Increased Plasma Levels of Soluble CD27 among HIV/HCV Co-infected and HIV/HCV/GBV-C Triply Infected Subjects.

Alireza Najafi[1], Mostafa Haji Mollahoseini[*1], Shahram Samiee[2], Sedigheh Amini Kafi-Abad[2], Arash Memarnejadian[3]

[1] Department of Immunology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] Research Center Laboratory, Iranin Blood Transfusion Organization, Tehran, Iran
[3] Department of Hepatitis and AIDS, Pasteur Institute of Iran, Tehran, Iran

*Corresponding author: e-mail address: m.mollahoseini@sbmu.ac.ir (M.Haji Mollahoseini)

ABSTRACT

CD27 is a biomarker associated with both T-cells and B-cells activation .Plasma soluble CD27 (sCD27) was identified as a marker of disease outcome in Human Immunodeficiency Virus (HIV) infection .Testing of plasma sCD27 represents a good tool to monitor the change of immune activation during HIV infection.We sought to analyses role of Hepatitis C Virus (HCV) and also GB Virus type C (GBV-C) co-infections on HIV-related immune activation, through measuring sCD27 plasma levels. Blood samples from a total of 86 patients with HIV infection were taken. Plasmas were analyzed for HCV using serologic test and GBV-C by reverse transcriptase polymerase chain reaction (RT-PCR). CD4+ and CD8+T-cell counts were evaluated by CD3/CD4+ and CD3/CD8+ double staining of whole blood followed by flow cytometric analysis .Then Cross-sectional comparison of sCD27 plasma levels was carried out among patients : HIV (n=20), HIV/ GBV-C (n=14), HIV/ (HCV) (n=26) and HIV/HCV/GBV-C (n=26).Plasma level of sCD27 was higher in HIV/HCV/GBV-C patients as compared to HIV mono-infected patients (p= 0.006) and based on results there was significant differences in the plasma levels of sCD27 between HIV-infected individuals with and without HCV coinfection (P=0.017) and also correlation between sCD27 and percent of CD4+T-cells was in highest level among HIV/HCV co-infected patients group [r= -0.59 (p=0.001)]. High levels of sCD27 among HIV/HCV patients argues in favor of sCD27 plasma level determination for monitoring of clinical features among HIV/HCV coinfected patients.

Keywords: HIV; HCV; GBV-C; CD27; Coinfection

INTRODUCTION

Considering that immune activation contributes to the HIV pathogenesis, parameters reflecting the activation of immune response are attractive candidates as HIV surrogate markers .Surrogate markers can reflects other parts of HIV pathogenesis that no detectable based on just CD4+ T cell counts and viral load measurements [1].

CD27 was associated with HIV progression in other setting and was identified as a HIV surrogate marker [2]. In chronic clinical conditions such as persistent virus infection and autoimmune disease that are associated with an enhanced activation of the immune system, an increase in plasma soluble CD27 (sCD27) level has been documented [3]. CD27 regulates cellular activity in subsets of immune cells. In normal immune responses, CD27 signaling appears to be limited. However, continuous CD27–CD70 interactions may cause immune deregulation in condition of chronic immune activation [4].

GBV-C is a flavivirus closely related to HCV, with approximately 30% amino acid sequence homology that initially reported to be associated with post-transfusion hepatitis in humans. However, several research groups demonstrated that persistent GBV-C infections are asymptomatic and there is no relation between virus infection and human disease. Nowadays GBV-C introduces as a harmless virus or orphan virus. Many clinical and epidemiological studies have suggested a beneficial effect of GBV-C co-infection on the course of HIV-1 infection, however not all studies confirmed the results [5, 6]. Definitive mechanisms of effect of GBV-C -renamed as

human pegivirus [5] - on immune system of HIV patients need to be identified yet.

HCV coinfection is associated with a higher mortality among HIV patients and so more research is needed to clarify mechanism of HIV/HCV interaction to improve treatment options for co-infected individuals [7, 8].

Main objective of this study was to demonstrate the impact of co-infection with pathogenic (HCV) vs. non-photogenic (GBV-C) viruses on immune system among HIV-infected individuals and in this context to evaluate plasma sCD27 as marker for immune activation. We demonstrated increased plasma levels of soluble CD27 among HIV infected patients with HCV co-infection.

MATERIAL AND METHODS

Under study Subjects

Study subject were patients being seen at the Iranian Blood Transfusion (IBTO) during April and September 2011 in Tehran. Sample were serologically determined as HIV positive via ELISA assay and subsequently confirmed by Western blotting in the Central Research Laboratory of IBTO. The patients were at various stages of HIV infection, ranging from asymptomatic to those fitting the CDC case definition of AIDS. Study participants signed informed consent approved by the institutional review board for human studies at IBTO.

Blood samples from a total of 86 patients with HIV infection were taken and were enrolled in the sCD27 plasma levels determination. They were stratified in to four groups according to the GBV-C and HCV coinfection status: HIV (n=20), HIV/ GBV-C (n=14), HIV/ HCV (n=26) and HIV/HCV/GBV-C (n=26).

Laboratory Procedures

Analysis of lymphocyte Subpopulations

Whole blood analyzed for the percentage of CD4+ and CD8+ T-cells by CD3/CD4+ and CD3/CD8+ double staining method and flow cytometric analysis on lymphocyte population gate.

Detection of GBV-C

Plasma GBV-C RNA was detected by RT-PCR as previously described (9) with some modifications. Total RNA was extracted from 200 µL of plasma using the high pure viral nucleic acid kit (Roche, Germany), according to the manufacturer's instructions .Five µL of extracted RNA was added to RT mix containing dNTP, random hexamer primer, RNase inhibitor

and murine leukemia virus reverse transcriptase. cDNA synthesis was performed at 40ċ for 40 min. cDNA was then added to PCR mix containing Taq polymerase, dNTP and primer pairs(forward, flanking NS5a 77-101 region : 5-CTC TTT GTG GTAGTA GCC GAG AGA T-3, and reverse,flanking NS5a 211- 188 region: 5- CGA ATG AGT CAG AGG ACG GGG TAT-3),designed based on the previously reported viral genome . Amplification was performed at 95ċ for 5 min, and then 45 cycles of 94ċ for 45 sec, 55ċ for 45 sec and , 72ċ for 45 sec with a 5-min final extension at 72 ċ. Amplicons were detected by gel electrophoresis on an ethidium bromide-stained 2% agarose gel under UV light. Negative (HGV RNA negative plasma) and positive (HGV RNA positive plasma) controls were included in each run. Finally, the product band was selected and purified with the illustra microspin columns Kit (GE health care, USA), and positive cases were reconfirmed by PCR products sequencing.

SCD27 plasma level

To measure the plasma levels of sCD27, a sandwich enzyme-linked immunosorbent assay (human sCD27 ELISA, eBioscience, UAS) was used. Briefly, subjects' plasma was added to mouse anti- human CD27 coating antibodies that were concomitant with a biotin-conjugated anti-human sCD27 antibody and Streptavidin-HRP. After 3 h incubation and washing, to remove unbound materials, substrate solution was added to the wells. A colored product was formed in proportion to the amount of human sCD27 present in the sample. The reaction was terminated by addition of acid and absorbance was measured at 450 nm. A standard curve was prepared from seven sCD27 standard dilutions and human sCD27 sample concentration determined. This assay detected sCD27 quantities as low as 0.2 U/ml.

Anti-HCV assay

Plasma samples were screened for HCV infection using a commercial ELISA kits (Acon Laboratories, USA) to detect anti-HCV antibodies based on the manufacturer's protocol.

Statistical Analysis

All statistical analyses were carried out using the statistical program SPSS (version 17; SPSS, Chicago, IL); p Values of 0.05 or less were considered significant and p values were two-sided throughout. Kolmogorov- Smirnov tests were performed to determine whether variable

distributions were Gaussian. Because of non-normal distributions, nonparametric statistics were used. The Mann-Whitney U –test was used for dual group comparison .Correlations between sCD27 concentration and other parameters were performed by the Pearson or Spearman's rank test.

RESULTS

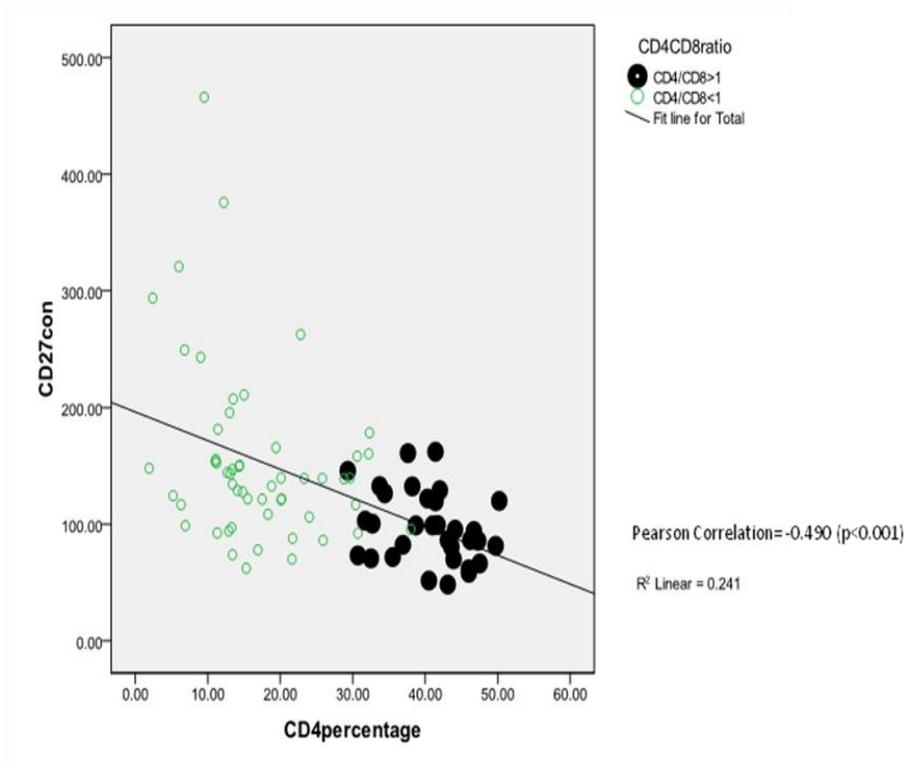*Plasma sCD27 in relation to CD4 positive T cell percentage, sex and age*

Patients with CD4/CD8<1 had higher sCD27 plasma levels than patients with CD4/CD8 ≥1 (Fig.1).According to our finding the plasma level of sCD27 was moderately higher in males as compared to females; however, this difference was not statically significant. The lowest levels of sCD27 in females (51.67 U/ml) belonged to an HIV/GBV-C co-infected 31years old patient with CD4/CD8= 40.5/38.3 and the highest levels (466.12 U/ml) was seen in a 32 years old female with CD4/CD8 = 9.5/66.3 that

belonged to   HIV/HCV/GBV-C coinfected patients. The lowest levels of sCD27 in males was 48.27 U/ml was seen in a 25 years old man with CD4/CD8 = 43.1/31.9 that belonged to HIV/HCV coinfected patients and the highest levels was 320.85 U/ml which belonged to an HIV/HCV co-infected 53years old patient with CD4/CD8= 6/58.5.

Our study did not show a significant correlation of alterations between the level of plasma sCD27 and age of patients (r= 0.109, p = 0.35), confirming data reported previously (10). Negative correlation was seen between the percentages of CD4 positive T cells and plasma sCD27 concentration [Correlation coefficient = -0.490 (p<0.001)]. R^2 (Determination coefficient)   of the linear model was 0.24 (p<0.001) that means twenty four percent of changes in CD4 count might be predictable with knowing sCD27 concentration among HIV positive patients (Fig.2).
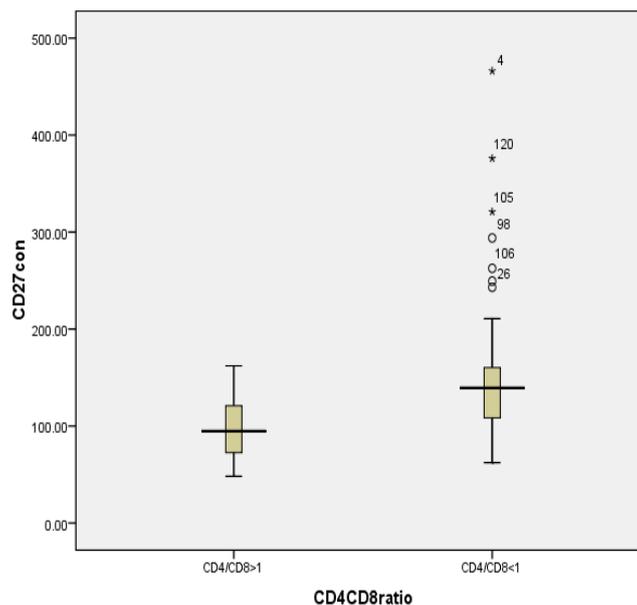
Figure 1. Evaluating of sCD27 plasma levels in relation to CD4 positive T cell percentage. Patients with CD4/CD8<1(n=54) have higher sCD27 plasma levels (154.07 ±75.95 U/ml) than 32 patients with CD4/CD8 ≥1 (97.37±30.42 U/ml) (p < 0.001).

Figure 2. There is a correlation between sCD27 plasma levels in relation to CD4 positive T cell percentage (Correlation coefficient = -0.490) ($p<0.001$).
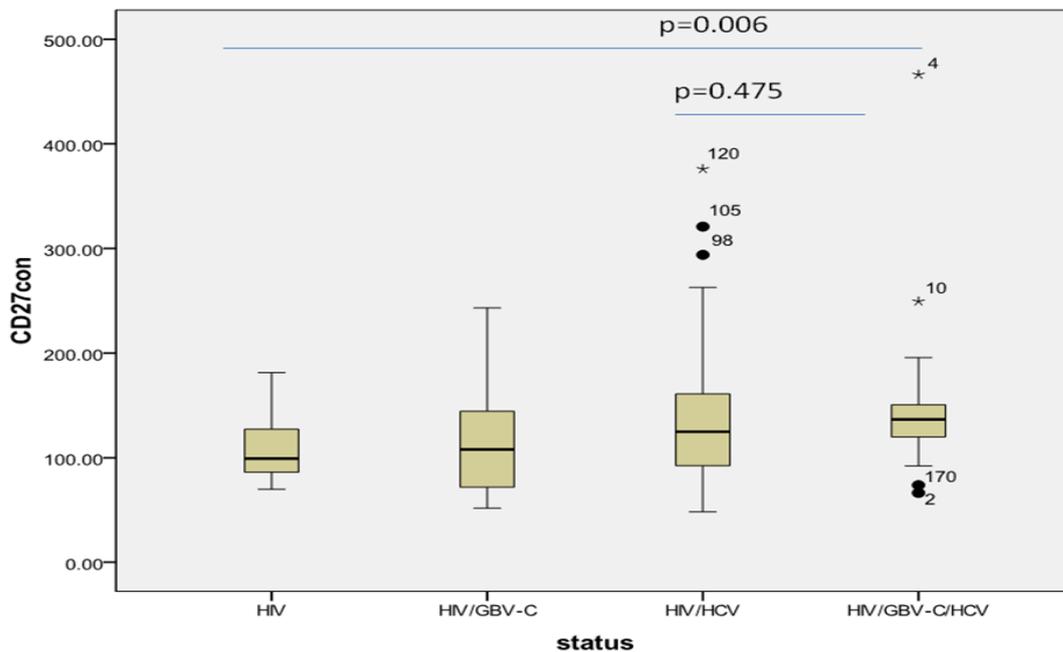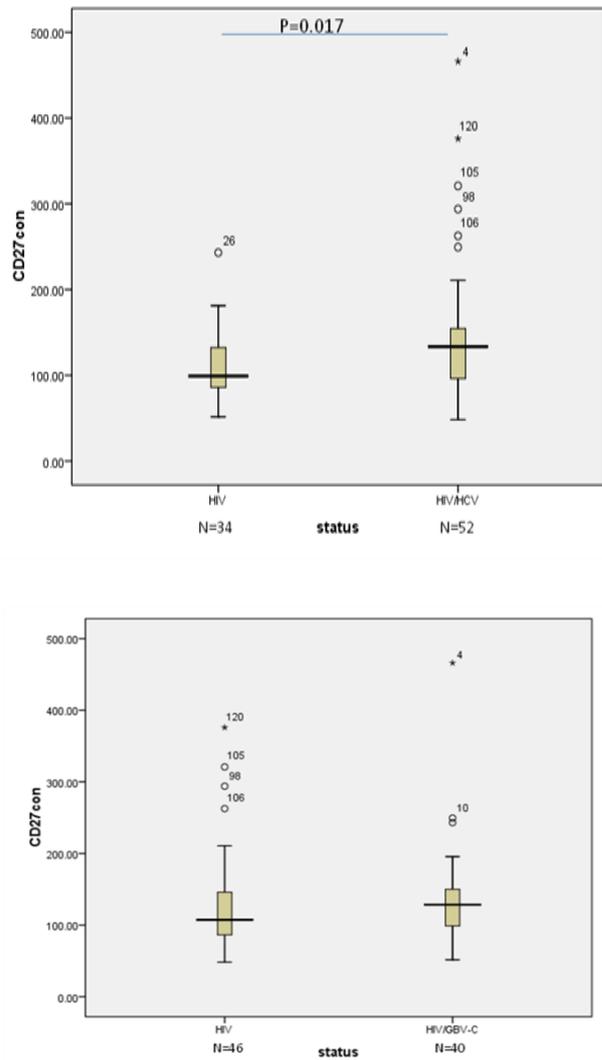

Figure.3. Dependency of sCD27 concentration and co-infection. Box plots represent the 10th, 25th, 50th, 75th, and 90th percentiles for the sCD27 concentration.
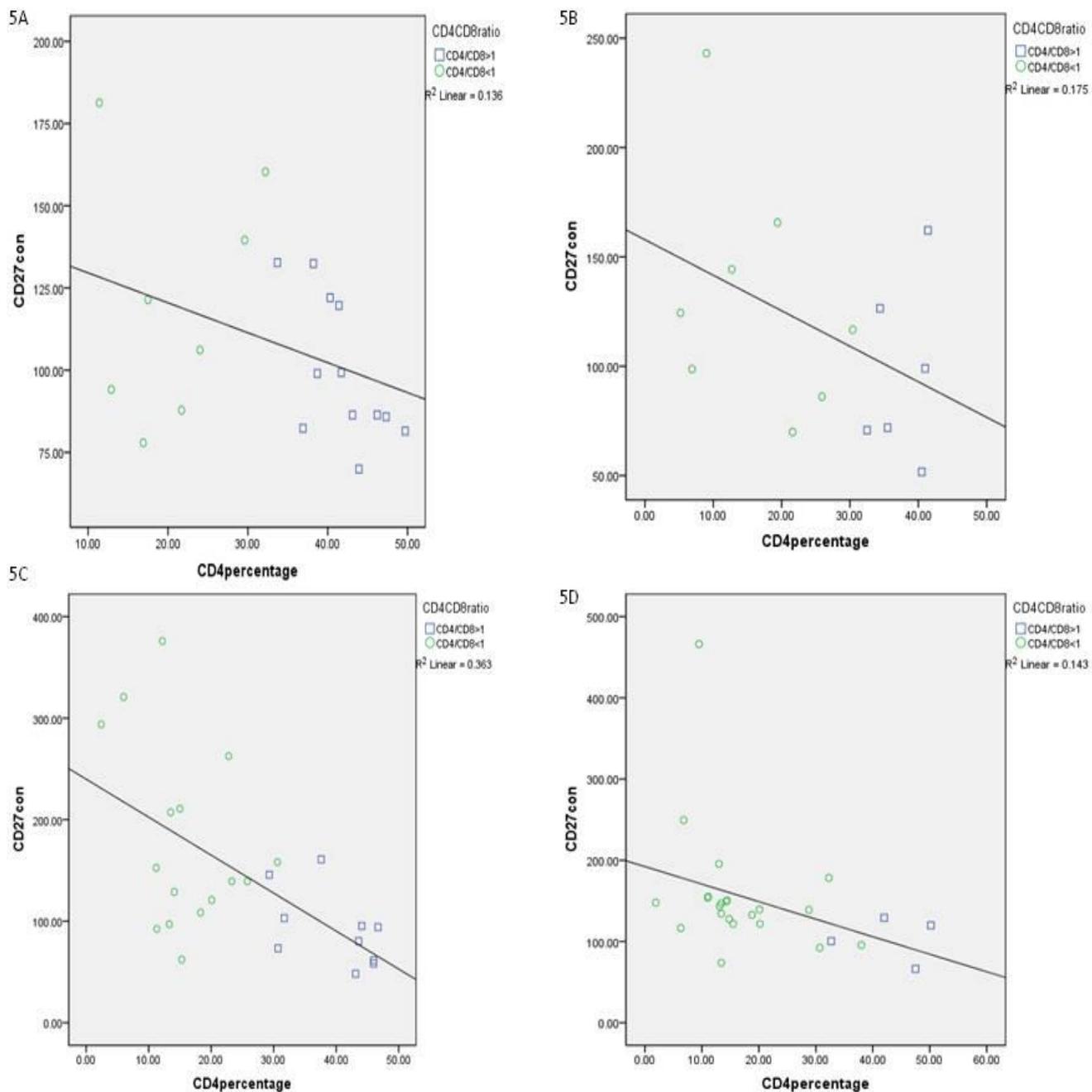
Dependency of sCD27 concentration and Coinfection

Based on the simultaneous infection of HIV positive patients with GBV-C and HCV, eighty six under study cases were stratified into four groups: HIV monoinfected (n=20), HIV/GBV-C (n=14), HIV/HCV (n=26) and HIV/HCV/GBV-C (n=26) co-infected patients. Box plots represent the 10th, 25th, 50th, 75th, and 90th percentiles for the sCD27 concentration and are shown in figure 3. The lowest levels of sCD27 (48.27 U/ml) was seen in a 25 years old man with CD4/CD8 = 43.1/31.9 that belonged to HIV/HCV co-infected patients and the highest levels (466.12 U/ml) was seen in a 32 years old female with CD4/CD8 = 9.5/66.3 that belonged to HIV/HCV/GBV-C co-infected patients. Plasma level of sCD27 was higher in HIV/HCV/GBV-C patients as compared to HIV mono-infected patients this difference was statically significant (p=0.006).

There was significant difference in the sCD27 concentration between HIV-infected individuals with and without HCV coinfection (P=0.017) but, there was no significant difference in the sCD27 concentration between HIV-infected individuals with and without GBV-C coinfection (Figure 4). We also investigate the correlation of sCD27 in relation to CD4 positive T cell count for groups separately and found that sCD27 determination coefficient R^2 of the linear model were approximately similar in HIV monoinfected, HIV/GBV-C and HIV/HCV/GBV-C co-infected patients (0.136, 0.175 and 0.143 respectively) but among HIV/HCV co-infected patients group was in highest level (0.363) (Fig 5).

Figure.4 Levels of sCD27 concentration according to HIV infection and HCV and/or GBV-C co-infection. Significant difference in the sCD27 concentration between HIV-infected individuals with and without HCV coinfection unlike GBV-C coinfection was seen (Figure.4A&B).

Figure.5. Dependency of sCD27 concentration and Coinfection status. (A)HIV monoinfected, (B) HIV/GBV-C, (C) HIV/HCV and (D) HIV/HCV/GBV-C coinfected patients. Strength of correlation among HIV/HCV coinfected patients was the best (R^2= 0.36, P=0.001).

DISSCUSSION

Remarkable efforts have been put into association of plasma biomarkers with HIV-1 disease progression to finding markers, which is relatively easy to perform, as a good alternative of CD4 count and/or viral load for monitoring HIV disease progression. Soluble CD27 is proposed to be the most suitable marker for monitoring HIV-1 disease progression by Messele et al [2].

In this report in agreement with other studies our results indicated negative correlation between the CD4 positive T cell count and plasma sCD27 concentration among HIV infected patients [4, 10-15].

We sought to analyses role of HCV and GBV-C co-infections on HIV-related immune activation, through measuring sCD27 plasma levels in HIV/GBV-C, HIV/HCV and HIV/HCV/GBV-C co-infected patients in comparison with monoinfected patients. We demonstrated increased plasma levels of soluble CD27 among HIV infected patients with HCV co-infection. Based on results there was significant differences in the plasma levels of sCD27 between HIV-infected individuals with and without HCV coinfection (P=0.017). Correlation between sCD27 and number of CD4+T-cells was in highest level among HIV/HCV co-infected patients group (Fig5C). CD27 can be shed from the cell surface upon cell activation. Therefore, higher sCD27 plasma levels and also lower CD27 cell surface expression in HCV infection are attributable to increased shedding by response to immune stimulation (16). Our findings confirm impaired induction of CD27 in chronic HCV infection reported by Yonkers et al [16].Regulation of CD27 is thought to be crucial for immune response formation in which impaired CD27 signaling is known to contribute to lack of a sustained memory T cell pool [16]. Therefore deregulation of CD27 levels among HIV/ HCV co-infected patients might be contribute to inadequate formation of virus specific T cell responses during chronic infection and reduced responsiveness to immunomodulator therapy among HIV/HCV co-infected subjects.

Given the high prevalence of HCV coinfection in HIV patients, there has been a high demand in finding new biomarkers of HIV/HCV pathogenesis and also finding new markers for improving treatment options for coinfected individuals under these chronic infection statuses. The magnitude of sCD27 plasma level alterations in relation to clinical conditions like post-HCV infection glomerulonephritis and other non-AIDS-related causes of death among HCV co-infected HIV subjects need to be defined. For instance sCD27 is a more sensitive marker for renal tubule apoptosis than plasma creatinine [17]. Therefore, in certain clinical scenarios including post-HCV infection glomerulonephritis increased level of sCD27 seems to be indicative, however, further studies should be conducted on HIV/HCV coinfected patients with renal dysfunction in order to verify this hypothesis.

As the transmission routes are shared by HIV, HCV and GBV-C infections, coinfection is common but there is remarkably little published about the potential complex interactions among GBV-C, HCV, and HIV in triply infected subjects and results are lacking for HIV infected patients who were infected by both HCV and GBV-C [6, 18]. Our data indicate that in higher plasma level of sCD27 among our HIV/HCV/GBV-C patients (Fig.3), which leaves unanswered the question, is GBV-C a beneficial infection? Longitudinal studies are needed to define the role of GBV-C-associated modulation of HIV disease. However existing data indicate that in contrast to HCV, GBV-C does not induce a more sCD27 plasma level in HIV positive patients (Fig.4B). This finding supports the theory that GBV-C is not pathogenic [18, 19].

We confirm that there is some limitation in cross sectional study by studying in a single time point; however our work generates some preliminary finding in role of co-infections on HIV-related immune activation.

CONCLUSION

Increased plasma levels of soluble CD27 among HIV infected patients with HCV but not with Human Pegivirus coinfection.sCD27 seems to be a valuable marker for monitoring of clinical features in HIV/HCV coinfection, where the immune activation plays a role in pathogenesis. More studies are needed to define the magnitude of sCD27 plasma level alterations in relation to the clinical conditions like post infectious glomerulonephritis and lymphoma among HIV/HCV patients.

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