Original Article

Correlation of Interleukin-21 Levels With Clinical and Laboratory Parameters in ART Recipients

Manoj Kumar Meena*, Shukla Das, Kuldeep Kumar and Praveen Kumar Singh

Department of Microbiology and Department of Medicine, UCMS & GTB Hospital, Delhi

DOI: 10.21276/APALM.3250

***Corresponding Author**:
Dr. Manoj Kumar Meena
drmanojmeena005@gmail.com

Submitted: 02-Feb-2023
Final Revision: 04-May-2023
Acceptance: 03-Jul-2023
Publication: 02-Aug-2023

Abstract

Background: Interleukin-21 (IL-21) is a relatively new immune-enhancing, multifunctional and pleiotropic cytokine that plays an essential role in controlling chronic viral infections. It is a protein that in humans is encoded by IL-21 gene, mapped on chromosome 4 and 180 kb from IL-2 gene and the mRNA encoding the product is 616 nucleotides long. IL-21 is expressed in activated human CD4+ T cells but not in most other tissues. In addition, IL-21 expression is up-regulated by Th2 and Th17 subsets of T helper cells, as well as T follicular cells. Further IL-21 is expressed in NKT cells regulating the functions of these cells. IL-21 may be a critical factor in the control of persistent viral infections such as HIV. Present study aims to explore the levels of IL-21 in a select segment of ART recipients of East Delhi covered by NACP, Govt. of India.

Methods: Estimation of levels of IL-21 was done using ELISA test employing commercially available kit (QAYEE-BIO Kit for Interleukin-21). The method of ELISA used for the present study was sandwich ELISA.

Results: The mean levels of IL-21 in the study of 40 ART recipients were 1178.21±927.063. There were no controls included in the study. The range of IL-21 were 150-6100. In one patient IL-21 levels was not detected.

Conclusion: This study attempts a clinical correlation with the levels of a cytokine with clinical and laboratory parameters which could help in a better understanding of the immunobiology of the disease. In the current study, the levels of IL-21 in samples of ART recipients were detected. A significant positive clinical correlation was seen between the levels of IL-21 and CD4 cell counts. IL-21 levels were high in those patients whose CD4 cell count was raised. There was a positive correlation between CD4 cell count and CRP. Therefore, further studies with a higher sample size may be required to arrive at a statistically significant correlation.

Keywords:

IL-21; Correlation; ART recipient; CD-4 count; CRP; Laboratory parameters

Introduction

IL-21 may be a critical factor in the control of persistent viral infections such as HIV. HIV infection is characterized by a progressive qualitative and quantitative deficiency of CD4 T cells and broad immunological defects that include immune

suppression in concert with increased inflammation and immune activation. CD4 T cell depletion is evident in circulation as well as in lymphoid tissue. Since CD4 T cells are the main source of IL-21, there is considerable interest in determining whether and how HIV infection alters IL-21 production.

Since its discovery in 2000, several studies have demonstrated that IL-21, alone and/or in combination with other cytokines such as IL-2, IFN- or IL-15, activates NK cells and enhance their proliferation. IL-21 is one of a group of cytokines including IL-2, IL-4, IL-7, IL-9 and IL-15 whose receptors' complex share a common chain (c). Furthermore, plasma cells are the antibody secreting cells.[1] The generation of these long lived plasma cells depends on a series of highly orchestrated interactions between antigen-specific CD4 T cells and B cells and the formation of germinal centres. IL-21 is a critical cytokine for the generation of virus-specific long lived plasma cells.[2]

IL-21 receptor (IL-21R) is expressed on the surface of T, B and NK cells. IL-21R is similar in structures to the receptors for other type I cytokines like IL-2R or IL-15 and requires dimerization with the common gamma chain (c) in order to bind IL-21. When bound to IL-21, the IL-21 receptor acts through the JAK/STAT pathway, utilizing Jak1 and Jak3 and STAT3 homodimer to activate its target genes.[3]

IL-21 is produced mainly by CD4+ T cells, which are also the main targets of HIV-1 and often depleted in HIV infected individuals.[4]

Unfolding the spectrum of activity of this cytokine has stimulated interest in the search for ways to harness it in strategies to prevent HIV infection as a vaccine adjuvant and as a therapeutic tool in acute and chronic phases of the disease in infected host. It is a unique cytokine that targets a wide range of immune cells thus offering an interesting perspective of its potential clinical utility.[4]

The intrinsic ability of IL-21 to promote the longevity of B cells should benchmark its role in sustained production of neutralizing antibodies by proliferating and differentiating B cells committed to cognate stimulating antigenic determinants.[5] Nonetheless, immune response to identical or related antigenic insults are quite heterogeneous enlisting complex factors including ethnic and HLA elements in determining the final outcome. Present study aims to explore the levels of IL-21 in a select segment of ART recipients of East Delhi covered by NACP, Govt. of India.

Materials and methods

This study was conducted in Departments of Microbiology and Medicine, University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi-110095 during November 2013 to April 2015.The 40 HIV reactive Patients of age more than 18 years, who were ART recipients for a period of 6 months to 1 year having a CD4 Count of <350 cells /µl were enrolled as subjects in this study. ART recipients with SLE and other autoimmune diseases, HIV and TB coinfection and pregnant females were excluded from the study. This study was undertaken after written informed consent from patients and clearance from Institutional Ethical Committee of UCMS & GTB Hospital, Delhi.The sample 3-5 ml of venous blood was collected from each patient under aseptic precautions. Sera were separated and stored at -70° C for estimations of IL-21 levels using ELISA test employing commercially available kit (QAYEE-BIO Kit for Interleukin-21). Routine Investigations including Heamoglobin (Hb), Total Leucocyte Count (TLC), Differential Leucocyte Count (DLC), Thrombocyte count / platelet count, Erythrocyte Sedimentation Rate (ESR), Blood sugar estimation (RBS), C - reactive protein (CRP) were also included and recorded each patient.

Statistical analysis: Data which was obtained from ART clinic were studied as continuous variable data. For continuous variable

data, two types of tests were used. Parametric and/or non-parametric tests. Pearson's correlation or Spearman correlation was applied between IL-21 and other clinical and laboratory parameters depending on normality of these parameters. Spearman correlation was applied to correlate levels of IL-21 with CD4 Count.

Results

Levels of Interleukin-21 in the ART recipients were as follows: 1. In 24 patients, serum levels of IL-21 less than <1000 pg/ml.

2. In 15 patients, serum levels of IL-21 more than> 1000 pg/ml. 3. There was a single patient in which IL-21 level was not detected.

IL-21	Mean±SD
IL-21 in 39 patients	1178.21±927.063 (150-6100)*
In 1 patient IL-21 not detected	*Range of IL-21

S.NO.	IL-21*	cd4 cell count#	CRP$	S.NO.	IL-21*	CD4 Cell Count	CRP
1	1100	338	4.8	21	150	136	26
2	1050	212	6.5	22	750	190	21
3	850	160	12	23	700	220	16
4	800	152	9	24	1100	288	11.5
5	1850	342	4.5	25	950	310	9
6	1100	332	5	26	900	388	12
7	1250	210	7	27	700	392	8.5
8	1000	170	9	28	1100	318	10
9	800	192	8.5	29	950	312	12.5
10	900	210	6.5	30	850	180	21
11	950	188	8	31	950	196	17
12	1100	308	5.2	32	900	178	23
13	900	280	7.5	33	950	224	11
14	700	240	8	34	900	236	9
15	2250	348	6.5	35	950	290	13.5
16	1650	312	16	36	Nd	126	24
17	6100	346	7	37	2800	340	9
18	1550	296	9.5	38	850	318	11.5
19	1100	248	11	39	750	362	8.5
20	1150	290	19	40	600	280	16.5
Nd – not Detectable * pg/ml# Cell/ul $mg/dl							

Salient Features of Laboratory parameters are as follows:

1. The mean haemoglobin level amongst ART patients was 11.17 ± 2.0687 pg/ml.
2. The mean ESR level amongst patients was 49.33 ±19.753 mm in first hr.
3. The mean blood sugar estimation level amongst patients was 103.82±16.849 mg/dL.
4. The mean Blood urea level amongst patients was 28.65 ± 11.459 mg/dL.
5. The mean Total Bilirubin Level amongst patients was 0.615 ± 0.3085 mg/dL.

Results of serum lipid profiles of the ART recipient patients are been shown in Table 2.

Table 1: Serum lipid profile of ART patients

Parameters (mean± SD) (Range) (mg/dl)	**PATIENTS (n=40)**
Total Cholesterol	149.65±36.302 (70-210)
Triglyceride	152.05±47.754 (81-259)
Data has been described as mean ± SD and range	

Salient features of serum lipid profile of ART recipients patients:

1. All the parameters of lipid profile in the subjects were estimated.

2. Mean values of total cholesterol and triglycerides were not raised in the ART recipients according to Asian standards.

There were no specific observations or correlation found In TLC, Platelet count, ALT and AST. Certain findings suggest that ALT and AST were abnormally raised in few ART recipients but these were probably due to improper visits to the ART centre.

Certain patient´s clinical and laboratory parameters were different at different visits. These were not due to the progression of HIV disease but main reason was that they did not follow the counseling and dietary guidelines properly.

There were no other specific findings in clinical and laboratory parameters in ART recipients. Out of 40 recipients, 3 subjects were positive for HBsAg and VDRL.

Special Investigations

These include two investigations

1. CD4 cell count

2. Interleukin-21(IL-21)

CD4 Cell Count

Majority (77.14%) of the patients amongst ART recipients had CD4 count <200 cells/mm3.

Only 1 subject has CD4 cell count <100.

Table 2: CD4 cell count in ART recipients

CD4 Cell Count	**Cases (n=40) (%)**
50-100	1 (2.50%)
100-200	5 (12.50%)
200-350	34 (84.00%)
CD4 Cell Count (cells/µl)	**mean± SD (Range)**
40 patients	271.10±67.646 (76-348)

Salient features of CD4 cell count in the subjects are:

1. As per the inclusion criteria, all the patients had CD4 count <350 cells/µl

2. The values of CD4 cell count ranged from 76 to 348 cells/µl.

Parameters	**IL-21 (Spearman Correlation)**	
	r-value	p-value
IL-21	1.000	–
Hb	-0.302	0.062
TLC	-0.002	0.989
ESR	0.144	0.382
Platelet count	0.106	0.519
Blood urea	-0.337	0.036
Serum creatinine	-0.243	0.142
Bilirubin	-0.11	0.946
AST (SGOT)	-0.154	0.348
ALT (SGPT)	-0.122	0.459
Alkaline phosphatase	-0.340	0.034
Serum amylase	-0.046	0.782
Blood sugar	0.066	0.689
Serum cholesterol	-0.144	0.383
Triglycerides	-0.273	0.093
CD4 count	-0.051	0.757

As shown in Table 4, all laboratory parameters were independent variables when correlated with IL-21, except blood urea and alkaline phophatase. Both these parameters (blood urea and alkaline phophatase) showed negative correlation with IL-21. When IL-21 levels were increased, corresponding blood urea levels in the serum decreased. Similar observation was also observed with alkaline phosphatase. Blood urea and alkaline phosphatase showed negative correlation with IL-21. Only these two laboratory parameters showed significant correlation. Further large scale studies are required to estimate their exact correlation with IL-21 and whether they will serve as surrogate markers or not.

Correlation of Blood Sugar Estimation with CD4 cell counts. No significant correlation was found between RBS with CD4 cell count.

Correlation of Hemoglobin with CD4 cell counts. No significant correlation was found between Hemoglobin and CD4 cell count.

Correlation of Blood urea with CD4 cell counts. There was no correlation between blood urea with CD4 cell count.

Correlation of Lipid Profile with CD4 cell counts. It was observed that in 13 subjects, Total cholesterol and Triglycerides level were high suggesting a strong correlation between Lipid Profile and CD4 cell count.

Correlation of Total Leucocyte Counts with CD4 cell counts. It was observed that in nine patients TLC counts were low. The CD4 counts of these subjects were also low suggesting a strong correlation between TLC count and CD4 cell count.

Correlation of SGPT (ALT) and SGOT (AST) with CD4 cell counts. No significant observation was found between ALT and AST with CD4 cell counts.

Interleukin-21

The levels of IL-21 in samples were detected by commercial kit which used a double antibody sandwich enzyme-linked immune sorbent one-step process assay (ELISA).

Correlation of IL-21 levels with Blood Sugar Estimation (random blood sugar - RBS). No significant correlation was found between IL-21 and Blood Sugar of ART recipients.

Correlation of Hemoglobin with IL-21 levels. No significant correlation was found between Hemoglobin and IL-21.

Correlation of Blood urea and Alkaline phosphatase with IL-21levels. Both these parameters (blood urea and alkaline phosphatase) showed negative correlation with IL-21. When IL-21 levels were increased, corresponding blood urea levels in the serum decreased. Similar observation was also observed with alkaline phosphatase.

Correlation of lipid profile with IL-21 levels. There was no significant correlation between IL-21 and Lipid profile.

Correlation of ALT and AST with IL-21. No significant correlation was found between ALT and AST with IL-21.

Correlation of CD4 cell counts with IL-21 levels. There was strong clinical correlation between CD4 cell counts and IL-21. The levels of IL-21 were high in ART recipients whose CD4 counts were improved while receiving ART.

But on statistical analysis, when Spearman's correlation was applied between these two parameters no significant correlation was observed.

Discussion

Clinical and laboratory parameters

All the Clinical and laboratory parameters including Hemoglobin, TLC, DLC, Platelet count, ESR, Blood sugar estimation, CRP, AST and ALT, Liver function tests and Kidney function tests were included in our study. Beside these, two special investigations, CD4 cell count and IL-21 were compared and correlated with the clinical status of ART recipients.

The mean hemoglobin level was 10.75±1.027 gm/dl in HIV patients. Lipids (Total cholesterol & triglycerides) and CD4 cell count

On statistical Analysis no correlation was observed between total cholesterol and CD4 cell count. But strong clinical correlation observed between triglycerides and CD4 cell count.

Table 3: Correlation of serum cholesterol with and CD4 cell count and triglyceride

	CD 4 cell count		**Triglyceride**	
	r-value	**p-value**	**r-value**	**p-value**
Cholesterol	0.094	0.564	0.370	0.019

There was a strong clinical correlation between CD4 cell count and lipids. The clinical importance of the above 2 parameters and other abnormal lipoprotein levels lies primarily in the greater risk they confer to the development of cardiovascular diseases (CVDs). There is a growing concern about premature CVDs in both untreated and treated HIV+ persons. Premature atherosclerosis has been reported in young adults with HIV infection in the pre-highly active antiretroviral therapy (HAART) era[6]. Hypercholesterolemia and hypertriglyceridemia have been associated with the use of HAART[7,8] but untreated HIV infection

generate similar changes in lipids metabolism - increased LDL cholesterol and decreased HDL cholesterol, with high levels of triglycerides (TGs), in advanced stages of the disease[9].

Patients with HIV infection have lower BMI and hemoglobin even in early stages of infection. Also in early stages of infection patients have lymphadenopathy. Further large scale studies are required for a definitive opinion.

Correlation of total leucocyte count with cd4 cell count. On statistical analysis there was no correlation between TLC and CD4 count. In nine patients TLC counts were low. CD4 counts of these patients were also low suggesting a clinical correlation between ***TLC count and CD4 cell count in ART recipient.*** TLC as an entity to arbitrate the initiation of prophylaxis is not accepted universally though[10]. The low sensitivity and specificity and the contrived presumption that TLC of <1200 cells/mm3 related to an absolute CD4 count o <200 cells/mm3 was not optimal in targeting patients requiring HAART. Moreover, data on the relationship between CD4 cell count and total leucocyte count is limited in resource poor settings.

The CD4 cell count of ≤ 200 cell/μL proved to be a vital marker in the management of HIV/AIDS patients. Although CD4 cell count was considered the best laboratory marker of HIV infection, it was an expensive test and not widely available because of lack of sophisticated equipment. This problem was more in resource-constrained developing countries where the majority of people infected with HIV live[11].

Correlation of IL-21 with cd4 cell count. There was strong clinical correlation between CD4 cell counts and IL-21. The levels of IL-21 were high in ART recipients whose CD4 counts were initially low but improved one year after while receiving ART.

But on statistical analysis, when Spearman's correlation was applied between these two parameters no significant correlation was observed.

On statistical analysis IL-21 levels had no significant correlation with laboratory parameter CD4 cell counts. But there is a clinical correlation between IL-21 and CD4 cell count. It was observed that in 7 subjects, serum IL-21 levels were high and their CD4 counts were also improved while received ART.

In 3 ART recipients serum IL-21 levels were low and their CD4 counts were also low and not improved after one year while received ART.

So these observations suggesting level of IL-21 in these subjects were directly proportional. As IL-21 is secreted by CD4 cell count so this suggested that when CD4 cell counts increase, IL-21 concentrations in serum also increase.

A strong clinical correlation was also observed between levels of IL-21 and CD4 T cell. The intrinsic ability of IL-21 to promote the longevity of B cells should benchmark its role in sustained production of neutralizing antibodies by proliferating and differentiating B cells committed to cognate stimulating antigenic determinants. Nonetheless, immune response to identical or related antigenic insults are quite heterogeneous enlisting complex factors including ethnic and HLA elements in determining the final outcome. The impact of IL-21 on antibody responses to pathogens is not well defined, but given the roles of IL-21 in promoting B cell responses and plasma cell formation, it is likely that humoral immunity to infections will be influenced by the availability and kinetics of induction of IL-21. The necessity for IL-21 in ensuring durable T cell immunity to acute infections appears to be subtle.

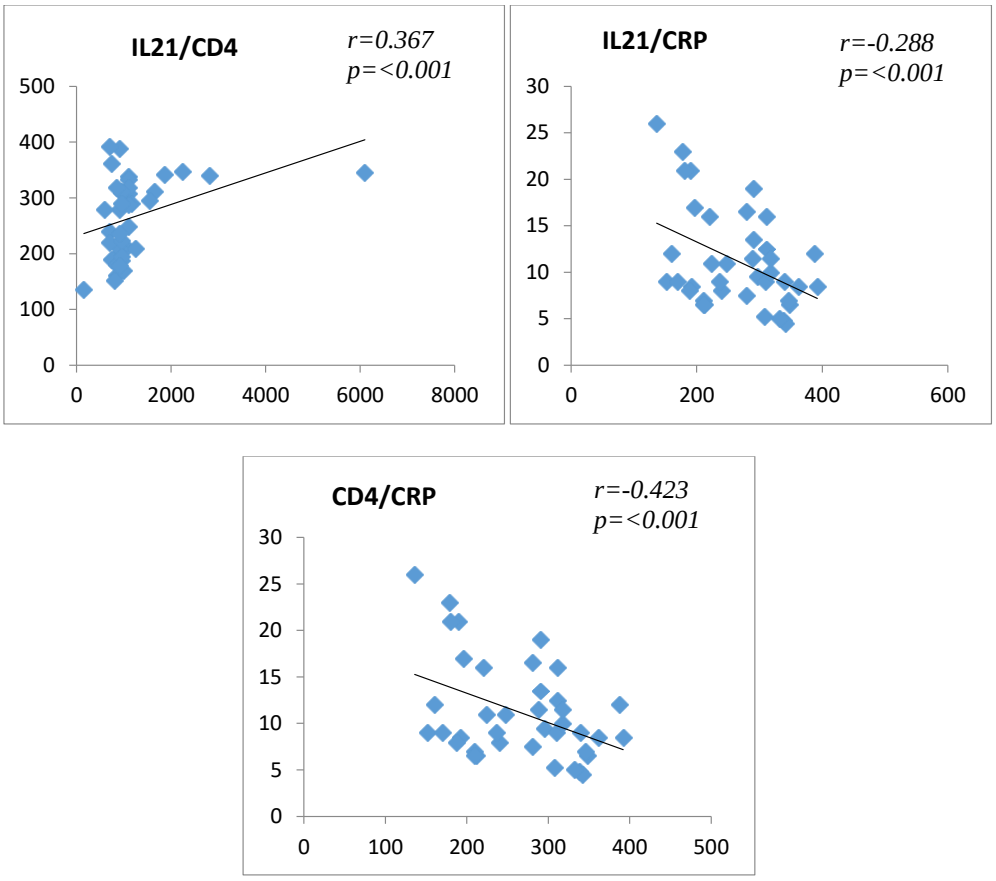

Fig 1: Correlation between IL21/CD4, IL21/CRP and CD4/CRP

There is also significant negative correlation between CD 4 cell count and CRP. It shows that these two above mentioned variables are not related to each other. Their p value is <0.001 as mentioned in Figure 3. A significant positive clinical correlation was seen between the levels of IL-21 and CD4 cell counts. IL-21 levels were high in those patients whose CD4 cell count was raised. These in conjunction with other parameters could serve as important biomarkers, indicating the course of progression of the disease.

Conclusion

This study attempts a clinical correlation with the levels of a cytokine which could help in a better understanding of the immunobiology of the disease. A significant positive clinical correlation was seen between the levels of IL-21 and CD4 cell counts. IL-21 levels were high in those patients whose CD4 cell count was raised. This correlation shows that IL-21 is a potential candidate to evaluate the clinical pathophysiology of people living with HIV/AIDS (PLHA). The correlation shows that IL-21 level is a potential indicator in determining prognosis of ART recipients. There was a positive correlation between CD4 cell count and CRP. Therefore, further studies with a higher sample size may be required to arrive at a statistically significant correlation.

Acknowledgement: None

Declaration of conflicts of interest: The authors have no conflict of interest to declare for this study.

References

1. Iannello A, Boulassel MR, Samarani S, Tremblay C, Toma E, Routy JP, et al. IL-21 enhances NK cell functions and survival in healthy and HIV-infected patients with minimal stimulation of viral replication. J Leukoc Biol. 2010;87(5):857-67.
2. Kapil A, Bhaskaran CS. Human immunodeficiency virus: AIDS. In: Kapil A, Bhaskaran CS, editors. Textbook of Microbiology, 9th ed. Hyderabad: Universities Press (India) Pvt Ltd.; 2013. p. 570-588.
3. Iannello A, Boulassel MR, Samarani S, Debbeche O, Tremblay C, Toma E, Routy JP, Ahmad A. Dynamics and consequences of IL-21 production in HIV-infected individuals: a longitudinal and cross-sectional study. J Immunol. 2010;184(1):114-26.
4. Pallikkuth S, Parmigiani A, Pahwa S. Role of IL-21 and IL-21 receptor on B cells in HIV infection. Crit Rev Immunol. 2012;32(2):173-95.
5. Gallo RC, Salahuddin SZ, Popovic M, et al. Frequent detection and isolation of cytopathic retroviruses (HTLV III) from patients with AIDS and at risk for AIDS. Science. 1984;224:500-3.
6. Tabib A, Greenland T, Mercier I, et al. Coronary lesions in young HIV-positive subjects at necropsy. Lancet. 1992;340:730.
7. Echevarria KL, Hardin TC, Smith JA. Hyperlipidemia associated with protease inhibitor therapy. Ann Pharmacother. 1999;33:859-63.
8. Mirete G, Masia M, Gutierrez F, et al. Acute pancreatitis as a complication of ritonavir therapy in a patient with AIDS. Eur J Clin Microbiol Infect Dis. 1998;17:810-11.
9. Hellerstein MK, Grunfeld C, Wu K, et al. Increased de novo hepatic lipogenesis in human immunodeficiency virus infection. J Clin Endocrinol Metab. 1993;76:559-65.
10. Selik RM, Starcher ET, Curan JW. Opportunistic diseases reported in AIDS patients: frequencies, associations, and trends, and trends. AIDS. 1987;1:175-182.
11. Sax PE. HIV Infection. In Cunha BA, editor. Antibiotic E. New York: Physicians; 2005. p. 227-33.