Bangladesh Journal of Medicine (BJM)
ISSN : 1023 - 1986
eISSN : 2408 - 8366

ORIGINAL ARTICLE

mRNA EXPRESSION OF GENES INVOLVES IN INTRINSIC APOPTOTIC PATHWAY DURING HIV INFECTION AND THERAPY AND ITS RELATION IN CIRCULATING CD4 T-LYMPHOCYTES

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Abstract:

Background: *CD4 T-lymphocyte apoptosis in Human Immunodeficiency Virus infection may occur due to activation of the intrinsic apoptotic pathway or triggered via death receptors. In this study, the differential expression of the genes involved in the intrinsic apoptotic pathway was investigated on circulating CD4 T-lymphocytes of HIV-infected patients and healthy controls.* ***Methods:*** *The study included 55 HIV patients who were (1) asymptomatic antiretroviral therapy naive, (2) symptomatic antiretroviral therapy naive, and (3) on antiretroviral therapy. As control 20 healthy persons were included. CD4 T-lymphocytes were isolated from blood samples of HIV patients and healthy controls using density gradient centrifugation method and by negative selection from peripheral blood mononuclear cells (using CD4+ T Cell Isolation Kit followed by total RNA extraction and cDNA synthesis. Genes involved in the intrinsic apoptotic pathway of CD4+ T-lymphocyte including BAX, BAK, BIM, MCL-1 and BCL-2, Caspase-3, Caspase-9 and Calpain-1 were selected and analyzed by real-time PCR to detect their expression. The fold changes of gene expression of PLHIV groups were compared with that of healthy controls.* ***Results:*** *Among the genes under study, no significant upregulation in the expression of BAX, BAK, Caspase-3, Calpain-1 and no significant downregulation in BIM, MCL-1 expression was observed with the exception of upregulation of BCL-2 and downregulation of Caspase-9 expression which were statistically significant ($p < 0.05$). None of the studied genes showed any significant correlations between the gene expressions and CD4 T-lymphocyte count.* ***Conclusion:*** *Gene expression of intrinsic apoptotic pathway may not have significant effect on peripheral CD4 T-lymphocytes count in HIV infection and treatment.*

Keywords: *HIV, Apoptosis, CD4 T-lymphocytes; ART*

Received: 16-02-2022 *Accepted: 06-04-2022*
DOI: https://doi.org/10.3329/bjm.v33i2.59291

Citation: *Mahboob M, Munshi SU, Tabassum S. mRNA expression of genes involves in intrinsic apoptotic pathway during HIV infection and therapy and its relation in circulating CD4 T-Lymphocytes. Bangladesh J Medicine 2022; 33: 176-185.*

Introduction:

Apoptosis or programmed cell death is a physiological process that occurs in multicellular organisms, responsible for removal of unnecessary, damaged, mutated and/or elderly cells. The principal morphological features of apoptosis are shrinkage of nuclei and cytoplasm, condensation of nuclear chromatin, dilatation of endoplasmic reticulum and membrane blebbing.[1] This process is triggered by sequential activation of caspase family via two different but congregating pathways known as extrinsic and intrinsic pathways.[2,3] In extrinsic

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pathway, death receptor ligands like TNF-", TRAIL activates death receptors and thus formation of a death-inducing signaling complex (DISC) followed by activation of procaspase-8/10 and subsequent initiation of pro-apoptotic cascade of caspases.[4,5] On the other hand, the intrinsic pathway which can be triggered by various stresses, including DNA-damaging agents, activation of oncogenes, overload of Ca^{2+}, deprivation of growth factors and can be dominantly controlled by Bcl-2 protein family.[6] This mitochondria-controlled cell death is a two-step process. In the initial step, upon appropriate stimulus mentioned above, an increase in mitochondrial permeability occurs which results in release of apoptogenic factors through the outer membrane and disrupts the electrochemical gradient of inner membrane of mitochondria. This disorder is sensed by a multiprotein complex called mitochondrial permeability transition pore (mPTP) which resides at the junction of inner and outer mitochondrial membranes.[7] This mitochondrial dysfunction causes disruption of plasma membrane integrity and/or the activation of specific apoptogenic proteases i.e., caspases by mitochondrial proteins like cytochrome c that leaked into the cytosol.[8] To complete apoptosis, this released cytochrome c assembled with apoptotic protease activating factor 1 (APAF1) and pro-caspase 9 and produce 'apoptosome' (a caspase activating multiprotein complex). This 'apoptosome' activates caspase-3 and executes apoptosis.[9]

Deregulation of these apoptotic pathways is central to various diseases, including Human Immunodeficiency Virus (HIV) infection being one of them. HIV principally infects T helper (Th) cells and cells of the monocyte or macrophage lineage, which express the CD4 cell surface protein. The gradual and selective loss of the CD4 subset of T-lymphocytes, the key component of innate and adaptive immune response, is the central feature of the pathogenesis of HIV which correlates with the progression from asymptomatic HIV infection to Acquired Immune Deficiency Syndrome (AIDS).[10] It has been suggested that apoptosis plays a dominant role in CD4 T-lymphocyte depletion during HIV-1 infection.[11] Though it has been reported that CD4 T-lymphocytes turnover is rapid in HIV-1-infected individuals and correlates with a high degree of viral replication,[12,13] the majority of dying CD4 T-lymphocytes are not productively infected and appear to undergo bystander apoptosis.[14,15] There is relatively little information concerning the potential role of intrinsic pathway of apoptosis in human primary peripheral CD4 T-lymphocytes in HIV-infected patients. Some microarray studies demonstrated the apoptosis-regulating genes in intrinsic pathway were not differentially expressed in a consistent pattern in activated CD4 T-cells from HIV positive versus healthy donors.[16-18]

Intrinsic apoptotic pathway relies largely on the B-Cell Lymphoma-2 (BCL-2) family related pro-apoptotic genes e.g., BCL-2-associated X protein (BAX), BCL-2 homologous antagonist/killer (BAK), BCL2-Interacting mediator of cell death (BIM), the anti-apoptotic genes e.g., Myeloid Cell Leukemia-1 (MCL-1) and B-cell lymphoma-2 (BCL-2) proteins.[19,20] The expression of genes plays a key role in the response of cells to death-inducing stimuli. A growing body of evidence indicates that the levels of numerous death-related genes can be induced during apoptosis.[21] The integration of cellular signals from diverse apoptotic pathways requires the finely balanced expression of pro- versus anti-apoptotic proteins. Gene expression patterns of pro- and anti-apoptotic genes, established by the levels of transcription[22] as well as alternative splicing,[23] can dictate the life-or-death decisions of cells. Presently, there is no or limited data on expression of genes involved in the intrinsic apoptotic pathway in peripheral CD4 T-lymphocytes during different stages during HIV infection and therapy. Much of the experimental data of present date relies on *in vitro* or *ex vivo* cellular or tissue models, which necessarily do not mimic the immunologic complexity in peripheral CD4 T-lymphocytes of an HIV-infected person.[24] However, previous studies aimed at investigating the HIV-1-induced modulation of host gene expression have been mostly performed in established human cell lines.[11] Studies have identified numerous viral components, Tat, Nef, Vpr and gp120 that can induce apoptosis via different pathways at least under *in vitro* conditions.[25-28] In fact, gene profile analysis has shown that a number of genes associated with both extrinsic and intrinsic pathways[29,30] are modulated after infection of cell lines with HIV *in vitro*, while data *ex vivo* from patients have only partially revealed which genes could be involved in the apoptotic response to HIV infection. In particular, previously investigations in HIV patients were focused on single or strictly related extrinsic apoptotic pathway.[18,31]

In addition, there is lack of information about the changes in gene expression of intrinsic apoptotic pathway after the initiation of antiretroviral therapy (ART). Thus, this study was taken to observe whether there was any difference between the expressions of gene involved in intrinsic apoptotic pathway of circulating CD4 T lymphocytes in blood of HIV patients and whether that expression was changed after the initiation of ART or not. This is the first study in our country to measure and compare the levels of mRNA expression profile of genes involved in the intrinsic apoptotic pathway of CD4 T-lymphocytes - the pro-apoptotic genes BAX, BAK, and BIM, the anti-apoptotic gene BCL-2, MCL-1 and the protease genes e.g., Caspase-9, Caspase-3, Calpain-1 during the course of HIV infection among patients on ART and compared their gene expression with those of healthy controls.

Methods:

Study period and subjects:

This cross-sectional analytical study was conducted from July 2015 to June 2016 at Department of Virology, BSMMU. The selected study participants comprised of 55 Serologically confirmed (by both rapid test and ELISA) HIV infected patients and 20 healthy adult individuals negative for HIV antibody and clinically free from any signs of acute or chronic infection for > 3 months. The purposive sampling technique was applied to recruit HIV infected patients and healthy controls for the study. The HIV patients were divided into 3 groups based on their clinical data, history of ART and CD4 T-lymphocyte count. They were as follows: (1) Asymptomatic (CD4+ T-cells >200/ il, n=16) ART naive, (2) Symptomatic (CD4+ T-cells <200/il, n=14) ART naive, (3) patients on ART (>2 years, n=25) irrespective of symptom. The ART receiving patients were either on a regimen of AZT (Zidovudine) + 3TC (Lamivudine) + NVP (Nevirapine) or AZT + 3TC + EFV (Efavirenz) according to the National ART guidelines.[32] Those patients who were on anti-tubercular therapy or with other co infections like viral hepatitis were excluded from the study to avoid the dilemma as *apoptosis* of T cells is enhanced in patients with *Tuberculosis and Hepatitis*.

Specimen collection and laboratory work

Blood samples of HIV positive patients were collected from Ashar Alo Society, a community based non-government registered organization of People Living with HIV/AIDS (PLHIV), Dhaka and blood samples of healthy controls were collected at the Department of Virology. The laboratory works were performed at the Department of Virology, BSMMU, Shahbag, Dhaka. Following aseptic precaution, approximately 5 ml of venous blood was collected from all the study subjects. After collection, each blood sample was divided in to two parts – 4 ml was collected in sodium heparin containing Vacutainer (BD Biosciences, USA) for separation of CD4 T-lymphocytes and 1 ml in K+EDTA coated vacutainer for CD4 T–Lymphocyte count.

Measurement of CD4 T-lymphocytes:

CD4 T-lymphocyte count was performed (Beckman coulter, USA) commercially at Armed Forces Institute of Pathology (AFIP), Dhaka.

Isolation of CD4 T-lymphocytes:

Isolation of CD4 T-lymphocytes was performed according to the procedure described by Dieckmann *et al.* and Dagur *et al.*[33,34] Peripheral blood mononuclear cells (PBMCs) were separated from blood samples by density gradient centrifugation using Ficoll Histopage-1077 (Sigma-Aldrich, St. Louis, MO, USA). Afterwards, cell counting was performed in a Neubauer chamber and number of cells was adjusted to10^6 cells/ml in all samples and the cell pellet was re-suspended in phosphate buffer saline. CD4 T-lymphocytes were isolated from PBMCs with a negative CD4 T cell isolation kit. Antibody mixture (mouse IgG antibodies, Life Technologies, California) against the non-CD4+ T-lymphocytes were added and incubated for 20 minutes. Pre-washed Dynabeads (Depletion MyOne™ Dynabeads®, Life Technologies, California) were added and incubated 15 minutes. The tube was placed in the magnetic rack for 2 minutes and then supernatant containing the untouched human CD4 T-lymphocytes was transferred, to a new larger tube. The cell suspension was proceeded for extraction of total mRNA.

Total RNA isolation from CD4T-lymphocytes:

Total RNA was extracted from the isolated CD4T-lymphocytes by Total RNA mini kit (Geneaid, Taiwan). Tube containing isolated CD4T-lymphocytes was centrifuged at 300g for 5 minutes. Supernatant was discarded and the cell pellet was mixed with RB Buffer and ß-mercaptoethanol and incubated for 5 minutes for lysis of cells. The sample lysate was mixed with 70% ethanol prepared in ddH2O, shaken vigorously and transferred to the RB Column and centrifuged. The flow-through was discarded and RB Column was washed by Wash Buffer W1 and Wash Buffer (absolute ethanol). Finally, RNase-free water was added and centrifuged to elute the purified RNA. Quantity and purity of the isolated RNA were measured using a NanoDrop 2000 UV-Vis spectrophotometer (Thermo Scientific, USA).

Quantitative real-time PCR (qPCR):

For the cDNA synthesis, 500 ng total RNA was reverse transcribed using M-MuLV Reverse Transcriptase (Solis BioDyne, Estonia) and oligo (dT) for first strand cDNA synthesis. After mixing extracted mRNA with oligo(dT) incubation was done in water bath at 70æ%C for 10 minutes and then placed in ice for 10 minutes. Mastermix was added gently and again incubated at 37æ%C for 60 minutes in water bath. Gene expression was performed by Step One PCR machine (Applied Biosystem, USA) using the HOT FIREPol EvaGreen qPCR Mix Plus (ROX) (Solis Biodyne, Estonia). The forward and reverse primers and the annealing temperature for all the genes under the present study are given in Table I.

Table-I

Primer sequences

Gene	Forward (5^1-3^1)	Reverse(5^1-3^1)	Annealing temperature
GAPDH	ATCCCATCACCATCTTCCAG	ATGAGTCCTTCCACGATACC	55°C
BAX	TTCTGACGGCAACTTCAACTGG	GAGGAAGTCCAATGTCCAGC	52°C
BAK	GCTCCCAACCCATTCACTAC	TCCCTACTCCTTTTCCCTGA	55°C
BIM	TGGCAAAGCAACCTTCTGATG	GCAGGCTGCAATTGTCTACCT	55°C
MCL-1	GGGCAGGATTGTGACTCTCATT	GATGCAGCTTTCTTGGTTTATGG	55°C
Calpain-1	CCGGGACTTCATACGTGAGT	AGGTGCCCTCGTAAAATGTG	52°C
Caspase-3	TTCAGAGGGGATCGTTGTAGAAGTC	CAAGCTTGTCGGCATACTGTTTCAG	52°C
Caspase-9	TCAGGCTCTTCCTTTGTTCAT	CTTTGCTGCTTGCCTGTTAG	55°C

All the PCR reactions were as follows: One cycle of denaturation for 15 min at 95°C, then 40 cycles were as follows: (i) denaturation at95°C for 15 seconds, (ii) annealing at specific temperature for each gene mentioned above for 30 seconds, and (iii) extension for 30 seconds at 72°C. The Glyceraldehyde 3-phosphatedehydrogenase (GAPDH) was used as internal control to normalize the PCR reactions of selected genes. All the real-time PCR reaction for each gene of a sample was run in triplicate. The C_T value of GAPDH (for validity of test procedure) ranged from 18.5 to 19.32, and mean difference between different study groups were not significant ($p>0.05$). In every set of reactions, no template control (NTC) was included as negative controls.

Statistical analysis:

The threshold cycle values (CT) were measured to detect the threshold of each of genes of interest and GAPDH gene in all samples. Data expressed as CT values of the genes under study were normalized to GAPDH and fold change values were determined via the $2^{-\Delta Ct}$ method.[35] To correct inter assay variance, mean of studied genes of healthy control group was set to 1 for each gene, and the fold change values of the genes in HIV subjects were expressed relative to this set mean of 1 and were compared. Results were expressed as Mean±SEM. The mean differences of gene expression between groups and within groups were analyzed by one-way analysis of variance (ANOVA). Statistical analysis was performed using the SPSS 19.0 software, and results with $P<0.05$ were considered as significant.

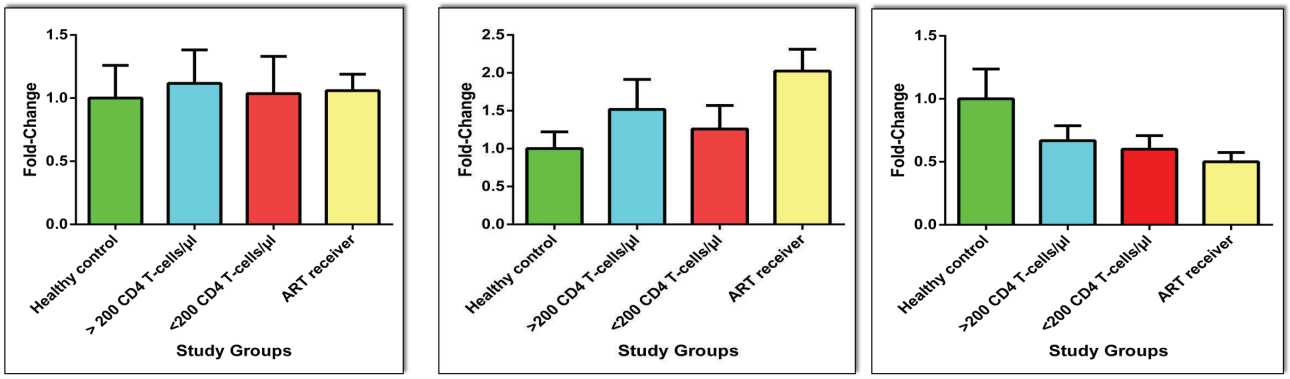
Ethics statement:

The study was approved by the Institutional Review Board (IRB) of Bangabandhu Sheikh Mujib Medical University (BSMMU), Bangladesh (BSMMU/2015/14245). Before collection of blood sample from the study subjects, informed written consent was obtained from each of the participants. The data was collected through a predesigned questionnaire and the identities of the participants were kept anonymous.

Results:

The present study included a total of 55 HIV infected patients among which 32 (58.18%) were males and 23 (41.82%) were females. A total of 20 healthy subjects of which 8 (40%) were males and 12 (60%) were females were included as control. The mean ages (mean± SD) of healthy controls, asymptomatic group, symptomatic group and patients on ART were 33±10.86, 30.875±9.17, 37.5±10.13 and 39.92±10.49 respectively. The range of CD4 T-lymphocyte count among asymptomatic group was 215-1530 cells/µl, 8-94 cells/µl among symptomatic group and 266-1563 cells/µl among ART receiver groups. Though there was no specific clinical signs and symptoms reported from the asymptomatic patients and patients who were on ART, the symptomatic patient group had several complaints. The common complaints among patients of the symptomatic group were- oral infections (55%) followed by skin infections (40%), fever (10%), respiratory tract infections (20%), sexually transmitted infections (10%), diarrhea (35%) and fever with diarrhoea (10%). The minimum duration of ART intake was >2 years among ART receiving patients

Changes in mRNA expression of BCL-2 pro apoptotic gene family: In CD4 T-lymphocytes, there was no significant difference in the expression of BAX, BAK-1 and BIM gene. The expression of BAX gene was slightly up regulated 1.11 (±0.26) fold in > 200 CD4 T-cells/µl group or unchanged 1.03 (±0.29) fold in < 200 CD4 T-cells/µl group and 1.05 (±0.12) fold in ART receiver group ($p > 0.05$) (Figure-1a). BAK gene expression was slightly up-regulated 1.51 (±0.39) fold, 1.25 (±0.31) fold and 2.02 (±0.28) fold respectively among the 3 PLHIV groups in comparison to healthy controls ($p > 0.05$) (Figure-1b). However, BIM gene expression was slightly down regulated 0.66 (±0.11) fold, 0.60 (±0.10) fold, and 0.51 (±0.07) fold respectively among the 3 PLHIV groups (Figure-1c).

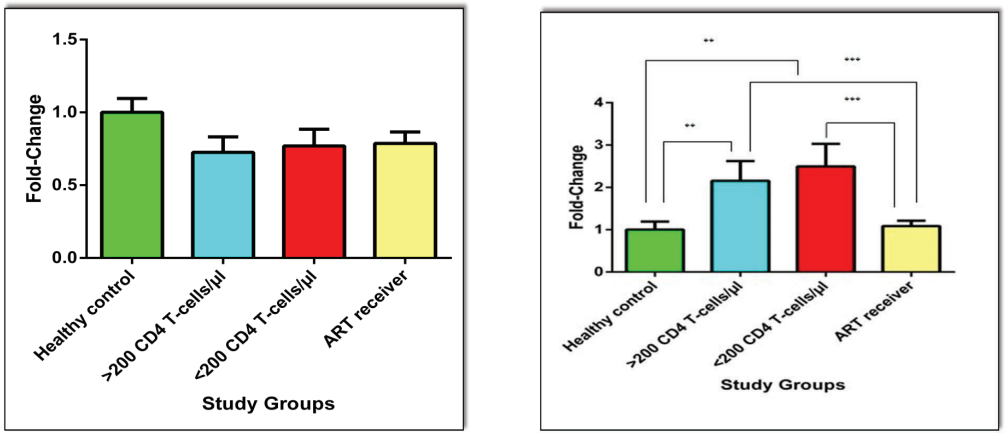


1a (Bax) 1b (Bak-1) 1c (BIM)

Fig.-1: *Changes in the Bcl-2 family pro-apoptotic gene expression among the study groups.*

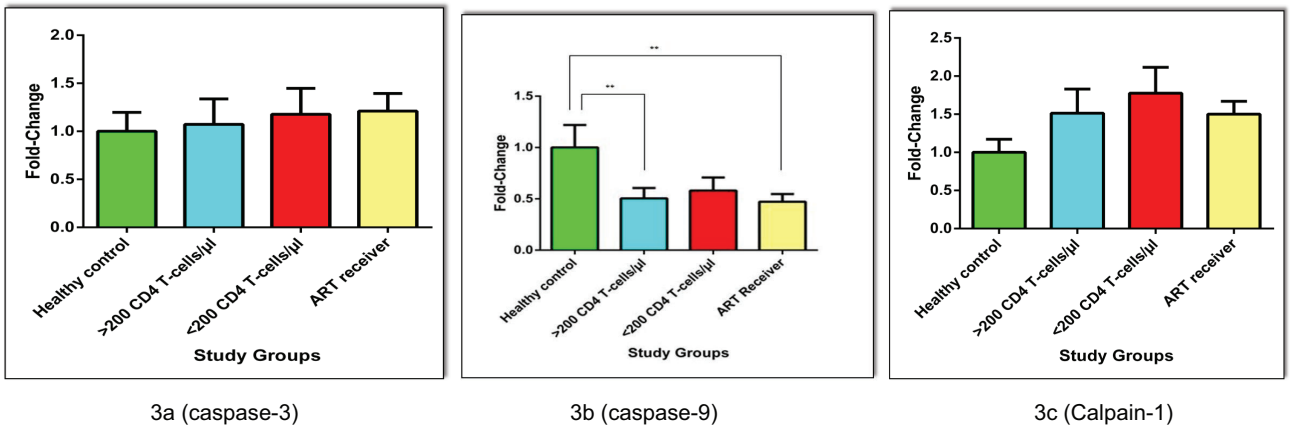
Changes in expression of anti-apoptotic gene MCL-1 and BCL-2: The anti-apoptotic gene MCL-1 expression showed slight downregulation of 0.72 (±0.10), 0.76 (±0.11), and 0.78 (±0.07) fold among all 3 PLHIV groups respectively, and these expression changes were not significant when compared with healthy controls ($p > 0.05$) (Figure- 2a). Bcl-2 expression was significantly up-regulated 2.15 (±0.46) fold in > 200 CD4 T-cells/µl group, 2.49 (±0.53) fold in < 200 CD4 T-cells/µl group and 1.08 (±0.12) fold in ART receiver group. In comparison with healthy control, the differences in > 200 CD4 T-cells/µl group and < 200 CD4 T-cells/µl group were statistically significant ($p < 0.05$). The ART receiver group showed down regulation of expression compared to > 200 CD4 T-cells/µl group and < 200 CD4 T-cells/µl group and the differences in expression were statistically significant ($p < 0.05$) (Figure- 2b).

Changes in expression of genes of proteases family (Caspase and Calpain 1): Caspase-3 expression was slightly up regulated 1.07 fold (±0.26), 1.17 (±0.27) fold and 1.21 (±0.17) fold among all 3 PLHIV groups respectively, and these expression changes were not significant when compared with healthy controls ($p > 0.05$) (Figure-3a). Caspase-9 gene expression showed significant down-regulation 0.50 (±0.10) fold in > 200 CD4 T-cells/µl group and 0.47 (±0.08) fold in ART receiver groups ($p<0.05$), whereas, 0.58 (±0.12) fold change in < 200 CD4 T-cells/µl group which was not significant ($p > 0.05$) (Figure- 3b). Calpain-1 gene expression was slightly up-regulated 1.51 (±0.31 fold), 1.77 (±0.33) fold and 1.50 (±0.17) fold respectively among the 3 PLHIV groups ($p > 0.05$) (Figure- 3c).



2a (Mcl-1) 2b (Bcl-2)

Fig.-2: *Anti apoptotic gene Mcl-1 and Bcl-2 expression among the study groups.*



3a (caspase-3) 3b (caspase-9) 3c (Calpain-1)

Fig.-3: *Proteases gene (Caspase-3, Caspase-9 and Calpain-1) expression among study groups.*

Correlation of studied gene expressions with CD4 T-lymphocyte count: The Pearson's correlation (r) test for expression of studied genes in different groups of patients with the CD4 T-lymphocyte counts revealed no statistically significant correlation ($p > 0.05$)

Discussion:

HIV-1 infection is characterized by a slow but progressive death of CD4 T-lymphocytes, which are the key players of the immune system that coordinate the humoral and cellular responses.[11] However, the exact mechanisms leading to such a dramatic depletion of CD4 T-lymphocytes *in vivo* is not well understood, although it has been proposed that this phenomenon is multifactorial. It has been suggested that apoptosis plays a dominant role in the observed HIV-1-mediated CD4 T-lymphocyte depletion.[11] In HIV-positive patients, less than 1% of CD4 T-lymphocytes are infected in peripheral blood[12] that constitutes only a minor contributing source.

Though the pathways of CD4 T-lymphocyte death in HIV infection are many, the response to death signals varies depending on cell type, activation or developmental stage of the cell, as well as the chemical or physical environment.[36] The phenotypic and molecular definitions of apoptosis have evolved significantly over time, and there is increasing recognition of overlap between alternate mechanisms of cell death.[24] There is relatively little information concerning the potential role of intrinsic pathway of apoptosis in human primary peripheral CD4 T-lymphocytes in HIV-infected patients. Thus, one of the key questions is whether apoptosis is dependent on the modulation of pro- or of anti-apoptotic genes involving intrinsic apoptotic pathway or not. Moreover, currently there is no data regarding these gene expressions in peripheral CD4 T-lymphocytes among Bangladeshi people living with HIV.

In present study, expressions of the major genes of intrinsic apoptotic pathway were analyzed in CD4 T lymphocytes isolated from HIV-infected patient. In CD4 T-lymphocytes, no significant change in the expression of BAX, BAK, Caspase-3, Calpain-1, BIM and MCL-1 was observed in any of the groups with the exception of significant upregulation of BCL-2 and down regulation of Caspase-9 expression. Therefore, these findings indicate that during HIV infection from the point of gene expression, the intrinsic apoptotic pathway in peripheral CD4 T lymphocyte is not altered. It has been established that HIV-1 can induce apoptosis in CD4+ T lymphocytes by distinct pathways.[37] However, there is still controversy about the precise mechanism and whether apoptosis is occurring in infected cells,[38,39] uninfected bystander cells,[40] or both. The vast majority of T cells undergoing apoptosis in peripheral blood of HIV patients are uninfected[41] even though apoptosis is seen in peripheral CD4 lymphocytes.[42] Apoptosis of uninfected bystander T cells is caused by activation-induced cell death of mature T cells following chronic immune activation, in addition to HIV-1-mediated mechanisms.[43,44] Apoptosis of circulating CD4T cells has not been consistently found to correlate with HIV viral load.[45] It has been observed in lymph nodes collected from HIV-infected children and SIV-infected macaques that apoptosis occurs predominantly in bystander cells and not in the productively infected cells themselves.[46]

The majority of the genes of intrinsic apoptotic pathway of peripheral CD4 T-lymphocytes demonstrated small changes or no change in CD4 T-lymphocytes isolated from HIV positive individuals

in the present study. In present study, the expression of pro-apoptotic BAK in CD4 T-lymphocytes, gene was not significantly up regulated among all HIV patient groups which was also observed in a similar kind of microarray study.[16] Though previous study demonstrated increased gene expression of Caspases-9 in a CD4 T- cell line infected with HIV,[47] significant down regulation of Caspase-9 was observed in peripheral CD4 T-lymphocytes in the present study. Caspase activation is the central event in the intrinsic apoptosis pathway with activation of the caspase cascade via activation of Caspase-9 as an initiator caspase (Parrish *et al.*, 2013).[48] A previous study demonstrated increased gene expression of Caspases-9 in a CD4 T- cell line.[29] But in the present study, significant downregulation of Caspase-9 displayed no significant hallmarks of intrinsic apoptosis in peripheral CD4 T-lymphocyte in HIV-1-infection.

There is no data regarding Mcl-1 gene expression of CD4 T-lymphocytes in HIV infection. In the present study, no significant down regulation was observed in the expression of Mcl-1 gene in CD4 T-lymphocytes. The expression of calpain is particularly low in resting lymphocytes; however, it has been shown that synthesis and secretion of calpain occurs in active lymphocytes, particularly in T cells Interferon-gamma induced calpain mRNA and protein in U-937 and THP-1 cells.[49] In the present study, it was observed that the expression of anti-apoptotic gene Bcl-2 was significantly up-regulated, whereas, Bax was unchanged. A previous study performed in peripheral CD4 T-lymphocyte demonstrated similar findings that support the present study.[17] The Primary CD4 T-lymphocytes infected with HIV showed damages in mitochondria induced by the intrinsic pathway of apoptosis where it was proposed that permeabilization of mitochondrial membrane may be a consequence of the activation of the p53 pathway which upon phosphorylation, induces up-regulation of Bax and may finally translocate to the mitochondrial membrane and promote apoptosis.[39] In fact, HIV-1 infection induces genotoxic stresses linked to p53 activation in CD4+ T cells due to by integration mediated-dsDNA strands break, secretion of interferons and expression of HIV-1 proteins.[50-52] The higher levels of expression and activation of p53 were reported in both primary CD4+ T cells and latently infected cell lines,[11,53] which in turn may activates targets p53genes, leading to cell apoptosis.[39] HIV-1 envelope induces activation of the proapoptotic proteins p53 and Puma as transcriptional activator, leading to Bax/Bak activation and inhibition of Bcl-2.[39,54] Interestingly, this up-regulation of p53 occurs exclusively in the HIV expressing primary CD4+ T cells population but not in uninfected bystander primary CD4 T cells.[11] Therefore, it is speculated that the observed unchanged level of BAX in CD4 T-lymphocytes may be due to no significant change in level of p53 in bystander primary CD4 T cells. A previous study performed in peripheral CD4 T-lymphocytes demonstrated similar findings that support the present study.[17] Increase gene expression of Bcl-2 in the present study may be explained by some previous studies performed in HeLa and Jurkat cells, which demonstrated the ability of endogenous expression of Tat protein of HIV to differentially modulate cellular responses to apoptotic signaling with increased expression of gene Bcl-2, that enhances cellular survival.[55,56] The inhibitiory effect of Bcl-2 gene in apoptosis may be inefficient because HIV protease may cleave and inactivate Bcl-2 and cause apoptosis.[57]

The features of gene expression of intrinsic apoptotic pathway of peripheral CD4 T lymphocytes observed in present study were a little bit puzzling. Thus, the observed results of the present study, displayed no significant hallmarks of intrinsic apoptosis in peripheral CD4 T-lymphocytes in HIV-1-infection. The observed changes in expression of genes do not suggest any obvious trend toward either increased or decreased intrinsic apoptosis in peripheral CD4 T-lymphocytes in different stages of HIV infection and even after initiation of ART. Therefore, the findings of the present study showed that altered gene expression of intrinsic apoptotic pathway may not be true for ongoing apoptosis of peripheral CD4 T-lymphocytes. The differential gene expression at cellular level was not confirmed by protein level activity which is one of the limitations of the present study.

Conclusion:

Differential expression of genes related with intrinsic apoptotic pathway in CD4 T lymphocytes was seen as expected. However, gene expression of intrinsic apoptotic pathway in peripheral CD4+ T-lymphocytes may not be responsible for apoptosis in HIV infection. More studies are needed to investigate the effects of gene expression at cellular level and must be confirmed by protein level activity. Our study is limited by the fact that we did not analyse the activity of relevant transcription factors and post-transcriptional regulation. These questions need to be examined in a follow-up study.

Conflict of Interest:
The authors stated that there is no conflict of interest in this study.

Funding:
No specific funding was received for this study.

Acknowledgements:
We gratefully appreciate the help of Department of virology, *Bangabandhu Sheikh Mujib Medical University, Dhaka*

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