (eGFR) and CD4+ T cell count of treatment naïve patients were compared

with the same patients on cART after six months duration. GFR was calculated by Modification of Diet in Renal Disease (MDRD) equation. Results were analysed using the Statistical Package for the Social Sciences (SPSS) software for Windows Version 17.0.

Results: Out of 54 patients, 53.7% (n=29) were males and 46.3% (n=25) were females. The mean CrCl of HIV positive patients on cART (79.09±25.705 mL/min) was higher than treatment naïve (69.65±25.506 mL/min) patients and was highly significant (p-value=0.003). The mean eGFR of HIV positive patients on cART (102.711±26.9424 mL/min/1.73 m²) was higher than treatment naïve (90.189±28.2575 mL/min/1.73 m²) patients and was highly significant (p-value=0.003). The mean serum urea of HIV positive patients on cART (25.78±4.721 mg/dL) was lower than HIV positive treatment naïve (26.19±4.742 mg/dL) patients but was non-significant (p-value=0.640). The mean serum creatinine of HIV positive patients on cART (0.815±0.1393 mg/dL) was lower than HIV positive treatment naïve patients (0.906±0.1687 mg/dL) and was also highly significant (p-value=0.003). The mean CD4+ T cell count of HIV positive patients on cART (401.63±225.816 cells/μL) was higher than HIV positive treatment naïve (287.13±198.263 cells/μL) patients and was very highly significant (p=0.001).

Conclusion: Renal impairment (CrCl <60 mL/min) and eGFR (<60 mL/min/1.73 m²) were higher in HIV positive treatment naïve patients than those on cART. Radiological parameters like size of the kidney and cortical echogenicity became normal after six months on cART.

Keywords: Cluster of differentiation, Creatinine clearance, Estimated glomerular filtration rate, Highly active antiretroviral therapy, Human immunodeficiency virus

INTRODUCTION

Human Immunodeficiency Virus (HIV) and Acquired Immunodeficiency Syndrome (AIDS), is a serious global health issue in many developing and under developed countries including India, where opportunistic infections and poor medical facilities contribute to significant morbidity and mortality. India has reported its first case of AIDS in 1986. By the year 2006, it was estimated that there were around 5.6 million cases of HIV in the country, concentrated mainly in Maharashtra, Tamil Nadu, Karnataka, Andhra Pradesh, Manipur and Nagaland [1]. States of Odisha, Bihar, West Bengal, Uttar Pradesh, Rajasthan, Madhya Pradesh, Gujarat together account for 41% of new cases of AIDS and in Odisha, Ganjam district has the highest (38%) incidence in South Odisha [2]. India has a low HIV prevalence of 0.22%. Even with this low prevalence in terms of absolute numbers, India has the third highest burden of HIV in the world with an estimated 2.14 million people living with HIV, 87,000 new infections and 69,000 AIDS related deaths annually [1].

Infection with HIV has been associated with many types of renal diseases including acute renal failure, acute tubular necrosis and

HIV Associated Nephropathy (HIVAN) which ultimately may progress to ESRD [3,4]. HIV positive persons are also exposed to life long ART, with the possibility to cause or aggravate kidney injury. Newer guidelines recommending earlier initiation of ART may further reduce the incidence of HIVAN, but in general risk advantage for kidney health is unidentified [5]. Since the introduction of Highly Active Antiretroviral Therapy (HAART), also known as cART in 1995, patients with HIV infection have improved the quality of life, delayed the progression of AIDS and have been shown to reduce mortality and morbidity rates. The prevalence of HIVAN decreased with use of ART, but there remains near a four fold increased risk of kidney disease including CKD, in the people living with HIV compared with the general population [6,7]. CKD that may progress to ESRD requiring dialysis and renal transplant which can be diagnosed in its early stage through routine screening and careful attention to changes in renal functions [8]. Renal function is mostly assessed by GFR and CrCl. GFR is the flow rate of filtered fluid through the kidney. CrCl is the volume of blood plasma that is cleared of creatinine per unit time. Several antiretroviral agents are removed partially; by the kidneys

and dose adjustments is essential in patients with reduced GFR. Renal failure is defined as increased in serum creatinine level more than 30% of basal level, or blood urea nitrogen 20 mg/dL and serum creatinine level 1.3 mg/dL (in females) or 1.5 mg/dL (in males).

The aim of this study was to assess the renal function abnormalities in HIV infected patients and to compare the change in renal function of treatment naïve patients with those on cART.

MATERIALS AND METHODS

A prospective longitudinal cohort study was designed and conducted in the Departments of General Medicine and Radiodiagnosis in MKCG Medical College and Hospital, Berhampur, Odisha, India, over a period of two years from August 2017 to September 2019. The study was conducted after the study protocol was approved by the Institutional Ethics Committee (No 536/Chairman-IEC, MKCG Medical College, Brahmapur, Odisha, India). Informed consent was obtained from all the patients and study was done in accordance with the guidelines of the Declaration of Helsinki 2008.

A detailed medical history and clinical examination were obtained from each patient. A total number of 54 (49 treatment naïve+5 from ART centre) patients, diagnosed as HIV positive confirmed by positive ELISA test, belonging to the age group of 18-70 years of both genders, admitted to different wards of Medicine Department or patients attending ART centre of the hospital for routine clinical check up were taken for the study who fulfilled the inclusion exclusion criteria.

Inclusion criteria: HIV infected patients naïve to cART and HIV infected patients on cART for a minimum period of three months were included in this study.

Exclusion criteria: Patients known to have Acute Kidney Injury (AKI) or CKD, diabetes mellitus, hypertension, pregnancy, Hepatitis B surface Antigen (HBsAg) positive, Hepatitis C Virus antibody (HCV Ab) positive and patients taking nephrotic drugs were excluded from the study.

All the 49 treatment naïve patients were investigated with routine and specific laboratory tests including baseline investigations like Complete Blood Count (CBC), fasting plasma glucose, post prandial plasma glucose, glycated haemoglobin, lipid profile, liver function test, serum urea, serum creatinine, serum electrolytes, serum calcium, serum potassium, serum phosphorous, CD4+ T cell count, HBsAg, HCV Ab, urine for routine and microscopy, urine for microalbumin, Electrocardiogram (ECG) and chest X-ray. Ultrasonography of abdomen was done in the Department of Radiodiagnosis to see the kidney size, Corticomedullary Differentiation (CMD) and thus to rule out CKD and later to assess HIV Associated Nephropathy (HIVAN) with the findings of enlarged, echogenic kidneys. Height, weight, pulse, Blood Pressure (BP) and Body Mass Index (BMI) of all the patients were recorded. Five patients who met the clinical and laboratory criteria were also included in this study from the ART centre of the hospital after verification and review of the records. The same investigation reports were collected when they were treatment naïve.

Out of 54 HIV positive patients, 42 patients were treated with triple therapy regimen of tenofovir disoproxil fumarate 300 mg+lamivudine 150 mg+efavirenz 600 mg (TDF+3TC+EFV) and 12 patients were treated with zidovudine 300 mg+lamivudine 150 mg+nevirapine 200 mg (AZT+3TC+NVP). All patients were selected randomly and took the drug regimen at bedtime. All the investigations were repeated after six months of treatment on cART. As present cohort was a prospective study with internal comparisons, renal functions of 54 (49+5 from ART centre) treatment naïve patients were compared with the renal functions of the same 54 patients after six months on cART/HAART.

A pilot study was conducted in the ART centre in the hospital, where records of eight HIV positive patients on HAART were verified. Percent

of exposed with outcome was found to be 25 with an improvement in renal parameters in two patients after six months on HAART.

Sample size calculation: The sample size was calculated by using an online sample size calculator (Open Epi version 3.01) and assuming percentage of exposed with outcome to be 25%, at 5% precision and 95% level of confidence interval. The minimum required sample size was calculated to be 51 in each group.

Creatinine Clearance (CrCl) was calculated from the patients age, sex and serum creatinine according to the formula derived from Cockcroft-Gault equation [9,10]:

$$\text{CrCl} = \{140 - \text{age (years)}\} \times \text{weight in kg} / \text{serum creatinine} \times 72 \text{ (male)}$$

$$\text{CrCl} = \{140 - \text{age (years)}\} \times \text{weight in kg} \times 0.85 / \text{serum creatinine} \times 72 \text{ (female)}$$

GFR was estimated from serum creatinine according to the study equation of MDRD [11].

$$\text{GFR} = 186 \times (\text{SCr} - 1.154) \times (\text{Age} - 0.203) \times \{(0.742) \text{ if female}\} \text{ and } \{(1.210) \text{ if black/male}\}$$

This MDRD study equation calculator is for use with SCr reported in mg/dL. Hepatitis B (HBsAg), Hepatitis C (HCV Ab) serology tests was done using Enzyme-Immuno Assay (EIA) kits. CD4+ T cell count were determined by Fluorescence-Activated Cell Sorting (FACS) count version 1.00/08 medicine.

STATISTICAL ANALYSIS

Data were entered using Microsoft Excel and exported to SPSS version 17.0. Comparison of parameters was done using Analysis of Variance (ANOVA) and correlations between parameters were analysed using Pearson correlation analysis. Values were expressed as mean±SD/or proportion (percentage) with statistical significance set at p-value less than 0.05 (p<0.05) were considered statistically significant.

RESULTS

A total number of 54 HIV positive patients were included in the study, out of which 29 (53.7%) were males and 25 (46.3%) were females, of age ranges between 18-70 years with mean age of 39.83±10.73 years [Table/Fig-1].

Age group (in years)	No. of males N1	No. of females N2	Total no. of patients N1+N2	Percentage (%)
18-30	4	5	9	16.67
31-40	8	12	20	37.03
41-50	11	6	17	31.48
51-60	3	2	5	9.26
61-70	3	0	3	5.56
Total	29	25	54	100

Mean±SD (39.83±10.73)

[Table/Fig-1]: Age group and gender distribution of HIV positive patients in the study.

The mean BMI in study was (18.827±4.593 kg/m²). The mean of CD4+ cell count of HIV positive treatment naïve patients and on Highly Active Antiretroviral Therapy (HAART) was higher and was very highly significant (p=0.001) [Table/Fig-2].

Variable	Mean±SD		p-value
	HIV positive (Treatment-naïve) N=54	HIV positive (On HAART) N=54	
CD4+ (cells/μL)	287.13±198.263	401.63±225.816	0.001

[Table/Fig-2]: Mean change in CD4+ count after treatment.

(Analysis of variance, ANOVA: p-value <0.05: Significant)

The mean serum creatinine of HIV positive patients on HAART (0.815±0.1393 mg/dL) was lower than HIV positive treatment naïve patients (0.906±0.1687 mg/dL) and was also significant (p=0.003) [Table/Fig-3].

Variables	Mean±SD		p-value
	HIV positive (Treatment-naïve) N=54	HIV positive (On HAART) N=54	
Sr. Urea (mg/dL)	26.19±4.742	25.78±4.721	0.640
Sr. Creatinine (mg/dL)	0.906±0.1687	0.815±0.1393	0.003
CrCl (mL/min)	69.65±25.506	79.09±25.705	0.003
eGFR (mL/min/1.73 m ²)	90.189±28.2575	102.711±26.9424	0.003

[Table/Fig-3]: Comparison of renal function tests with treatment. (Analysis of variance, ANOVA: p-value <0.05; Significant); CrCl: Creatinine clearance; eGFR: Estimated glomerular filtration rate

There was a positive and non-significant correlation of eGFR with BMI. There was a positive and non-significant correlation of eGFR with CD4+ count [Table/Fig-4].

Variable		BMI	CD4+ Count
eGFR (mL/min/1.73 m ²)	r	0.190	0.059
	p-value	0.168	0.672

[Table/Fig-4]: Correlation of eGFR with BMI and CD4+ count among treatment naïve patients. (Pearson correlation analysis was used)

There was a positive and significant correlation of CrCl with CD4+ count among treatment naïve patients. There was a negative and non-significant correlation of CrCl with CD4+ count among HIV positive patients on HAART [Table/Fig-5].

	Correlation of CD4+ count with CrCl	
	r	p-value
Treatment naïve patients	0.297	0.029
Patients on HAART	-0.031	0.825

[Table/Fig-5]: Correlation of CD4 + count with CrCl among treatment naïve patients and patients on HAART. (Pearson correlation analysis was used)

There was a negative and non-significant correlation of serum urea of CD4+ count. There was a positive and non-significant correlation of serum urea with CD4+ count among HIV positive patients on HAART [Table/Fig-6].

	Correlation of CD4+ count with serum urea	
	r	p-value
Treatment naïve patients	-0.169	0.223
Patients on HAART	0.128	0.356

[Table/Fig-6]: Correlation of CD4 + count with serum urea among treatment naïve patients and patients on HAART. (Pearson correlation analysis was used)

There were two regimens taken under study for HAART (ZLN regimen and TLE regimen).

The mean CrCl of HIV positive patients on ZLN regimen was higher than HIV positive treatment naïve patients but was non-significant (p-value=0.085). The mean CrCl of HIV positive patients on TLE regimen was higher than HIV positive treatment naïve patients but was non-significant (p-value=0.337) [Table/Fig-7].

Regimen	CrCl (Mean±SD) (mL/min)		p-value
	Treatment- naïve (N=54)	On HAART (N=54)	
ZLN (n=12)	59.25±16.880	73.83±23.068	0.085
TLE (n=42)	72.62±26.904	80.60±26.475	0.337

[Table/Fig-7]: Mean change in Creatinine Clearance (CrCl) according to treatment regimens of HIV positive treatment-naïve patients with on HAART patients. (Analysis of variance, ANOVA: p-value <0.05; Significant)

The mean eGFR of HIV positive patients on ZLN regimen was higher than HIV positive treatment naïve patients but was non-significant (p=0.088). The mean eGFR of HIV positive patients on TLE regimen

was higher than HIV positive treatment naïve patients but was non-significant (p=0.869) [Table/Fig-8].

Regimen	eGFR (Mean±SD) (mL/min/1.73 m ²)		p-value
	Treatment-naïve (N=54)	On HAART (N=54)	
ZLN (n=12)	76.525±16.0324	95.217±26.3206	0.088
TLE (n=42)	94.093±29.8826	104.852±27.0434	0.869

[Table/Fig-8]: Mean change in eGFR according to treatment regimens of HIV positive treatment-naïve patients with on HAART patients. (Analysis of variance, ANOVA: p-value <0.05; Significant)

The percentage of patients with CrCl ≤60 mL/min decreased from after treatment showing improvement in CrCl. Then there was increase in percentage of patients with CrCl in range of 60.01-90 mL/min treatment indicating improvement of patients with CrCl ≤60 mL/min to this range of 60.01-90 mL/min. There was also increase in percentage of patients with CrCl ≥90.01 mL/min after treatment indicating patients improved in CrCl after treatment [Table/Fig-9]. But the change in mean values of CrCl in those three ranges were not statistically significant [Table/Fig-10].

CrCl (mL/min)	No. of HIV positive naïve patients	No. of HIV positive patients on HAART
≤60	23 (42.59%)	14 (25.93%)
60.01-90	21 (38.89%)	25 (46.29%)
≥90.01	10 (18.52%)	15 (27.78%)

[Table/Fig-9]: Change in no. of patients of different CrCl range of HIV positive after treatment.

CrCl (mL/min)	Mean±SD (mL/min)		p-value
	HIV positive (Treatment naïve)	HIV positive (On HAART)	
≤60	48.217±6.8649	52±6.3019	0.1
60.01-90	70.8095±10.0504	72.36±6.9390	0.33
≥90.01	111.2±17.4974	104.8±8.6038	0.23

[Table/Fig-10]: Change in mean values of CrCl of different CrCl range of HIV+ve patients after treatment. (Analysis of variance, ANOVA: p-value <0.05; Significant)

Changes in Radiographic Parameters

All the 54 patients presented with normal CMD and renal thickness. Nephromegaly was observed in 16 (29.6%) treatment naïve patients with mild increase in size, out of them (n=9) were males and (n=7) were females. Hyperechogenic kidneys were found in 47 (87.03%) treatment naïve patients where number of males was 26 and number of females was 21. After three months on cART, no changes were observed. But after six months on cART, all the above patients regained normal size and cortical echogenicity.

DISCUSSION

The objective of this study was to assess the renal function abnormalities in HIV infected patients and to compare the change in renal function of treatment naïve patients with those on cART. Combination cART, also known as HAART is now recommended in all people with confirmed HIV infection, irrespective of CD4 count or clinical status. Two nucleoside or Nucleotide Reverse Transcriptase Inhibitors (NRTI) together with a Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI), Protease Inhibitor (PI) or integrase inhibitor is standard cART. In the present study, 42 patients were treated with dual NRTI combinations of tenofovir and lamivudine along with one NNRTI efavirenz and 12 patients treated with dual NRTI combinations of zidovudine and lamivudine along with one NNRTI nevirapine. It is now known that there are several renal syndromes and diseases associated with HIV infection [12]. In US African Americans have approximately 3-4 folds higher rates of ESRD compared to European Americans [13]. The prevalence of HIVAN among African Americans was estimated at between 3.5-10% in

a primary care setting [14] and at 12% in an autopsy based study from patients treated with HAART [15]. This study shows serum urea of HIV positive patients after six months on HAART was lower 25.78 ± 4.721 mg/dL than HIV positive treatment naïve patients at recruitment baseline 26.19 ± 4.742 mg/dL but was non-significant ($p=0.640$). Low serum urea in patients on HAART can indicate liver dysfunction. Serum creatinine level was lower (0.815 ± 0.1393 mg/dL) and highly significant ($p=0.003$) in HIV positive on HAART in comparison to HIV positive treatment naïve patients (0.906 ± 0.1687 mg/dL). The low serum urea and low serum creatinine concentration in HIV positive on HAART patients found in this study is similar to the studies done internationally by Eneyew K et al., in Ethiopia and Emem C et al., in Nigeria [16,17]. These results differ from previous studies where they found high urea concentration with normal serum creatinine [18].

Serum creatinine is inversely proportional to CrCl. This study shows increase in CrCl in patients on HAART (79.09 ± 25.705 mL/min) and highly significant ($p=0.003$) in comparison to treatment naïve patients (69.65 ± 25.506 mL/min). This was similar to study Kirchner J [19], where benefit of HAART was reported in two African American patients with two NRTI and antiprotease. Lower GFR signifies a true loss of kidney function even if reduced GFR is expected with ageing. It is required to estimate GFR to stage CKD, rather than relying on serum creatinine concentration. This study shows eGFR increased (102.711 ± 26.9424 mL/min/ 1.73 m²) on HAART and highly significant ($p=0.003$) in comparison to HIV positive treatment naïve patients (90.189 ± 28.2575 mL/min/ 1.73 m²). This was similar to an observational prospective multicentre cohort study where GFR was improved regardless of ethnicity, CD4+T cell counts and baseline renal function with or without viral suppression [20]. There was no proteinuria in any patient of both treatment naïve and on HAART.

Limitation(s)

The primary limitation to the generalisation of these results is; it was a single centre study with a smaller sample size.

CONCLUSION(S)

This study showed that HIV positive patients on triple drug cART of both the regimens of tenofovir, lamivudine with efavirenz and zidovudine, lamivudine with nevirapine had change in mean renal function and all biochemical and radiological parameters improved after treatment with significant change.

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