Original Research Article

Assessment of the correlation of CD4 cell counts to C-reactive protein and lipoproteins in subjects with HIV infection: A clinical study

BH. V. K. Praveen Varma[1], Ashok Vankayala[2], K. N. S. V. Chalapathi Rao[3], D.S.S.K. Raju[4*]

[1]Associate Professor, Department of General Medicine, Maharajah's Institute of Medical Sciences, Nellimarla, Vizianagaram, Andhra Pradesh, India

[2]Professor, Department of General Medicine, Maharajah's Institute of Medical Sciences, Nellimarla, Vizianagaram, Andhra Pradesh, India

[3]Professor, Department of General Medicine, Maharajah's Institute of Medical Sciences, Nellimarla, Vizianagaram, Andhra Pradesh, India

[4]Assistant Professor, Department of Biochemistry, Maharajah's Institute of Medical, Sciences, Nellimarla, Vizianagaram, Andhra Pradesh, India

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Abstract

Aims-The present study was conducted to assess any correlation existing between changes in C - reactive protein and lipoproteins with CD4 cell counts in HIV Positive patients. **Materials and methods**- The study was conducted on 102 HIV-positive subjects. C-reactive protein, Lipid profiles, CD4 cell counts were assessed for all subjects. The collected data were subjected to evaluation and results were formulated. **Results**- 102 HIV positive subjects were grouped based on CD4 cell counts in four groups, the group I CD4 count <200/µL, group II CD4 count 201-350/µL, group III CD4 count 351-500/µL, and group IV has CD4 count>500/µL. In the majority of subjects with decreased CD4 count increased CRP levels were seen with a significant inverse correlation between CRP and CD4 count. It indicates that CRP level increases with increased HIV infection severity, and with decreased CD4 count. HDL decreased in HIV-positive patients and VLDL was increased with decreased CD4 cell count. No significant association was seen in serum Triglycerides and LDL with CD4 cell count. **Conclusion**- The present study concludes that C-reactive protein and lipid profiles are affected in HIV-positive subjects with decreased CD4 count. Hence, regular assessment of C-reactive protein and lipid profile help in assessing disease progression, forming a treatment plan, and decreasing the risk for cardiovascular disease in HIV-positive subjects, when CD4 cell counts cannot be assessed.

Keywords: AIDS, CD4 cell count, C-reactive protein, HIV, Lipoproteins, lipid profile.

Introduction

Acquired immunodeficiency syndrome (AIDS), is a deadly disease, caused by a human immune-deficiency virus, which affects the body's immune system, initially attacking CD4 cells and exposing the affected subjects to various life-threatening opportunistic infections including malignancies. Human immune-deficiency virus HIV-1, HIV-2 is identified. AIDS is seen globally with increasing incidence in every country including India. Nearly half of the Indian population is sexually active, and approximately 5 million Indians have HIV with rapidly increasing numbers every day, majority of the cases are due to STIs (Sexually Transmitted Diseases)[1].

In subjects with HIV, clinical latency makes diagnosis and management of HIV difficult in affected subjects. Widely used viral load assays as disease markers are not utilized in India owing to economic hindrance, fewer facilities, and limited resources. Hence, CD8+ and CD4+ cell counts with their ratios are used for assessing the disease status as CD4 cells accurately judge immune deficiency in HIV-affected subjects. CD4+T lymphocytes levels also help in staging and grading of HIV following CDC classification[2].

Increased plasma triglycerides are seen in HIV-infected subjects by decreased lipoprotein clearance, which leads to hepatic lipid synthesis and decreased lipoprotein lipase by increasing reesterification of fatty acid (FA) or hepatic fatty acid synthesis.

*Correspondence

Dr. D.S.S.K. Raju

Assistant Professor, Department of Biochemistry, Maharajah's Institute of Medical, Sciences, Nellimarla, Vizianagaram, Andhra Pradesh, India.

E-mail: dsskraju@gmail.com

First dyslipidemia seen in HIV-infected subjects was Hyper triglyceridaemia. However, other lipid abnormalities including hypo HDL cholesterolemia and hypo-cholesterolemia are also seen associated with HIV. Disturbance in lipid profiles and metabolism in subjects with HIV act as an increased risk factor for cardiovascular events such as atherosclerosis[3].

CRP is an acute-phase protein synthesized in the hepatocytes, whose levels increase with infection and inflammation. CRP is synthesized after response to cytokines such as IL-6. A negative correlation is established between CD4 count and CRP[4]. Increased CRP levels are usually seen in malignancies especially with metastasis, acute rheumatic fever with or without carditis, bacterial and viral infections, rheumatoid arthritis, and/or myocardial infarction. HIV infection is usually associated with CD4 cells depletion and immune system destruction leading to opportunistic infections and inflammation increasing CRP levels to nearly 1000 folds. These increased CRP levels are detected in HIV-affected subjects nearly 5 hours following infection[5].

The present study was conducted to assess any correlation existing between changes in C-reactive protein and lipoproteins with CD4 cell counts in HIV Positive patients.

Materials and methods

The present study was conducted to assess any correlation existing between changes in C - reactive protein and lipoproteins with CD4 cell counts in HIV Positive patients. The study was conducted at Maharajah's Institute of Medical Sciences, Nellimarla, Vizianagaram, Andhra Pradesh, India after obtaining clearance from the concerned Ethical committee. The study population was comprised of the subjects visiting the ART center of the Institute for treatment of HIV

infection. The study included a total of 102 subjects from both genders. After obtaining the detailed study design, informed consent was taken from all the subjects verbally and in written form before final inclusion.

The subjects who were not willing to participate in the study were excluded. After final inclusion, the blood sample from all study subjects was collected under aseptic and sterile conditions. Normal fasting blood samples were collected to allow accurate testing and comparison for the normal value establishment. To get the blood sample, venous blood was considered and collected from study participants by vein puncture in a clean glass vial. The vial was plain without any anticoagulant or additive. The collected sample was allowed to form serum by clotting. This was followed by centrifugation to separate plasma from the cells. The serum was then subjected to laboratory analysis.

To confirm and diagnose HIV in the collected sample, NACO recommendations for HIV testing were followed in the present study. By SD Bioline Rapid card, Triline, and Trispotcard test. SD Bioline HIV1/23.0 is an immune-chromatographic rapid test for the qualitative detection of all antibodies of all is types (IgG, IgM, IgA) specific to HIV–1 including subtype and HIV-2 simultaneously, in human serum, plasma, or whole blood.

To estimate CD4 cell counts, FACS (Fluorescence-activated cells otter) count System (Becton Dickinson, USA). Single Plate form Flow cytometry method. Lipid profile analysis-Serum total cholesterol, triglycerides, HDL-C, LDL-C, VLDL-C were estimated by a fully automated chemical analyzer machine (I-lab 650) by enzymatic method. To assess CRP levels, the nephelometry method using the automatic machine (IMMAGE 800).

The collected data were subjected to statistical evaluation and the results were formulated. The data were expressed as number and percentage, and mean and standard deviation. The level of significance was kept at a p-value of ≤0.05.

Results

The present study was conducted to assess any correlation existing between changes in C - reactive protein and lipoproteins with CD4 cell counts in HIV Positive patients. The study included a total of 102 subjects from both genders. C-reactive protein, Lipid profiles, CD4 cell counts were assessed for all subjects.

The distribution of total cholesterol in the study subjects based on CD4 cell counts was assessed in the study subjects. It was seen that in CD4 cells count of <200 counts/µL were seen in total 57 subjects with 34 subjects of cholesterol <150mg/dl, 19 subjects with cholesterol of 150-200mg/dl, and in 4 subjects with cholesterol >200mg/dl. For CD4 cells of 201-350 counts/µL, 25 subjects were there. 14 subjects of cholesterol <150mg/dl, 8 subjects with cholesterol of 150-200mg/dl, and in 3 subjects with cholesterol >200mg/dl. In CD4 cells of 351-500, a total of 11 subjects were there, where 9 subjects of cholesterol <150mg/dl and 2 subjects with cholesterol of 150-200mg/dl. In CD4 cells of >500 counts/µL, a total of 9 subjects were there, where 8 subjects of cholesterol <150mg/dl and 1 subject with cholesterol of 150-200mg/dl (Table 1).

Table 1: Cholesterol distribution ion the study subjects based on CD4 cell levels

CD4 Cell counts/ µL	Total Cholesterol (mg/dl) (n)			Total
	<150	150-200	>200	
<200	34	19	4	57
201-350	14	8	3	25
351-500	9	2	-	11
>500	8	1	-	9
Total	65	30	7	102

Concerning HDL-cholesterol distribution based on CD4 cell counts, it was seen that for CD4 cells of >200 counts/ µL, there were 57 subjects, where 34 subjects had HDL of <30mg/dl and 23 subjects had HDL of 31-60mg/dl. In CD4 cells of 201-350 counts/µL, there were a total of 26 subjects, where 14 subjects had HDL of <30mg/dl and 12 subjects had HDL of 31-60mg/dl. In CD4 cells of 351-500 counts/ µL, a total of 11 subjects were there, where 4 subjects had HDL of <30mg/dl and 7 subjects had HDL of 31-60mg/dl. For >500 counts/ µL of CD4 cells, there were 8 subjects in total where, 3 subjects had HDL of <30mg/dl and 5 subjects had HDL of 31-60mg/dl. A total of 55 subjects had HDL of <30mg/dl and 47 subjects had HDL of 31-60mg/dl (Table 2).

Table 2: HDL-Cholesterol distribution ion the study subjects based on CD4 cell levels

CD4 Cell counts/ µL	HDL cholesterol (mg/dl) (n)		Total
	<30	31-60	
<200	34	23	57
201-350	14	12	26
351-500	4	7	11
>500	3	5	8
Total	55	47	102

On assessing the VLDL-cholesterol distribution based on CD4 cell counts, it was seen that for CD4 cells of >200 counts/ µL, there were 57 subjects, where 23 subjects had VLDL of 1-35mg/dl and 34 subjects had VLDL of >35mg/dl. In CD4 cells of 201-350 counts/µL, there were a total of 11 subjects, and all 11 subjects had VLDL of >35mg/dl. In CD4 cells of 351-500 counts/ µL, a total of 8 subjects were there, where 2 subjects had VLDL of 1-35mg/dl and 6 subjects had VLDL of >35mg/dl. For >500 counts/ µL of CD4 cells, there were 8 subjects in total where, 2 subjects had VLDL of 1-35mg/dl and 6 subjects had VLDL of >35mg/dl. A total of 35 subjects had VLDL of 1-35mg/dl and 67 subjects had VLDL of >35mg/dl (Table 3).

Table 3: VLDL-Cholesterol distribution ion the study subjects based on CD4 cell levels

CD4 Cell counts/ µL	VLDL- cholesterol (mg/dl) (n)		Total
	1-35	>35	
<200	23	34	57
201-350	10	16	26
351-500	-	11	11
>500	2	6	8
Total	35	67	102

CRP association to CD4 cell levels was also assessed in the present study. CRP level of <5mg/L was seen in 2.94% (n=3) subjects with CD4 cells of <200, 6.86% (n=7) subjects with 201-350 CD4 cell counts, 4.90% (n=5) subjects with 351-500 CD4 cell counts, and 6.86% (n=7) subjects

with CD4 cell counts of >500, and in total of 21.56% (n=22) subjects. CRP level of 6-10 mg/L was seen in 15.68% (n=16) subjects with maximum subjects with CD4 cells counts of <200 counts with 9.80% (n=10) subjects. In CRP of 11-20mg/L, there were 27.45% (n=28) subjects with majority of subjects having CD4 cell levels of >200 counts with 14.70% (n=15) subjects. For CRP levels of 41-50 and >50 mg/L there were 3.92% (n=4) and 19.60% (n=20) subjects respectively, and maximum subjects were having CD4 cell counts of >200 counts with 1.96% (n=2) and16.66% (n=17) subjects respectively (Table 4).

Table 4: CRP distribution ion the study subjects based on CD4 cell levels

CRP (mg/L)	CD4 cells/ml								Total	
	<200		201-350		351-500		>500			
	%	n	%	N	%	n	%	N	%	n
<5	2.94	3	6.86	7	4.90	5	6.86	7	21.56	22
6-10	9.80	10	2.94	3	2.94	3	-	-	15.68	16
11-20	14.70	15	8.82	9	1.96	2	1.96	2	27.45	28
21-30	6.86	7	-	-	-	-	-	-	6.86	7
31-40	2.94	3	0.98	1	0.98	1	-	-	4.90	5
41-50	1.96	2	0.98	1	0.98	1	-	-	3.92	4
>50	16.66	17	2.94	3	-	-	-	-	19.60	20
Total	55.88	57	23.52	24	11.76	12	8.82	9	100	102

Discussion

The present study was conducted to assess any correlation existing between changes in C - reactive protein and lipoproteins with CD4 cell counts in HIV Positive patients. The study included a total of 102 subjects from both genders. C-reactive protein, Lipid profiles, CD4 cell counts were assessed for all subjects. The distribution of total cholesterol in the study subjects based on CD4 cell counts was assessed in the study subjects. It was seen that in CD4 cells count of <200 counts/µL were seen in total 57 subjects with 34 subjects of cholesterol <150mg/dl, 19 subjects with cholesterol of 150-200mg/dl, and in 4 subjects with cholesterol >200mg/dl. For CD4 cells of 201-350 counts/µL, 25 subjects were there. 14 subjects of cholesterol <150mg/dl, 8 subjects with cholesterol of 150-200mg/dl, and in 3 subjects with cholesterol >200mg/dl. In CD4 cells of 351-500, a total of 11 subjects were there, where 9 subjects of cholesterol <150mg/dl and 2 subjects with cholesterol of 150-200mg/dl. In CD4 cells of >500 counts/µL, a total of 9 subjects were there, where 8 subjects of cholesterol <150mg/dl and 1 subject with cholesterol of 150-200mg/dl. These results were consistent with the results of Asztalos B. F et al[6] in 2005 and Suy A et al[7] in 2007 where authors reported similar CD4 cell counts and cholesterol distribution in HIV infected subjects.

CHDL-cholesterol distribution was assessed in the present study based on CD4 cell counts, it was seen that for CD4 cells of >200 counts/ µL, there were 57 subjects, where 34 subjects had HDL of <30mg/dl and 23 subjects had HDL of 31-60mg/dl. In CD4 cells of 201-350 counts/µL, there were a total of 26 subjects, where 14 subjects had HDL of <30mg/dl and 12 subjects had HDL of 31-60mg/dl. In CD4 cells of 351-500 counts/ µL, a total of 11 subjects were there, where 4 subjects had HDL of <30mg/dl and 7 subjects had HDL of 31-60mg/dl. For >500 counts/ µL of CD4 cells, there were 8 subjects in total where, 3 subjects had HDL of <30mg/dl and 5 subjects had HDL of 31-60mg/dl. A total of 55 subjects had HDL of <30mg/dl and 47 subjects had HDL of 31-60mg/dl. VLDL-cholesterol distribution based on CD4 cell counts was analyzed in the present study, it was seen that for CD4 cells of >200 counts/ µL, there were 57 subjects, where 23 subjects had VLDL of 1-35mg/dl and 34 subjects had VLDL of >35mg/dl. In CD4 cells of 201-350 counts/µL, there were a total of 11 subjects, and all 11 subjects had VLDL of >35mg/dl. In CD4 cells of 351-500 counts/ µL, a total of 8 subjects were there, where 2 subjects had VLDL of 1-35mg/dl and 6 subjects had VLDL of >35mg/dl. For >500 counts/ µL of CD4 cells, there were 8 subjects in total where, 2 subjects had VLDL of 1-35mg/dl and 6 subjects had VLDL of >35mg/dl. A total of 35 subjects had VLDL of 1-35mg/dl and 67 subjects had VLDL of >35mg/dl. These findings were in agreement with the studies of Hernandez J et al[8] in 2016 and Schlein C et al[9] in 2017 where HDL and VLDL distribution based on CD4 cell counts in HIV infected subjects was comparable to the present study.

The present study also assessed the CRP association to CD4 cell levels. SchleinCRP level of 6-10 mg/L was seen in 15.68% (n=16) subjects with maximum subjects with CD4 cells counts of <200 counts with 9.80% (n=10) subjects. In CRP of 11-20mg/L, there were 27.45% (n=28) subjects with majority of subjects having CD4 cell levels of >200 counts with 14.70% (n=15) subjects. For CRP levels of 41-50 and >50 mg/L there were 3.92% (n=4) and 19.60% (n=20) subjects respectively, and maximum subjects were having CD4 cell counts of >200 counts with 1.96% (n=2) and16.66% (n=17) subjects respectively. These results were comparable to the results by Guardo AC et al[10] in 2015 and Vaisar T et al[11] in 2015 where CRP distribution based on CD4 cell counts in the HIV infected subjects similar to the present study was reported by the authors.

Conclusion

Within its limitations, the present study concludes that C-reactive protein and lipid profiles are affected in HIV-positive subjects with decreased CD4 count. Hence, regular assessment of C-reactive protein and lipid profile help in assessing disease progression, forming a treatment plan, and decreasing the risk for cardiovascular disease in HIV-positive subjects, when CD4 cell counts cannot be assessed. However, the present study had a few limitations including small sample size, short study duration, retrospective nature, and geographical area biases. Hence, more longitudinal studies with a larger sample size and longer monitoring period will help reach a definitive conclusion.

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