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The relationship between the regulatory tat gene and the Foxp3 gene expression level in HIV-infected individuals with AIDS

Abstract

A major factor in the pathogenesis and progression of AIDS as an acquired immunodeficiency is a defect in immune responses through improved regulatory and inhibitory functions that can be used as one of the goals of immunotherapy in the treatment of these patients. An increase in the Foxp3 transcription factor as a marker of Treg and as a suppressor molecule of the immune system is directly related to T-cell activity regulation and the HIV disease progression. Their association with the progression and prognosis of HIV malignancies has been demonstrated in separate studies. This study aimed to investigate the relationship between the expression levels of the regulatory tat gene and Foxp3 in HIV patients.

Methods: This study examined peripheral blood mononuclear cells (PBMCs) from 46 imprisoned HIV-positive patients and 46 normal individuals (as controls). The expression levels of Foxp3 and tat viral genes were evaluated using the Real-Time PCR technique.

Results: The expression of the Foxp3 gene significantly ($P < 0.05$) increased in the samples of HIV patients, particularly those treated with HARRT drugs. The expression of tat viral gene increased significantly in patients undergoing HARRT treatment compared to those not using the drug. A direct and partial correlation was observed between the expression levels of Foxp3 and tat viral genes in individuals undergoing treatment.

Conclusion: An increase in the expression of Foxp3 as a key marker of Treg and the increased expression of the tat viral gene as an inducer of Treg seem to be useful in the more accurate examination of prognosis, particularly in individuals undergoing treatment.

Keywords: *HIV Patients, Treg, Foxp3 biomarker, regulatory tat gene*

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Introduction

Regulatory T cells (Tregs) are considered effective cells in the immune system that play an important role in cancers, autoimmune diseases, and infectious diseases (1). The following are the two main subgroups of Tregs:

(A) Natural Tregs (nTreg or tTreg) that form in the thymus during puberty, and their suppressive activity is essential for the establishment and maintenance of immune homeostasis.

B) Induced Treg cells that form from primary T cells following the detection of a native antigen outside the thymus.

The key elements in identifying these cells are transcription factors, among which the Foxp3 transcription factor plays a key role in the development and function of Treg cells.

Studies on Treg cells were facilitated by discovering the Foxp3 Forkhead box P3 transcription factor, which is encoded by the X chromosome, and loss-of-function mutations, leading to the severe immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome in humans and similar devastating lesions in mice (4-5). The expression level of Foxp3 protein in Treg cells is critical for inhibitory function. Reduced Foxp3 levels have been shown to result in a defective inhibitory function (6). In addition, enhanced expression of Foxp3 in mature Treg cells is essential for maintaining the phenotype and inhibitory function of Treg cells. Degradation of a selective Foxp3 allele in mature Treg cells, along with the permanent loss of inhibitory function, salient surface features of Treg cells, and acquisition of operational T cell characteristics, involves the production of IL-2, IL-4, and IFN cytokines promoting the immune response (7). These studies also suggest that Foxp3 is essential for the differentiation and inhibitory function of Treg cells and determines the Treg cell line (8).

Thymic Treg cells (nTreg)

The main group of regulatory T lymphocytes contains TCRαβ and their role is to regulate immune responses and maintain tolerance. A few Treg lymphocytes express the TCRϒδ receptor. The most important feature of the TregCD4 + CD25 + subgroup is that they are the precursors of T lymphocytes produced in the thymus. Following the detection of native antigens in the thymus, the following three results will happen depending on the TCR affinity:

1. Identification of the MHC + native antigen complex with an overall low affinity → formation of normal T lymphocytes relatively tolerant of native antigens.
2. Identification of the MHC + native antigen complex with an overall moderate affinity → formation of normal T lymphocytes
3. Identification of the MHC + native antigen complex with an overall high affinity → deletion of self-reactive T lymphocytes.

IL-2, TGF-B, and Foxp3/STAT-5 transcription factors play a key role in the development of this group of Treg lymphocytes. This group is characterized by CD4, CD25, CTLA-4, and GITR indicators, and directly reacts to and inhibits the immune system cells.

Induced Treg cells (iTreg)

This group of Treg lymphocytes is formed from mature T lymphocytes under the influence of cytokines in peripheral tissues following the entry of non-native antigens such as allergens, food, and commensal germs (9, 10, 11).

Methods

This case-control study based on measuring the expression levels of Foxp3 and viral tat genes was conducted on HIV-infected individuals in the group undergoing HAART treatment and in those with no treatment in the prisons of Qom province during 2015-16. The prisons in Qom province were selected as the research environment due to better access to sample cases. The study population consisted of HIV-positive patients whose ELISA diagnostic tests and Western blotting were approved by the Qom Province Health Center. This research was conducted on 23 HIV-infected people with no medical treatment and another 23 individuals undergoing medical treatment separately. In addition, healthy individuals with confirmed physical health by a physician and matched the patients' age and gender in this study were tested as a control sample. Blood samples were collected from healthy individuals as a control group after ensuring that they were not infected with other autoimmunity diseases.

Figure1.Viral tat gene replication curve

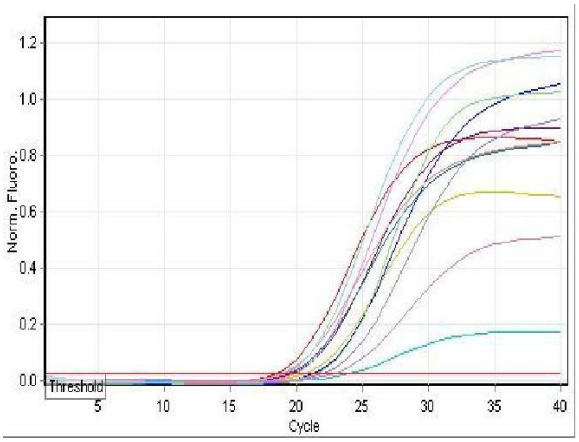
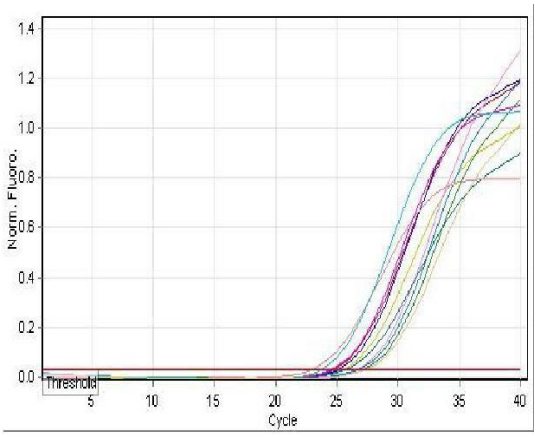


Figure2. FOXP3 gene replication curve



Melting point and specific melting temperature For the tat gene and Foxp3 gene

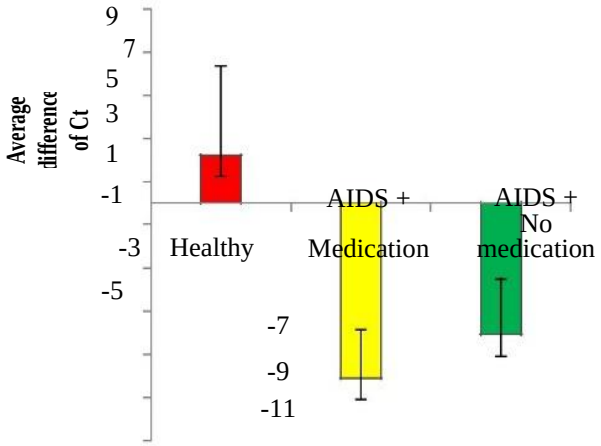


Figure3. Results of the statistical analysis of the FOXP3 gene expression

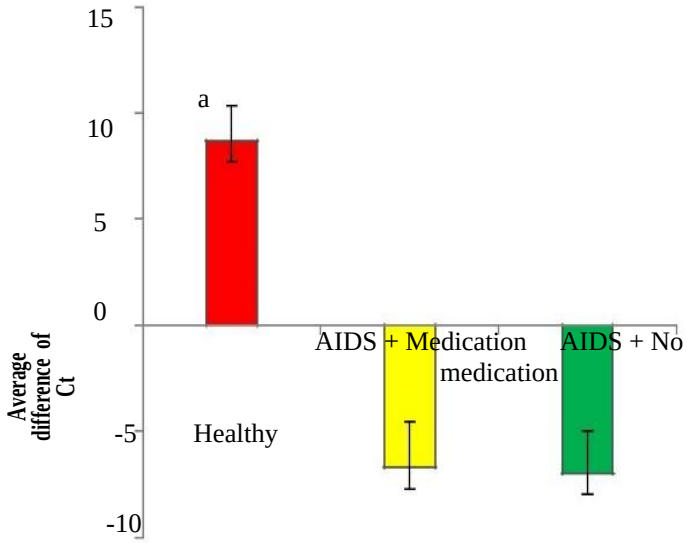


Figure4. Results from the study of the expression of the viral tat gene

The results of the correlation analysis between the expression levels of FOXP3/viral tat genes

Correlations

		FOXP3 HIV+DRUG	tat HIV + DRUG
Spearman's rho FOXP3 HIV+DRUG	Correlation Coefficient	1.000	0.551**
	Sig. (2-tailed)	.	0.006
	N	23	23
tat HIV + DRUG	Correlation Coefficient	.551**	1.000
	Sig. (2-tailed)	0.006	.
	N	23	23

**Correlation is significant at the 0.01 level (2-tailed).

Table1. There is a direct and partial correlation in the medication group.

Correlations

		FOXP3 HIV - DRUG	tat HIV - DRUG
Spearman's rho FOXP3 HIV - DRUG	Correlation Coefficient	1.000	0.324
	Sig. (2-tailed)	.	0.131
	N	23	23
tat HIV - DRUG	Correlation Coefficient	0.324	1.000
	Sig. (2-tailed)	0.131	.
	N	23	23

Table2. There is no correlation in the no medication group.

Results

Studies indicate increased levels of CD25+CD4+ Tregs in infected people. Tregs are a subset of TCD4+ cells that comprise 5-10% of TCD4+ cells in the peripheral circulation (10, 11), and their function is to inhibit immune responses and establish self-tolerance and immune homeostasis (12). A reduction in the number of TCD4+ lymphocytes through the direct apoptotic effects of HIV infection, on the one hand, and induced replication and a relatively increased frequency of Tregs in the disease chronic phase, on the other hand, raise the hypothesis that this increase is harmful in the disease chronic phase and plays a role in the pathogenesis of HIV. However, this increase in the disease acute phase leads to self-tolerance by controlling T-cell activation and partially reducing the number of HIV-suspected cells. The imbalance between TE and Treg creates a dominant Treg fragment, which reduces TE responses and raises the hypothesis that the increase in Tregs is harmful relative to its benefits (13). Although the number of Tregs and viral loads are not correlated in plasma (14, 54), they

are positively correlated. Therefore, an increase in Tregs seems to be more correlated to a reduction in TCD4 than to a viral load.

Data were analyzed using SPSS software, and the following are the obtained results:

- Foxp3 gene expression increased significantly in patients compared to controls ($p = 0.05$).
- The expression of the viral tat gene increased significantly in medicated patients compared to those with no medication ($p < 0.05$).

Conclusion

The main cause of HIV pathogenicity, as an acquired immunodeficiency, is a defect in immune responses through increased regulatory and inhibitory functions, which also account for an important factor in reducing the recovery rate of infected patients. To be used in the treatment of these patients. This can also be used as a goal of immunotherapy in the treatment of these patients. Previous studies suggest that an increase in the Foxp3 transcription factor as an indicator of increased Tregs is associated with their activity.

An increase in Foxp3 levels, particularly in people undergoing HARRT treatment compared to those with no medication, as an indicator of increased Tregs shown in HIV patients of this study and in previous studies, can play an important role in suppressing immune responses of bad prognosis in HIV patients, as described above (12, 15, 16). The present study has demonstrated that the increased expression of this molecule, particularly in patients undergoing treatment with HARRT drugs compared to non-medicated patients, as a T-cell suppressive agent leads to weakened immune responses (17). Studies conducted in the past and present also indicate that the increased Foxp3 factor in HIV patients undergoing treatment may be partially normalized or it may not be normalized by ART or HARRT (18-19-20).

According to the correlation analyses of the viral tat gene and the Foxp3 molecule, a direct and partial correlation was observed in patients treated with HARRT, whereas this correlation was absent in individuals with no medication. This demonstrates the ability of the tat gene to induce the expression of the Foxp3 gene, particularly in patients treated with HARRT.

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Acknowledgments

None.

Conflict of interest

None.

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Ethics statement

None

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