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Research

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Determination on the Expression of Activated RANTES G 28 in HIV-1 Infection and in Co Infection with Hepatitis B Virus and Hepatitis C Virus and Its Effect on Leukocytes

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ABSTRACT

Human Immunodeficiency Virus (HIV), coinfected with hepatitis B virus and hepatitis C virus challenges the immune system. Regulated on activation normal T cell expressed and secreted (RANTES) is one of the natural ligands for the chemokine, uses receptor which potently suppresses viral infection. We examined possible activation of RANTES G 28 in HIV-1 infection and in coinfection with HBV and HCV and its effect on leukocytes. Leukocytes from HIV-1infected HIV-1/HBV and HCV/HIV co-infected individuals were assessed by flow cytometry and Abacus 5 automated. RANTES G 28 was determined by Polymerase Chain Reaction. RANTES G 28 demonstrated positive correlation with HIV-1, HIV/HBV, HIV-1/HCV coinfection and leukocytes. The association presented difference in coefficient and predication of its occurrence. High expression of RANTES G 28 was indicated with stated the leukocytes. Difference in significance was indicated. There is need of more research to study and understand the functions of RANTES G 28 with reference to human system and its role in immunity.

KEYWORDS: HIV-1 infection; HIV-1/HBV coinfection; HIV-1/HCV coinfection; Leukocytes; Rantes G 28.

ABBREVIATIONS: RANTES: Regulated on activation normal T cell expressed and secreted; GAG: Glycosaminoglycans; CTL: Cytotoxic T Lymphocytes; HIV: Human Immunodeficiency Virus; CBRD: Department of Center for Biotechnology Research Development; CVR: Centre for Virus Research; SRC: Scientific Research Committee; KEMRI: Kenya Medical Research Institute; ERC: Ethical Research Committee.

INTRODUCTION

Many studies have reported variants in the genes encoding HIV-1 coreceptors and their natural ligands, which have been shown to modify HIV-1 infection and disease progression.[1] Due to their shared routes of transmission, HIV-1 and HCV coinfection (HCV/HIV-1) is common, affecting approximately 25-33% of HIV-1-infected persons.[2] HIV/HCV co-infection has been associated with a faster rate of hepatitis C disease progression, higher HCV viral loads, and a greater risk of developing severe liver damage.[3] Human immunodeficiency virus (HIV) infection has an adverse impact on HBV-related liver disease progression, with higher serum HBV DNA polymerase activity, lower rates of loss of serum hepatitis B e antigen, and increased risk of cirrhosis, liver-related mortality, and hepatocellular carcinoma at lower CD4 T-cell counts. Chemokine has been shown to play a role in immune responses to viral infections. RANTES (regulated on activation normal T cell expressed and secreted was originally considered a T cell-specific chemokine, it is now known to be expressed by a number of other cell types including epithelial cells and platelets and acts as a potent chemoattractant for many cell types such as monocytes, NK cells,[4] memory T cells,[5] eosinophils[6] and DCs.[7] RANTES is a CC chemokine which preferentially has chemoattractant properties for monocytes and mem-

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ory T cells.[8] It is a pro-inflammatory chemokine and is found at the sites of many inflammatory disorders. It is also intimately linked with the function of Cytotoxic T Lymphocytes (CTL), which are important in the control of many intracellular pathogens.[9] RANTES is present in CTL granules, where it is complexed to Glycosaminoglycans (GAG), and is secreted during degranulation.[10,11] The −28C/G polymorphism in the RANTES promoter region had been found to affect the transcription of the RANTES gene. In human cell lines, the −28G allele was shown to increase promoter activity of RANTES, suggesting that the polymorphism can regulate RANTES expression in the human body and may delay HIV-1 disease progression.[12] Together with the other chemokines which bind to CCR5, RANTES is able to suppress HIV-1 infection *in vitro*.[13] The potential for the use of RANTES in antiviral therapy for HIV-1 infection has led to more detailed studies of its biology. Recent studies have shown that RANTES at micromolar concentrations can activate T cells, apparently acting as an antigen-independent T cell activator: this activity is independent of binding to CCR5 and other chemokine receptors.[13]

MATERIALS AND METHODS

Blood samples were obtained from an ongoing national study project, CD4 testing a national testing service for HIV infected individuals served at department of Center for biotechnology research development (CBRD) in Kenya Medical Research Institute (KEMRI), established in 2008. The Ethical Research Committee (ERC) and Scientific Research Committee (SRC) of the Kenya medical research institute (KEMRI) approved the protocol for the ongoing national study project and informed consent was obtained from each participant with inclusion.

This was a cross-sectional study carried out in the Department of Center for biotechnology research development (CBRD) and Centre for Virus Research (CVR) at Kenya medical research institute (KEMRI).The study was approved by Jomo Kenyatta University of Agriculture and Technology, Institute of Biotechnology Research department. Blood samples from HIV positive individuals above 18 years who had consented. The blood sample size was determined using standard statistical formula, $n=Z^2\ pq/d^2$.[14]

Venous blood (5 mL) was obtained. CD4, CD3 counts was accomplished with cytometric method using FACS caliber machine of Becton Dickinson (CA, USA). Absolute lymphocytes, neutrophils, monocytes, eosinophils, basophils and platelets counts were accomplished with Abacus 5 automated Blood Analyzer technologies of DIATRON MI PLC GROUP (Budapest, Hungary) Anti-HCV antibody was assayed using HCV ELISA -MUREX anti-HCV (ABBOTT Diagnostics Division Murex Biotech Limited Dartford, UK). Anti-HBV antibody was assayed using HBV Elisa-Hepanostika HBsAg Ultra; Hepanostika® HBsAg Ultra (bioMérieux Diagnostics, Lyon, France). HIV-1 was determined by The COBAS® AmpliPrep/COBAS® TaqMan (Roche Molecular Diagnostics, Pleasanton, California®). DNA was extracted using QIAGEN (QIAGEN GROUP Kits plasmid© 2011 QIAGEN), according to the manufacturer instructions.

RANTES G 28, A cytosine/guanine transversion at position 28 in human was studied using following primers: Forward: 5"-ACA GAG ACT CGA ATT TCC GGA-3". Reverse: 5"-CCA CGT GCT GTC TTG TTG ATC CTC-3" (Eurofins MWG/ Operon Germany).

The PCR cycles were 94 °C for 5 min, 35 cycles each of 94 °C for 1 min, 62 °C for 1 min and 72 ºC for 1 min. A final extension was 72 °C for 5 min. Gene Amp PCR machine (Applied Biosystems®). Amplified products and Dna ladder (Eppendorf®, Germany), of 100 base pair were loaded to a 1.5% agarose gel, (Sigma®, USA) and subjected to an electric current of 70 volts for 50 min and stained using ethidium bromide (Sigma®, USA) at concentration of 10 µl concentrated ethidium bromide in 100 µl Tris boric acetate for 20 min.[15]

Data Analysis

The data was entered and analyzed using SPSS statistical software and the differences in association was evaluated by Pearson's correlation, ANOVA and Regression was considered for significant.

RESULTS

Sero prevalence of HBV and HCV

The sero prevalence of HBV and HCV among HIV positive blood samples demonstrated out of 226 blood samples was as follow; HIV-1/HCV 8%, HIV-1/HBV 3% and HIV-1/HBV/HCV 0.9% (Figure 1).

Infection with HIV-1 demonstrated occurrence of HBV (3%), HCV (8) % and both HBV and HCV infections (0.9%).

Distribution of RANTES G 28 among HIV-1-infection, HIV-1/HCV, HIV-1/HBV and HIV-1/ HBV/HCV coinfection.

Frequency occurrence of RANTES G 28 indicated out of the total blood sample; 167 HIV-1 expressed RANTES G 28, 6 HIV/HBV, 15 HIV/HCV and 2 HIV/HBV/HCV coinfection demonstrated RANTES G 28 expression. Failure RANTES G 28 expression was demonstrated with HIV-1 and coinfection with HBV and HCV (Table 1).

RANTES G 28 was highly expressed in presence of HIV-1 infection. Expression of RANTES G 28 was also demonstrated with HIV-1/HBV, HIV-1/HCV coinfection. Failure RANTES G 28 expression was indicated with HIV-1 and coinfection with HBV and HCV.

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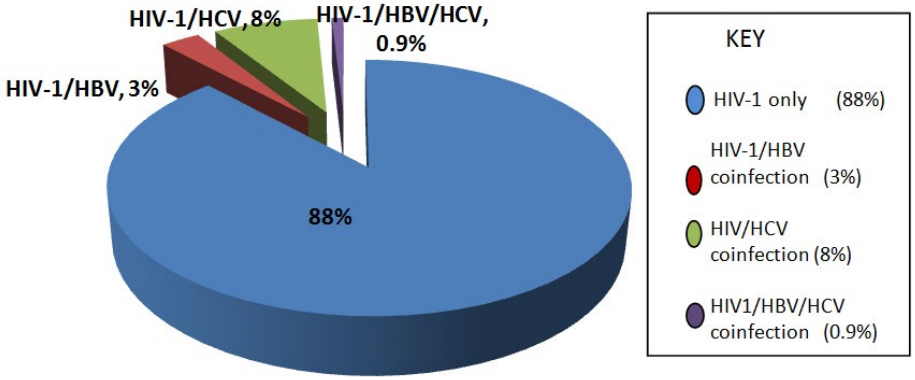

Figure 1: Sero prevalence among 226 blood samples collected from health centre of HIV-1/HCV, HIV-1/HBV and HIV-1/HCV/HBV coinfection.

	HIV-1(only) infection		HIV/HBV coinfection		HIV/HCV coinfection		HIV/HBV/HCV coinfection
	RANTES G 28 positive	RANTES G 28 negative	RANTES G 28 positive	RANTES G 28 negative	RANTES G 28 positive	RANTES G 28 negative	RANTES G 28 positive
Frequency occurrence(No)	167	32	6	1	15	3	2
% Distribution	76.9	10	86	14	83	17	100

Table 1: Frequency distribution of RANTES G 28 among HIV-1-infection, HIV-1/HCV and HIV-1/HBV coinfection.

Effect of RANTES G 28 on HIV-1, HIV-1/HBV, HIV-1/HCV and HIV-1/HBV/HCV coinfection.

A positive correlation was demonstrated between RANTES G 28 and HIV-1 infection, HIV/HBV, HIV-1/HCV and HIV-1/HBV/HCV coinfection .HIV-1infection, HIV/HBV and HIV-1/HBV/HCV coinfection indicated positive coefficient however HIV-1/HCV indicated negative coefficient. Positive predication of RANTES G 28 was indicated in presence HIV-1 infection, HIV/HBV, HIV-1/HCV and HIV-1/HBV/HCV co infection (Table 2).

	R	R^2	Un std coefficient	Sig
HIV-1	0.049	0.02	0.015	0.465
HIV-1/HBV	0.022	0.001	0.041	0.738
HIV-1/HCV	0.265	0.068	-0.329	0.000
HIV-1/HBV/HCV	0.040	0.002	0.152	0.552

Dependent variable: RANTES G 28 p=0.05

Table 2: Expression of RANTES G 28 on HIV-1, HIV-1/HBV, HIV-1/HCV and HIV-1/HBV/HCV coinfection.

A positive correlation between RANTES G 28 and HIV-1 infection, HIV/HBV, HIV-1/HCV and HIV-1/HBV/HCV coinfection was demonstrated. The size effect of RANTES G 28 with additional effect of HIV-1 infection, HIV/HBV and HIV-1/HBV/HCV coinfection was to increase but decrease in presence of HIV-1/HCV coinfection. Positive prediction of RANTES G 28 occurrence in presence of and HIV-1 infection, HIV/HBV, HIV-1/HCV and HIV-1/HBV/HCV coinfection was indicated.

Effect of RANTES G 28 on leukocytes (CD3, CD4, lymphocytes, neutrophils, monocytes, eosinophils, basophils and platelets).

A positive correlation was indicated between RANTES G 28 and all leukocytes (CD3, CD4, absolute lymphocytes, neutrophils, monocytes, eosinophils, basophils and platelets). Difference in coefficient was demonstrated between RANTES G 28 and all stated leukocytes and contrast in fraction of variation indicated (Table 3).

	R	R^2	Adjusted R	F static	UnStd coefficient (beta)	Sig.
CD3	0.021	0.000	-0.004	0.100	0.021	0.752
CD4	0.021	0.000	-0.004	0.096	0.021	0.757
LYM	0.103	0.011	0.006	2.400	0.103	0.123
NEO	0.106	0.011	0.007	2.538	-0.106	0.113
MON	0.015	0.000	-0.004	0.051	0.015	0.84
EOS	0.009	0.000	-0.004	0.019	-0.009	0.892
BAS	0.079	0.006	0.002	1.409	-0.79	0.186
PLT	0.003	0.000	-0.004	0.003	0.003	0.960

Dependent variable - RANTES G 28 P=0.05

Table 3: Effect of RANTES G 28 on CD3, CD4, lymphocytes, neutrophils, monocytes, eosinophils, basophils and platelets.

Positive correlation between RANTES G 28 and all

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leukocytes was demonstrated. The size effect of RANTES G 28 with additional effect of CD3, CD4, absolute lymphocytes, monocytes and platelets was to increase. In presence of neutrophils, eosinophils and basophils, RANTES G 28 size effect was to reduce. Positive prediction of occurrence of RANTES G 28 in presence of leukocytes was indicated.

Expression of RANTES G 28 in leukocytes infected with HIV-1.

At different cell distribution (cell/mm^3 and cells/uL), CD3, CD4, absolute lymphocytes, neutrophils, monocytes, eosinophils, basophils and platelets exhibited possibility of expressing RANTES G 28.

RANTES G 28 expression in the presence absolute lymphocytes and basophils: Frequency distribution (cells/mm^3) of absolute lymphocytes and basophils showed high percent of cells activated RANTES G 28 with difference in mean±SD.

Expression of RANTES G 28 on human absolute lymphocytes and basophils distribution (cell/mm^3) infected with HIV-1. Data present the mean±SD of the maximum response of each subject to RANTES G 28 of two experiments, basophils and absolute lymphocytes (Figures 2 and 3).

RANTES G 28 expression in the presence of Eosinophils and Neutrophils: Frequency occurrence (cells/mm^3) of eosinophils and neutrophils showed high percent of cells activated RANTES G 28 with difference in mean±SD.

Expression of RANTES G 28 activated by various cell distributions (cell/mm^3) of neutrophils and monocytes infected with HIV-1. Data represent the mean±SD of the maximum response of each subject to RANTES G 28 of two experiments with difference of human eosinophils and neutrophils (Figures 4 and 5).

RANTES G 28 expression in the presence of CD3 and Monocytes: Frequency distribution (cells/mm^3) of CD3 and Monocytes showed high percent of cells expression RANTES G 28 with difference in mean±SD.

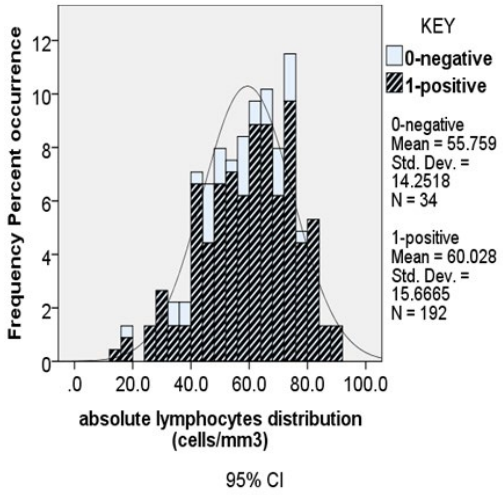
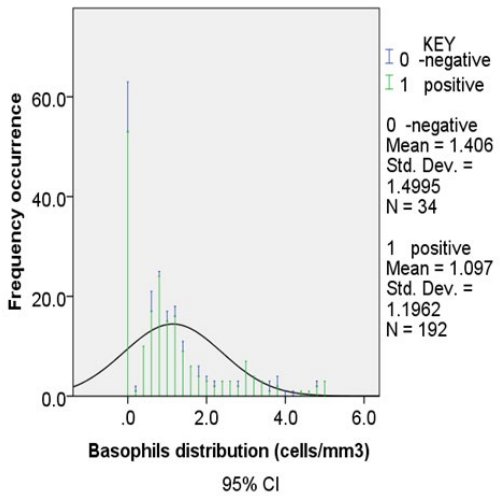


Figure 2



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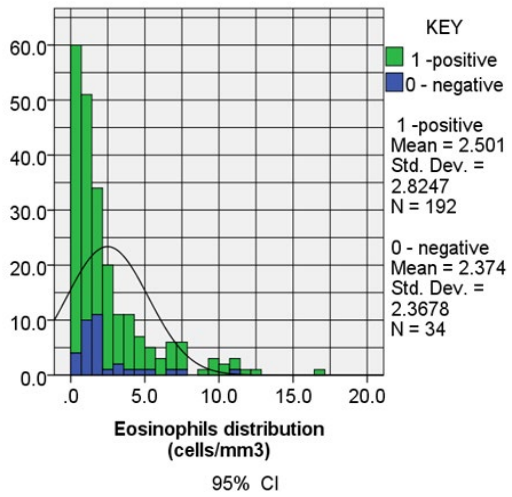

Figure 4

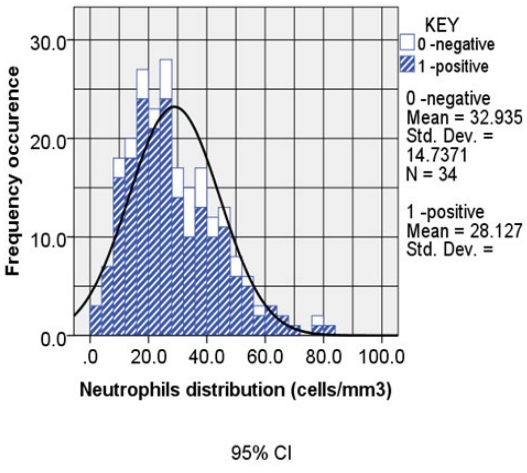

Figure 5

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Expression of RANTES G 28 on monocytes and CD3 expressed at various cell distribution (cells/mm^3) infected with HIV-1. Data represent the mean±SD of the maximum response of each subject to RANTES G 28 of two experiments with difference of human CD3 and monocytes (Figures 6 and 7) .

RANTES G 28 expression in the presence of CD4 and platelets: Frequency distribution (cells/mm^3) of CD4 and platelets (cells/ul) showed high percent of cells activated RANTES G 28 with difference in mean±SD.

Effect of RANTES G 28 on CD4 (cells/mm^3) and Platelets(cells/uL) expressed at various cell distribution infected with HIV-1. Data represent the mean±SD of the maximum response of each subject to RANTES G 28 of two experiments with difference of human CD4 and platelets (Figures 8 and 9).

DISCUSSION

There is evidence of RANTES G 28 expression in HIV-1, HIV-1/HBV, HIV-1/HCV and HIV-1/HBV/HCV coinfection and, in association with leukocytes. The RANTES-CCR5 pathway can influence immune responses in multiple ways during acute viral infections.[16,17]

The frequency occurrence of RANTES G 28 was shown to be highly expressed with HIV-1 infection this was also demonstrated by HIV-1/HBV, HIV-1/HCV and HIV-1/HBV/HCV coinfection. Few cells with HIV-1 infection, HIV/HBV and HIV/HCV coinfection failed to activate RANTES G 28.

A positive correlation between RANTES G 28 and HIV-1 infection, HIV-1/HBV, HIV-1/HCV and HIV-1/HBV/HCV coinfection was indicated and the additional effect of HIV-1 infection, HIV-1/HBV and HIV-1/HBV/HCV coinfection showed no significant size effect of RANTES G 28 was to increase. The additional effect of HIV-1/HCV co infection demonstrated no significant size effect of RANTES G 28 was to reduce.

In presence of HIV-1 infection, HIV-1/HBV, HIV-1/HCV and HIV-1/HBV/HCV coinfection a positive prediction

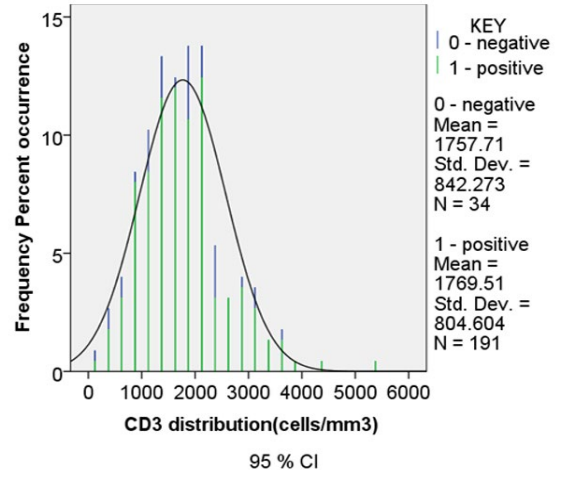

Figure 6

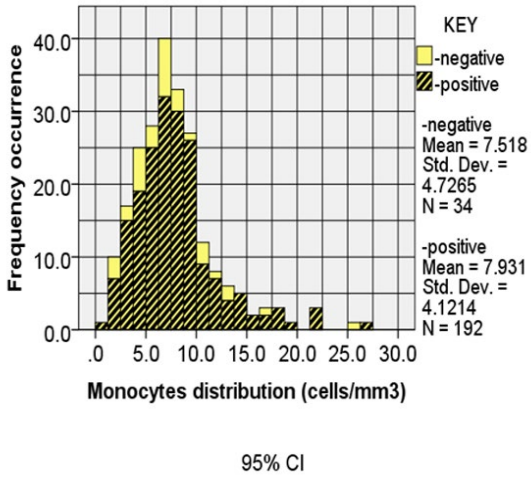

Figure 7

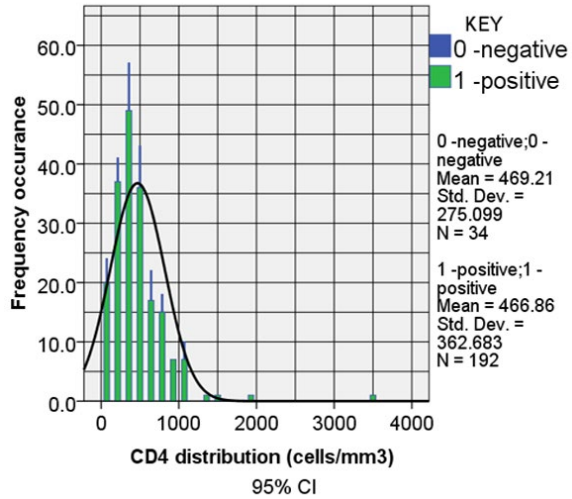

Figure 8

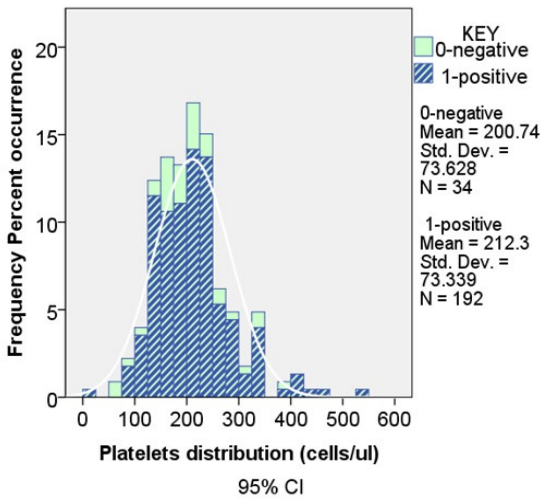

Figure 9

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of RANTES G 28 occurrence was demonstrated.It was reported RANTES - 28C/G demonstrated a marginal association with the susceptibility to HIV-1 infection and was highly activated with HIV-1 infected cells[18] and earlier study by Promrat, et al.[19] could not find any association of RANTES G–28 polymorphisms with susceptibility to HBV infection

A positive correlation was demonstrated between RANTES G 28 and CD3, CD4, absolute lymphocytes, neutrophils, monocytes, eosinophils, basophils and platelets. Chemokines recruit T cells with a Th1/Tc1 phenotype expressing specific chemokine receptors such as CCR5.[20]

The additional effect of CD3, CD4, absolute lymphocytes, monocytes, basophils and platelets showed no significant ($p>0.05$) size effect of RANTES G 28 was to increase. Hadida, et al.[21] demonstrated RANTES binds on lymphocytes *via* CCR5 receptor and enhances HIV-1–specific CTL activity. $CD4^+$ T cells with HIV-1 infection and increase RANTES expression *in vivo*.[19]

Positive predication of RANTES G 28 occurrence was demonstrated with absolute lymphocytes, CD4, basophils demonstrated. Platelets and CD3 failed to predict RANTES G 28 occurrence,[22] Activated platelets produced high quantities of RANTES[23] while pre-incubation of platelets with HIV gp41 enhances their release of RANTES.

At 95 CI, CD3, CD4, absolute lymphocytes, neutrophils, monocytes, eosinophils, basophils (cells/mm^3) and platelets (cells/ul) at different levels of distribution expressed of RANTES G 28. RANTES G 28 responded strongly to leukocytes activation in presence of HIV-1 infection, HIV-1/HBV, HIV-1/HCV and HIV-1/HBV/HCV coinfection. Appay, et al.[24] demonstrated RANTES could mediate enhancement of the cytotoxicity and at high concentrations and able to activate cells.

These results reported not all cells infected with HIV-1 infection, HIV-1/HBV, HIV-1/HCV and HIV-1/HBV/HCV coinfection expressed RANTES G 28, also the results demonstrated presence of extensive patterns of expression of RANTES G 28 with all leukocytes.

RANTES-induced T cell activation, which correlated directly with the amount of RANTES bound to the cells[25] at low concentrations.

In conclusion, RANTES G 28 is highly expressed in presence of HIV-1infection, and in coinfection with HBV and HCV. CD3, CD4, absolute lymphocytes, neutrophils, monocytes, eosinophils, basophils and platelets in presence of the HIV-1,HIV-1/HBV,HIV-1/HCV and HIV-1/HBV/HCV coinfection highly express RANTES G 28.[26] Not all cells in presence of HIV-1 and coinfections expresses RANTES G 28. The association presented difference in significant and predication of its occurrence which defines the pattern of responses. More research is required to understand the effects of RANTES G 28 with reference to HIV-1 infection, HIV-1/HBV, HIV-1/HCV and HIV-1/HBV/HCV coinfection and in presence of cells of immunity.

COMPETING INTEREST

The authors declare that they have no competing interest.

AUTHOR'S CONTRIBUTION

Contributions to perform the experiments; assisted in provision of reagents and experiment machines, data analysis and writing process of the paper.

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