Arch. Bas. App. Med. 8 (2020) 17 – 19
www.archivesbamui.com

Short Communication

Serum Chemokine Levels in HIV-infected Nigerian Patients

Onifade A.A, Rafiu A.T and *Arinola O.G
Department of Chemical Pathology and Immunology, University of Ibadan, Nigeria

Received: September 2019; Accepted: December, 2019

Abstract
The human immunodeficiency virus (HIV) is a retrovirus belonging to the family of lentiviruses that destroys CD4+ T-cells and resides in other immune cells that produces chemokines. Chemokines [such as Macrophage inflammatory protein-1α and 1 β (MIP-1α and MIP-1β), RANTES (Regulated on Activation Normal T expressed and Secreted Protein), and SDF-1 (Stromal Derived Factor 1)] are small proteins that attract leucocytes and play complex roles in coordinating immune responses. The interaction between chemokines and their receptors expressed on immune cells inhibits HIV-1 attachment and replication. However, studies on association between these chemokines and HIV produced different results and the underlying mechanisms of chemokines during HIV infection remain unclear. A total of 90 participants were recruited in this study which included 45 HIV infected and 45 apparently healthy HIV uninfected individuals that served as control. Serum levels of chemokines were determined using ELISA as described by the manufacturer of the kits. Appropriate statistical data were employed and $p \leq 0.05$ was considered significant. HIV infected individuals had insignificantly reduced serum levels of SDF-1, MIP1-α and RANTES compared with control. The results of this study is in agreement with previous work that chemokines are involved in the pathogenesis of HIV through their receptors and could be explore as option for therapeutic strategies

Key Words: *Cytokines, Chemokines, HIV, Therapeutic target.*

INTRODUCTION

Chemokines are potent activators and chemoattractants for certain leukocyte populations whose actions are mediated by a family of 7-transmembrane G-protein–coupled receptors. Significant advances have been made in understanding the regulation of chemokine receptor expression and the intracellular signaling mechanisms used in bringing about cell activation (Kalinkovich *et al*., 1999). Chemokine receptors have also been implicated in several disease states including allergy, psoriasis, atherosclerosis, malaria and human immunodeficiency virus Type 1 (Murdoch and Finn, 2000).

HIV infects immune cells of the macrophage and T-cell lineage. Circulating monocytes and resident macrophages secrete pro-inflammatory cytokines and chemokines which recruit and activate CD4+ T cells among other cells. HIV entry into these cells requires CD4 as a receptor in addition to a co-receptor which is frequently either chemokine receptor CCR5 or CXCR4 (Gorry & Ancuta, 2011; Wang *et al*., 2017). Binding and entry into human cells require the two HIV envelope glycoproteins gp120 and gp41 (Tagliamonte *et al*., 2011). To facilitate HIV-1 entry into human cells, Gp120 binds to human cellular CD4 with high affinity which causes a conformational change in Gp120 that reveals a co-receptor binding site. The binding to one of the chemokine receptors is facilitated to induce conformational change in the glycoprotein gp41 N-terminus. A fusion peptide portion of gp41 inserts into the host cell membrane and lowers energy that is required for fusion of the host and viral membranes (Tagliamonte *et al*., 2011) and the viral core is thus translocated into the cytoplasm of the host cell (Gorry & Ancuta, 2011).

Three CC-chemokines [MIP-1α (CCL3), MIP-1β (CCL4), and RANTES (CCL5)] bind to CCR5 whereas 1 CXC-chemokine [SDF-1] binds to CXCR4 (Colina *et al*., 2013; Moore and Stevenson, 2000). It was previously suggested that MIP-1α, MIP-1β and RANTES inhibits R5 HIV-1 infection of CD4 T-cells, peripheral blood monocytic cells, macrophages, and dendritic cells and that SDF-1 prevents infection by X4 strains and macrophages, CD4 and CD8 T-cells, and natural killer cells are major cellular sources of MIPα, MIP-1β, and RANTES (Brandt *et al*., 2002). Since these cells are involved in protection against HIV-1 infection, it is hypothesized by the present investigators that the serum levels of these chemokines may also be affected. This study determined serum levels of three CC-chemokines (MIP-1α, MIP-1β and RANTES) and one CXC-chemokine (SDF-1) which binds two critical chemokine receptors (CCR5 and CXCR4) in HIV pathogenesis.

MATERIALS AND METHODS

The study was approved by Oyo State Ethical Review Committee, Ministry of Health, lbadan and informed consent was obtained from each of the participants prior to specimen collection. A total of ninety participants (45 HIV-positive and 45 HIV-negative individuals) were recruited for the study. The study was carried out on patients attending PEPFAR Clinic,

**Author for Correspondence:* Tel: +234 8059638379 E-mail: abdufattahkunle@gmail.com

Adeoyo State Hospital, lbadan, Oyo State, Nigeria. Five (5) ml of venous sample collected into bottle without anticoagulants was centrifuged at 4000rpm for 10 min and serum collected was stored at -20$_o$C for two weeks till laboratory analysis for the chemokines using ELISA method as described by the manufacturer (Quantikine, R&D Systems, Minneapolis, MN). Briefly, 100 µl of standard or sample was added to wells of microtiter plates coated with antibody to the chemokines. Following incubation and washing, 200µl of enzyme (conjugated with the respective antibodies) was added. After incubation and washing, 200µl of substrate was added per well, and colour development was stopped by the addition of 2 N sulfuric acid. The plates were read by ELISA reader at 450 nm, and chemokine concentrations were calculated from a standard curve of corresponding chemokine.

The serum levels of chemokines were presented as Mean ± Standard Error of Mean and Mann-Whitney U test was used to compare the differences between the Means

RESULTS

The table below shows comparison of chemokines in HIV positive and HIV negative groups. The serum levels of SDF-1, MIP-1α and RANTES were significantly reduced in HIV positive individuals compared with HIV negative individuals.

Table 1:
Comparison of the mean chemokines (pg/ml) in HIV positive individuals and control.

Chemokines	HIV positive	Controls	U	P-value
SDF-1	570.5 ± 3.37	584.6 ± 5.22	2.171	0.03*
MIP-1α	16.1 ± 3.79	30.8 ± 5.94	3.131	0.002*
MIP-1β	11.7 ± 0.49	12.6 ± 1.33	0.234	0.815
RANTES	32.1 ± 7.50	49.7 ± 9.22	3.653	.000*

**Significant at p < 0.05*

DISCUSSION

Although chemokines and their receptors seem to be relevant to the pathogenesis of HIV infection, their exact role in disease progression *in vivo* has not been established (Kedzierska and Crowe 2001; Wang *et al*., 2017). Measurements of chemokines have been performed from serum and supernatants of stimulated cells however, the results have been contradictory. A study revealed that HIV patients have pronounced decline of serum CC-chemokines (Ye *et al*, 2004). Zanussi *et al*., 1996 reported that increased serum levels of RANTES and MIP-1s were associated with HIV progression, while others indicated that plasma levels of CC-chemokines have no correlation with disease progression or viral burden (Sriwanthana *et al*., 2001).

Moreover, the effect of HAART (Highly Active Anti-retroviral therapy) on the production of CC-chemokines remains unclear. Increased (Bisset *et al*., 1997), decreased and unchanged (Pierdominici *et al*., 2002) levels of circulating and inducible CC-chemokines have been observed during treatment. Therefore, there is need to determine the levels of chemokines in HIV patients in order to provide support for the basis of its therapeutic exploration. In the present study, significantly reduced levels of serum RANTES, SDF-1 and MIP-1a were observed in HIV patients compared with controls. These might be due to consumption of these chemokines through binding to CCR5 to block viral entry into uninfected cells, through downregulation of receptor expression on the host-cell surface and through induction of intracellular signaling to enhance HIV-1 replication.

Cocchi *et al*., (2000) suggested that RANTES, MIP-1a and MIP-1β may upregulate replication of HIV in macrophages and monocytes by recruiting activated target cells. Other chemokines found to affect HIV replication, although they are not involved in viral entry, are interferon-g-inducible protein 10 (IP-10/CXCL-10) and monocyte chemotactic protein (MCP)-1 (Lane *et al*., 2003). Thus, these chemokines act directly and specifically against HIV (Hernanz-Falcon *et al*., 2004). Therefore, the present study supported previous conclusion that the CD4 molecule is not sufficient for entry of HIV (Vila-Coro *et al*., 1999). CXCR-4 is a receptor for the (CXC) subclass of chemokines and mediates the entry/fusion of T-cell tropic strains of HIV (Rodriguez-Frade *et al*., 2004). Another receptor for the members of the 3 (CC) chemokine subclass, CCR-5, mediates entry and fusion of macrophage-tropic isolates of HIV (Neil *et al*., 2005). This molecule serves as a receptor for RANTES, MIP-lα, and MIP-1β and thus provides the basis for the observation that these β chemokines suppress HIV. Subsequently, a CXC chemokine called SDF-1, a ligand for CXCR-4, was shown to suppress replication of T-tropic HIV isolates, thus confirming the connection between chemokines, and HIV suppression (Mazzoli *et al*., 1997). Thus the use of multiple co-receptors and varieties of chemokines might be one of the mechanisms use by HIV to evade host immunity. The result of the present study supports the involvement of multiple chemokines in HIV pathogenesis and the level of these cytokines might be affected by antiretroviral therapy. Further studies should include increase of sample size, increase the number of chemokine types/subtypes, different HIV classes and HIV patients on anti-retroviral therapy.

REFERENCES

Bisset LR, Rothen M, Joller-Jemelka HI, Dubs RW, Grob PJ, Opravil M. (1997). Change in circulating levels of the chemokines macrophage inflammatory proteins 1agr; and 1β, RANTES, monocyte chemotactic protein-1 and interleukin-16 following treatment of severely immunodeficient HIV-infected individuals with indinavir. AIDS. 11: 485-491.

Cocchi F, DeVico AL, Garzino-Demo A, Arya SK, Gallo RC, Lusso P: (1995). Identification of RANTES, MIP-1agr;, MIP-1βas the major HIV-suppressive factors produced by CD8+ T cells. Science. 270:1811-1815.

Colina P, Bénureaua Y, Staropolia I, Wanga Y, Gonzalez N , Alcamic J, et al.(2013) HIV-1 exploits CCR5 conformational heterogeneity to escape inhibition by chemokines. PNAS 110 (23) 9475–9480

Gorry, P.R. and Ancuta, P. (2011). Coreptors and HIV-1 Pathogenesis. Curr HIV/AIDS Rep. 8(1):45-53.

Hernanz-Falcon P, Rodriguez-Frade JM, Serrano A, Juan D, delSol A, Soriano SF. (2004). Identification of amino

acid residues crucial for chemokine receptor dimerization. Nat Immunol. 5: 216-223.

Kalinkovich A, Weisman Z, Bentwich Z. (1999). Chemokines and chemokine receptors: role in HIV infection. Immunology Letters. Volume 68, Issues 2–3: 281-287

Kedzierska K and Crowe SM. (2001). Cytokines and HIV-1: interactions and clinical implications. Antivir Chem Chemother. 12(3): 133-50.

Lane BR, King SR, Bock PJ, Strieter RM, Coffey MJ, Markovitz DM. (2003). The C-X-C chemokine IP-10 stimulate HIV-1 replication. Virology 2003;307:122-34.

Mazzoli S, TrabattoMeyer L, Magierowska M, Hubert JB, Theodorou I, van Rij R, Prins M. (1999) . CC-chemokine receptor variants, SDF-1 polymorphism and disease progression in 720 HIV-infected patients. SEROCO Cohort. Amsterdam Cohort Studies on AIDS [Letter]. AIDS 1999; 13: 624-6.

Moore, J. P and M. Stevenson. (2000). New targets for inhibitors of HIV-1 replication. Nat. Rev. Mol. Cell Biol. 1:40-49).

Murdoch C and Finn A. (2000). Chemokine receptors and their role in inflammation and infectious diseases. Blood. 95: 3032-3043.

Neil SJ, Aasa-Chapman MM, Clapham PR, Nibbs RJ, McKnight A, Weiss RA (2005). The promiscuous CC chemokine receptor D6 is a functional co-receptor for primary isolates of human immunodeficiency virus type 1 (HIV-1) and HIV-2 on astrocytes. J Virol. 2005; 79: 9618-24.

Pierdominici M, Giovannetti A, Ensoli F, Mazzetta F, Marziali M, De Cristofaro MR. (2002). Changes in CCR5 and CXCR4 expression and beta-chemokine production in HIV-1–infected patients treated with highly active antiretroviral therapy. J Acquire Immune Defic Syndr. 29:122-31.

Rodriguez-Frade JM, del Real G, Serrano A, Hernanz-Falcon P, Soriano SF, Vila-Coro AJ. 2004). Blocking of HIV infection via CCR5 and CXCR-4 receptors by acting in trans on the CCR2 chemokine receptor. EMBO J. 2004; 23: 66-76.

Sriwanthana B, Hodge T, Mastro TD, Dezzutti CS, Bond K, Stephens HA (2001). HIV specific cytotoxic T lymphocytes, HLA A11 and chemokine-related factorsmay act synergistically to determine HIV resistance in CCR5 delta 32 negative female sex workers in Chiang Rai, Northern Thailand. AIDS Res Hum Retrov 2001; 17: 719-34.

Tagliamonte, M, Tornesello ML, Buonaguro FM, Buonaguro L. (2011). Conformational HIV-1 envelope on particulate structures: a tool for chemokine coreceptor binding studies. Journal of Translational Medicine. 9 (Suppl 1):S1.

Wang Z, Shang H and Jiang Y (2017) Chemokines and Chemokine Receptors: Accomplices for Human Immunodeficiency Virus Infection and Latency. Front. Immunol. 8:1274. doi: 10.3389/fimmu.2017.01274

Ye, P, Kazanjian P, Kunkel SL, Kirschner DE. (2004). Lack of good correlation of serum CC-chemokine levels with human immunodeficiency virus-1 disease stage and response to treatment. Journal of Laboratory and Clinical Medicine. 143: 310-31