

Plasma levels of Transforming Growth Factor Beta in HIV-1 patients with oral candidiasis

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Abstract

Background and Purpose: TGF- β is a potent regulator and suppressor of the immune system and overproduction of this cytokine may contribute to immunosuppression in HIV-infected patients. Increasing population of immunosuppressed patients has resulted in increasingly frequent of fungal infections, including oral candidiasis. The aim of this study was to evaluate the plasma levels of TGF- β under *in vivo* conditions.

Materials and Methods: Seventy-two samples were obtained from the oral cavities of HIV-positive Iranian patients and cultured on Sabouraud's dextrose agar and CHROMagar. Also blood samples were obtained to assess TGF- β levels using ELISA technique.

Results: Thirty-three out of 72 oral samples yielded *Candida* isolates, *Candida albicans* in 14 and non-*albicans candida* in 19. Fungal infection decreased significantly more TGF- β level than non-fungal infection also HIV negative were significantly more TGF- β than HIV positive.

Conclusion: Our findings suggest a significant interaction between fungal infection and HIV on expression of Transforming Growth Factor Beta.

Keywords: Transforming Growth Factor beta, Candidiasis, HIV

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Introduction

Cytokines are secreted proteins that regulate and determine the nature of immune responses, control immune cell trafficking and the cellular arrangement of immune organs [1-2]. TGF- β is a cytokine with an important role in enhancing regulatory T cell response and having both stimulatory and inhibitory effects on different cell types [3-4]. Various studies have reported this cytokine have a regulatory action on HIV replication thus, overproduction of TGF may contribute to immunosuppression in HIV-infected patients [5-6].

One of the main reasons of morbidity and mortality among HIV positive patients in late stages of HIV infection and when the count of CD4 cells were below 500/cumm, is opportunistic infection caused by agents that rarely infect health individuals [7].

Clearly specified that the increased incidences of localized and systemic infections caused by opportunistic fungi such as the *Candida* spp. and *Aspergillus* spp. during the past decade mainly because of the growing numbers of patients with diverse pathological and immunodeficient states such as acquired immunodeficiency syndrome (AIDS), neutropenia, neoplasia, malnutrition, Uncontrolled diabetes mellitus and organ transplantation [8-11].

Immune system is the main target of the Human Immunodeficiency Virus (HIV) that weakens the surveillance and defense system of the body against infections, resulting in HIV infected individuals becoming more susceptible to many infections that are easily be eliminated by the immune system of healthy individuals [12-13].

The immune response to pathogens is often characterized by either cell-mediated or humoral type effectors mechanisms as the phagocytic cells and lymphocytes (T&B both) are believed to function together in protecting the host against fungal pathogens[11, 14].

The cytokines produced by Th1 cells, such as TGF are known to activate phagocytic cells, thus leading them to transform into a candidacidal state also have a regulatory action on HIV replication[15] so here we investigated the effects of candidiasis in HIV-1–infected patients on the plasma concentration of TGF- β under *in vivo* conditions.

Material and Methods

Subject selection

A total of Seventy-two patients ranged between 18-50 years old referring to Center of Behavioral Disorders of Kerman (Kerman, Iran) participate in this study. Of the 72 individuals, 18 individuals were healthy, 18 were positive for HIV infection, 18 for oral candidiasis and 18 for HIV infection-oral candidiasis. The HIV status and oral candidiasis lesions of all the patients were confirmed by an infectious diseases specialist at the Center of Behavioral Disorders of Kerman. This study was approved by Ethics Committee of Kerman University of Medical Sciences and a written informed consent was obtained from all the participants prior to sample collection.

Collection of blood samples

72 samples were obtained from the oral cavities of HIV-positive patients according to the patient's clinical presentation. The oral candidiasis (OC) lesion samples were obtained from the tongue or the buccal mucosa by using sterile cotton swabs. The samples were collected under complete aseptic conditions and transported immediately to the medical mycology laboratory and processed specifically to ascertain candida infection.

These swabs were incubated in Sabouraud's dextrose agar with chloramphenicol (Merck, Germany) under aerobic conditions at 32 °C for 48 h and in CHROMagar™ Candida (CHROMagar, France) in the dark at 35 °C for

48 h for production of species-specific colors and were observed daily for the growth.

We used a 10% KOH preparation and Giemsa stain for microscopic examination of pseudohyphae and yeast cell forms.

Quantitation of plasma TGF- β

Plasma concentration of TGF- β was measured by ELISA kits (R&D Systems, USA) according to the manufacturer's instructions. Sensitivity of the kits was 2 pg/ml and inter- and intra-assay assessments of the reliability of the kit were conducted. All the standard safety precautions were taken at all times.

Statistical analysis

All statistical analyses were done by SPSS (ver. 20; SPSS Inc.). Two-way Analysis of Variance (ANOVA) was performed for comparing cytokines levels in plasma (in four subgroups and between groups). $P \leq 0.05$ was considered to be statistically significant.

Results

Out of the 72 oral samples, thirty-three samples yielded *Candida* isolates, *Candida albicans* in 14 and non *albicans Candida* in 19 (Table 1).

There was a statistically significant difference in the plasma TGF- β concentration between individuals who were healthy, positive for HIV infection, positive for oral candidiasis and positive for HIV infection-oral candidiasis, as assayed with ELISA. Fungal infection ($M=495.4 \pm 14.4$ pg/ml) decreased significantly more TGF- β level than non-fungal infection ($M=707.4 \pm 23.1$ pg/ml) ($P<.0001$). HIV negative were significantly more TGF- β ($M=734.2 \pm 21.2$ pg/ml) than HIV positive ($M=468.7 \pm 10.6$ pg/ml) ($P<.0001$) (Figure 1). There was a significant interaction between fungal infection and HIV on expression of this cytokine ($P<.0001$) (Figure 1).

Table 1. Frequency of fungal species isolated from clinical specimens of with HIV positive individuals in Kerman, Iran

	Frequency	
	Number	%
<i>Candida albicans</i>	14	42.4%
non <i>albicans candida</i>	19	57.7%
Total	33	100%

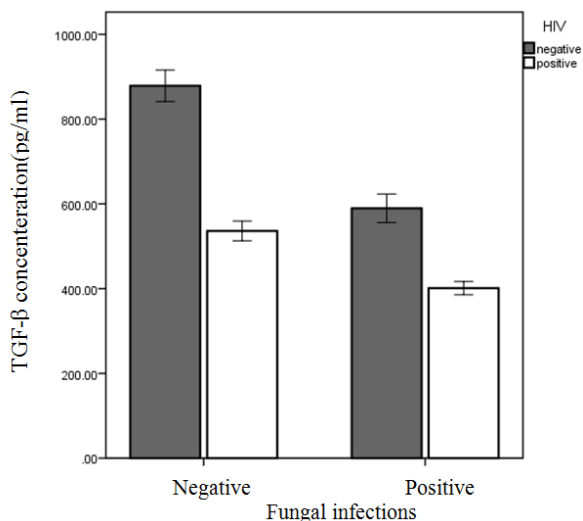


Figure 1. Concentrations of TGF- β in plasma from cases and controls. There was a main effect for fungal infection on plasma concentration of TGF- β . Fungal infection ($M=495.4\pm 14.4$ pg/ml) decreased significantly more TGF- β level than non-fungal infection ($M=707.4\pm 23.1$ pg/ml) ($p<.0001$). There was also a main effect of HIV on TGF- β expression, HIV negative were significantly had more TGF- β ($M=734.2\pm 21.2$ pg/ml) than HIV positive ($M=468.7\pm 10.6$ pg/ml) ($p<.0001$). Additionally, there was a significant interaction between fungal infection and HIV on the expression of this cytokine ($p<.0001$)

Discussion

The incidence of opportunistic fungal infections such as candidiasis has increased in recent years [8,16]. Oral candidiasis is one of the most common lesions in HIV-positive individuals [17]. On track to understanding the local immune mechanisms involved in resistance or susceptibility to infection was to evaluate the cytokines expressed in the subjects with and without HIV and candidiasis. For this, we measured and compared the TGF- β level using ELISA in subjects infected with HIV and candidiasis, comparing the cytokine levels with those found in subjects without these infections HIV. Based on our data, it is clear that there is a statistically significant difference among different groups.

The cytokines produced by Th1 cells, such as TGF has been shown to have a regulatory action on HIV replication and is a potent regulator and suppressor of the immune system thus, overproduction of TGF may contribute to immunosuppression in HIV-infected patients on the other hand; activate phagocytic cells against candidiasis [18-20]. Also this cytokine has been showed is anti-inflammatory cytokine that inhibits the secretion of pro-inflammatory cytokines and impair anti-fungal effector

functions by phagocytes [14] also, is considered to be essential in class-switch recombination to IgA that increases mucosal immunity [21].

In mice with candidiasis, CD4 + CD25 + T Reg cells, producing TGF- β , prevent complete elimination of the fungus from the gastrointestinal tract; fungal persistence allows the development of memory immunity [22].

Insignificant findings regarding the effects of fungal infection on TGF- β levels were available. According to our results, fungal infection decreased significantly more TGF- β level than non-fungal infection.

TGF- β was shown to be important for HIV pathogenesis by promoting virus production and impairing the host immune response. It has been shown that HIV antigens induce the expression of TGF- β in monocytes [15].

Because HIV infection weakens the cell mediated immune response and directs it to allow for its own survival, we would expect TGF- β , an immunosuppressive cytokine with roles in inhibiting the Th1 response, to increase in patients with HIV infection but our result showed the plasma levels of this cytokine in HIV subjects decreased significantly (Figure 1).

This finding should be considered in the management of HIV patients with opportunistic fungal infections. However, more studies are needed to improve our knowledge about the immune status in patients with weakened immune systems who are suffering from opportunistic fungal infections.

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Authors' contributions

AA.M. selected and presented the basic theme of the article, writing the paper & supervised the study, A.Izadi data collection and helping to write the manuscript, G.A. presented the basic theme of the immune articles and helping to do the most of the lab tests and N.N. Statistical Tests and edition of manuscript.

Conflicts of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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