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Plasma Levels of IFN-γ, IL-4, IL-6 and IL-17 in HIV-Positive Patients With Oral Candidiasis

Seyyed Amin Ayatollahi Mousavi,[1,2] Gholamreza Asadikaram,[3,4] Nouzar Nakhaee,[3] and Alireza Izadi[1,*]

[1]Department of Medical Mycology and Parasitology, School of Medicine, Kerman University of Medical Sciences, Kerman, IR Iran
[2]Tropical and Infectious Disease Research Center, Kerman University of Medical Sciences, Kerman, IR Iran
[3]Institutes of Neuropharmacology, Neuroscience Research Center, Kerman University of Medical Sciences, Kerman, IR Iran
[4]Department of Biochemistry, School of Medicine, Kerman University of Medical Sciences, Kerman, IR Iran

[*]*Corresponding author*: Alireza Izadi, Department of Medical Mycology and Parasitology, School of Medicine, Kerman University of Medical Sciences, Kerman, IR Iran. Tel: +98-3433221661, Fax: +98-3432480680, E-mail: alirezaizadi81@yahoo.com

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Abstract

Background: Cell-mediated immunity (CMI) by CD4+ Th-type (T helper-type) cells is the predominant host defense mechanism against oral candidiasis (OC) in HIV-infected individuals. Weakened CMI and depletion of CD4+ T cells are the main factor contributing to the output of OC in HIV-positive individuals. The cytokines produced by Th1, Th2 and Th17 cells play a role in mediating an increased susceptibility to OC during HIV infection.
Objectives: The present study investigated the plasma concentration of IFN-γ, IL-4, IL-6 and IL-17 in HIV-1 patients suffering from OC.
Patients and Methods: In total, 98 samples in four groups (HIV-positive and HIV-negative persons, with and without OC) were obtained from the oral cavities of participants and cultured on Sabouraud's dextrose agar and CHROMagar. Also, blood samples were obtained to assess plasma levels of IFN-γ, IL-4, IL-6 and IL-17, using the ELISA technique.
Results: There was a statistically significant difference in the plasma concentration of IFN-γ, IL-6 and IL-17 but not of IL-4. Our findings suggest a significant interaction between fungal infection and HIV on expression of assessed cytokines.
Conclusions: Fungal infection and HIV, alone and together, can seriously alter immune system function as assessed by measuring the levels of plasma cytokines. Therefore, these results provide important new information relative to the putative immune-based factors associated with resistance and/or susceptibility to OC in HIV-positive persons.

Keywords: Oral Candidiasis, HIV, IL-4, IL-6, IL-17, IFN-Gamma

1. Background

Human immunodeficiency virus (HIV) causes the incurable disease of adaptive immunodeficiency syndrome (AIDS), and is one of the major global health problems (1, 2). The world health organization (WHO) maintains that more than 35.9 million people worldwide are living with HIV/AIDS (3). The most common sources of morbidity and mortality among HIV-positive individuals in the late stages of the disease, when the count of CD4 reaches below 500/μL, are opportunistic infections resulting from agents, such as fungi, that rarely infect immunocompetent individuals (4). The incidence of opportunistic fungal infections has been progressively increasing in recent years, and invasive fungal infections have been reported among 26% of chronically and intensively immunosuppressed patients (5).

Infections with *Candida albicans* (perhaps the first indication of immunodeficiency), cryptococcal infection and penicillosis emerge when the CD4 count is between 500 - 200/μL, below 150/μL, and less than 100 /μL, respectively (6). *Candida albicans* is a commensal organism that is found normally in the gastrointestinal and reproductive tracts. Under immunocompromised conditions, *C. albicans* can convert from commensal to pathogen and cause symptomatic disease (7). Multi-species oral yeast colonization with inherently drug-resistant organisms is common in HIV-infected patients. While *C. albicans* is the predominant causative agent of all forms of mucocutaneous candidiasis, less frequent members of this genus, notably *C. glabrata*, *C. krusei*, *C. tropicalis*, *C. parapsilosi*, and *C. dubliniensis*, have been cited as the causative agents of oral candidiasis (OC) and have been isolated in HIV-associated OC (7, 8). Two components of the host immune system, the innate and adaptive immune responses, work in coordination as part of an integrated host immune response to prevent fungal infections (8).

A portion of the adaptive immune response is the adaptive T helper (Th) cell responses, which are classified as the protective type-1 (Th1) and the non-protective type-2 (Th2) (9). The type-1 response is characterized by the production of Th1 cytokines, such as IFN-γ. These cytokines stimulate

the differentiation of CD4 T cells to the Th1 profile, and stimulate macrophage activation, production of opsonizing antibodies and delayed type hypersensitivity. The type-2 response is characterized by the production of Th2 cytokines such as IL-4. IL-4 elicits the production of the IgE isotype, which is related to allergic reactions, and down regulates the Th1 profile (8, 9). IFN-γ, are known to activate phagocytic cells, and are thus more able to kill Candida. In contrast, the cytokines produced by Th2 cells, such as IL-4, induce alternative macrophage activation (10, 11).

TH17 is the third subset of effector cells that has recently been identified and characterized by the production of IL-17 (12). The responses of these cells have been implicated in driving the protective immune responses against a variety of microbes, including several bacterial and fungal pathogens. In addition, IL- 17 is probably implicated in the immunopathogenesis of autoimmune responses (13, 14).

Mononuclear phagocytic cells are the most important source of IL-6; however, IL-6 is also produced by T- and B-lymphocytes (15, 16). IL-6 mediates several anti-inflammatory and proinflammatory effects, and also has been found to serve as a growth factor for HIV (1).

2. Objectives

Numerous studies have evaluated the effects of HIV and fungal infection on the immune system in vitro and in animal models. To the best knowledge of the authors of this study, in vivo studies measuring the effects of HIV and fungal infection on cytokine levels in humans have rarely been conducted. Therefore, in this investigation, the levels of IFN-γ, IL-4, IL-6 and IL-17 were measured in patients infected with HIV-1 and suffering from OC.

3. Patients and Methods

3.1. Study Population and Sampling

This cross-sectional study was conducted in the department of medical mycology and parasitology between April 2013 and September 2014. This study was approved by the ethical committee of Kerman University of Medical Sciences. Written informed consent was obtained from each participant prior to the sample collection.

A total of 98 men ranged between 18 - 50 years old, who were referred to the Kerman behavioral disorders center (Kerman, Iran) were recruited and evaluated in this study. Subjects consisted of HIV-positive and HIV-negative persons with and without OC. Study groups were defined as: HIV-/OC- (control) (n = 32), HIV+/OC- (n = 24), HIV-/OC+ (n = 19) and HIV+/OC+ (n = 23). HIV infection was determined in plasma by ELISA, and confirmed by Western blot, at the Kerman blood transfusion organization in Kerman, Iran. The OC lesions were diagnosed on the basis of clinical presentation (white pseudomembranous plaques and/or erythematous areas), findings of direct microscopic examination and positive culture results (described below).

The clinical appearance was confirmed by an infectious diseases specialist at the center of behavioral disorders of Kerman, Iran. Oral candidiasis-positive subjects had clinically typical visible lesions of oral candidiasis with positive test results for oral Candida colonization. HIV-positive subjects were receiving highly active antiretroviral therapy (HAART), and none of the participants received antifungal drug treatment. Oral swabs of each subject were obtained from the tongue or buccal mucosa by using sterile cotton swabs. Samples were collected under complete aseptic conditions, transported immediately to the medical mycology laboratory and processed. Swabs were plated on Sabouraud-dextrose agar with choramphenicol (Merck, Germany) under aerobic conditions at 32°C and in CHROMagar Candida media (CHROMagar, France) in the dark at 35°C for 48 hours to produce species-specific colors. The samples were observed daily for growth. A 10% KOH preparation and Giemsa stain were used for microscopic examination of the samples.

The diagnosis of OC was confirmed by hyphae being present on a smear and a positive swab culture result that had characteristic colony morphology. Asymptomatic colonization was determined based on the presence of colonies after plate culture but without symptoms or a positive KOH smear result. Chromogenic culture media distinguish *C. albicans* from other yeast strains based on the color changes produced by the *Candida spp*. colonies. Fresh yeast colonies were processed for germ-tube formation (incubated with rabbit serum at 37°C for 3 hours). Colonies that formed germ tubes were classified as *C. albicans*. Colonies that did not form germ tubes were specified by carbohydrate assimilation tests with the RapID™ Yeast Plus System (Remel, USA), according to the manufacturer's instructions (6). Venus blood (10 mL) was collected in EDTA tubes and stored on ice until isolation of the plasma. Plasma was stored at -72°C until use. Plasma concentrations of cytokines were measured by ELISA kits (R and D Systems, USA), according to the manufacturer's guidelines.

3.2. Statistical Analysis

All statistical analyses were done using SPSS (ver. 20; IBM Inc.). The two-way analysis of variance (ANOVA) and the student's t-test were performed for the comparison of cytokine levels between groups. A value of $P \leq 0.05$ was considered to be statistically significant.

4. Results

The mean of CD4 T cell counts in HIV-positive participants was 456 cells/mm^3. Out of a total of 98 samples, a definitive diagnosis of OC was made in 42 individuals. All participants in this study were male, and the mean of age of the study population was 36.5 ± 0.81 years (Table 1).

4.1. Effect of HIV and OC on Plasma Concentration of IFN-γ

A two-way ANOVA revealed that a main effect exists for HIV on IFN-γ expression. HIV-positive subjects had significantly more IFN-γ expression than HIV-negative subjects (P = 0.0001). Also, there was a main effect for OC on plasma concentration of IFN-γ. Oral candidiasis-positive subjects elicited significantly more IFN-γ than the OC-negative subjects (P = 0.001). There was a significant interaction between OC and HIV in terms of the IFN-γ plasma levels (P = 0.005).

4.2. Effect of HIV and Oral Candidiasis on Plasma Concentration of IL-4

There is no main effect for HIV and OC on plasma concentration of IL-4 (P = 0.24, P = 0.17, respectively). Also there was not a significant interaction between HIV and OC in terms of IL-4 level (P = 0.28).

4.3. Effect of HIV and Oral Candidiasis on Plasma Concentration of IL-6

There was a main effect for HIV on IL-6 expression. HIV-negative subjects had significantly more IL-6 expression than HIV-positive subjects (P = 0.0001). However, there was not a significant effect for OC on plasma concentration of IL-6 (P = 0.65). Additionally, there was a significant interaction between HIV and OC in terms of the level of IL-6 (P = 0.001).

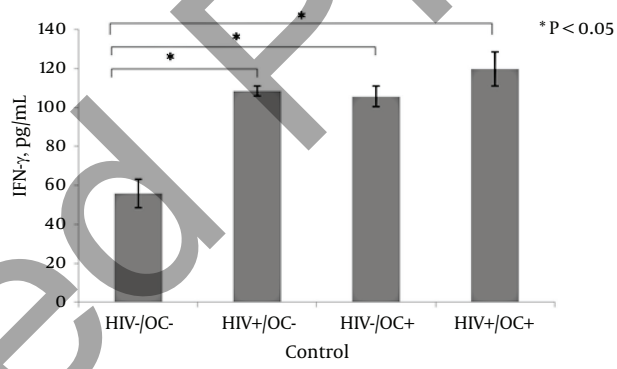
4.4. Effect of HIV and Oral Candidiasis on Plasma Concentration of IL-17

There was a main effect for HIV on IL-17 expression. HIV-negative subjects were significantly lower in terms of IL-17 than HIV-positive subjects (P = 0.001). Also, there was a main effect of OC on plasma concentration of IL-17. Oral candidiasis-positive subjects elicited significantly more IL-17 than OC-negative subjects (P = 0.03). There was a significant interaction between HIV and OC in terms of the expression of IL-17 (P = 0.0001).

4.5. Plasma Concentration of Cytokines

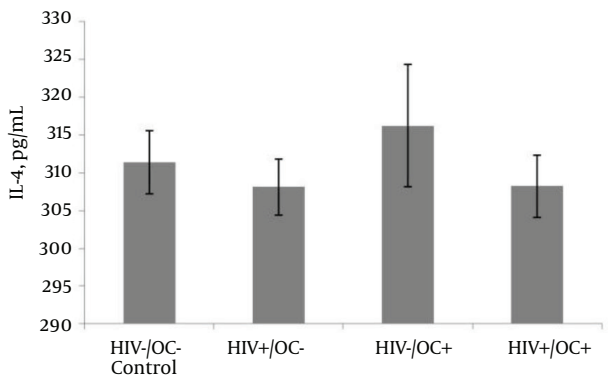
Analysis of plasma cytokine levels of HIV+/OC+, HIV+/OC-, HIV-/OC+ and HIV-/OC- revealed changes in the cytokine profiles. IFN-γ and IL-17 levels were determined to be statistically increased in HIV+/OC+, HIV+/OC- and HIV-/OC+ patients compared with the healthy controls (HIV-/OC-) (P = 0.03, P = 0.001, P = 0.0001, P = 0.01, P = 0.0001, P = 0.0001, respectively; Figures 1 and 4). IL-6 levels were determined to be statistically decreased in HIV+/OC- compared with HIV-/OC+ and HIV-/OC- (P = 0.001, P = 0.005, respectively; Figure 3). There was no significant difference in IL-4 level between groups (Figure 2).

Figure 1. Plasma Concentrations of IFN-γ in Infected Patients (HIV+) and Controls (HIV-) With (OC+) or Without (OC-) oral Candidiasis



IFN-γ was significantly increased in HIV+/OC-, HIV-/OC+ and HIV+/OC+ patients compared with controls (P < 0.05). There was no statistical difference between HIV+/OC-, HIV-/OC+ and HIV+/OC+. Data are shown as mean ± SEM of concentration of IFN-γ in each group.

Figure 2. Plasma Concentrations of IL-4 in Infected Patients (HIV+) and Controls (HIV-) With (OC+) or Without (OC-) Oral Candidiasis

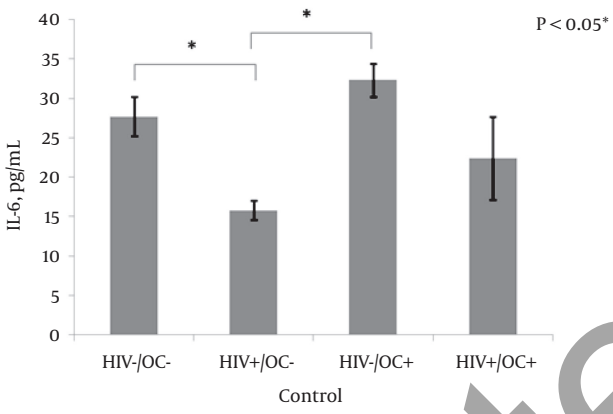


There was no statistical difference between HIV+/OC-, HIV-/OC+, HIV+/OC+ and HIV-/OC-. Data are expressed as mean ± SEM of concentration of IL-4 in each group.

Table 1. Demographic Characteristics of Studied Patients

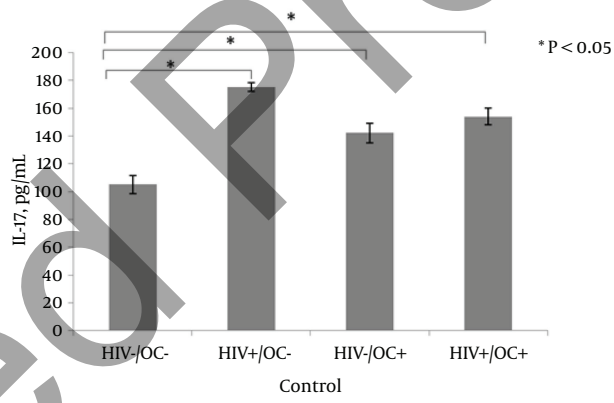
Group	No. of Patients	Mean of Age, y	Gender
HIV+/OC+	23	39.5 ± 2.72	male
HIV+/OC-	24	34.3 ± 1.83	male
HIV-/OC+	19	40.3 ± 2.92	male
HIV-/OC- (control)	32	31.4 ± 1.27	male
Total	98	36.5 ± 0.81	-

Figure 3. Plasma Concentrations of IL-6 in Infected Patients (HIV+) and Controls (HIV-) With (OC+) or Without (OC-) Oral Candidiasis



IL-6 was significantly decreased in HIV+/OC- patients compared with HIV-/OC+ and HIV-/OC- (controls) ($P < 0.05$). There was no statistical difference in HIV+/OC+ compared with HIV-/OC+, HIV+/OC- and HIV-/OC-. Data are shown as mean ± SEM of concentration of IL-6 in each group.

Figure 4. Plasma Concentrations of IL-17 in Infected Patients (HIV+) and Controls (HIV-) With (OC+) or Without (OC-) Oral Candidiasis



IL-17 was significantly increased in HIV+/OC-, HIV-/OC+ and HIV+/OC+ patients compared with controls ($P < 0.05$). There was no statistical difference between HIV+/OC-, HIV-/OC+ and HIV+/OC+. Values are presented as mean ± (SEM) of concentration of IL-6 in each group.

5. Discussion

In the present study, we evaluated the effects of HIV and Candida infection on cytokine levels and measured the plasma concentrations of IL-4, IL-6, IL-17 and IFN-γ, as well. The present findings demonstrated that the levels of IFN-γ in the plasma of patients with OC were significantly higher than those without OC. Also, the levels of this cytokine in HIV-positive individuals were significantly higher than HIV-negative individuals. Interestingly, according to the findings of this study, in patients without OC, the shift to OC+ caused a significant increase in the levels of IFN-γ in both HIV-positive and HIV-negative patients with different slops. The increased levels of IFN-γ suggest that inflammatory processes are enhanced. Results related to IFN-γ have been controversial, since both increases and decreases have been reported (17). Previous studies have also demonstrated that fungal infection and/or HIV infection can affect IFN-γ production. Lilly et al. showed HIV-positive subjects with oropharyngeal candidiasis (OPC) had changes in the levels of most tissue-associated cytokines. These included increased levels of IFN-γ (18). Shellito et al. assayed cytokine production by CD4+ T cells of normal mice challenged with pneumocystis and showed a rapid increase in CD4+ T cells that produced IFN-γ (19). Fatahinia et al. showed propolis treatments alone suppressed IFN-γ in the sera of mice when compared with mice that received propolis together with *C. albicans* (20). IL-4 is a Th2 cytokine that is predominantly produced by activated CD4+ T cells. It is also produced by NK cells, mast cells, and basophils and stimulates activation and differentiation of B-cells, secretion of IgG1 and IgE, activation of T cells, and MHC II expression on B-cells.

Macrophages seem to play a protective role in the mouse model of OC, since neutralization of IL-4 results in the delayed clearance of fungi (13). According to the results of the present study, OC and HIV infection did not alter the plasma concentration of IL-4, as the level of this cytokine in patients compared to non-infected controls

did not show a significant difference. Several studies have demonstrated that the increased secretion of IL-4 is related to in vivo HIV-1 infection. However, there are conflicting reports on the effect of HIV-1 infection on IL-4 production. In addition, reduced or normal IL-4 levels have also been reported in HIV-infected individuals (21). Increased IL-4 and IL-5 mRNA levels in *C. albicans* antigen-activated peripheral blood mononuclear cells (PBMC) from HIV-infected patients with OPC indicated a shift from a Th1 profile to a less polarized and protective Th2/Th0 profile, especially the patients who experienced a *C. albicans* infection. Therefore, in HIV or other immunosuppressive diseases, in which an increased level of IL-4 is observed, fungal infection can cause life-threatening diseases (21). IL-6 stimulates the acute phase response (innate immunity) and promotes B-cell proliferation (adaptive immunity).

Monocytes, macrophages, T lymphocytes, endothelial cells and fibroblasts are the source of this cytokine (22). As mentioned in the literature, IL-6 seems to be elevated in HIV infection, acting as a growth factor for HIV (to encourage HIV replication and proliferation) compared to healthy individuals (1). Studies have also have revealed that this cytokine enhances resistance to invasive aspergillosis and candidiasis in mice (16). This experiment did not detect any evidence of an effect of OC on IL-6 plasma concentration, and OC+ did not significantly alter IL-6 more than in the OC- subjects. On the other hand, HIV significantly alters the plasma level of IL-6, as the HIV-negative group had significantly more IL-6 than the HIV-positive group. In other words, HIV reduced the plasma concentration of IL-6. In HIV-negative and HIV-positive patients, following a shift from OC- to OC+, the plasma concentration of IL-6 did not change significantly.

This finding confirms the significant interaction between OC and HIV in terms of the level of IL-6. Th17 cells are a recently identified subtype of CD4+ T cells that respond to fungal antigens and are important in mucosal immunology. In addition, because HIV infection results in loss of CD4+ T, Th17 cells potentially play an important role in HIV pathogenesis (23). Studies have shown a role for IL-17 in immunity against extracellular pathogens and have shown that Th17 cell activation promoted deleterious inflammation and defective fungal clearance in pulmonary aspergillosis and gastrointestinal candidiasis (24). In mice, deficiency of IL-17 causes increased susceptibility to disseminated candidiasis (25, 26). Therefore it is expected that IL-17 levels rise in fungal infections. Results of this study showed OC significantly elicited the plasma concentration of IL-17 in HIV-negative subjects while, in HIV-positive patients OC decreased the plasma level of this cytokine.

Results of several studies on IL-17 levels in HIV infection have shown different results as to whether IL-17 levels are increased, decreased, or not affected in plasma after HIV infection (27, 28). For instance, one study has shown that in HIV-infected children there was a significant loss of IL-17, producing PBMC (29). In contrast to these findings, the results of our study showed that participants in the HIV-negative group were significantly lower in terms of IL-17 than HIV-positive subjects. According to the results presented here, it appears that IL-17 plays a significant role in defense against OC because it was more increased in OC patients in comparison to OC-negative subjects than other cytokines. Based on the fact that IL-17 is the first line of the adaptive immune response against infections, hence, it appears that adaptive immunity of HIV-positive patients also follows this pattern. This study is the first to have evaluated the plasma concentration of cytokines in the plasma of HIV-1 patients suffering from OC in a clinical setting. The key finding of this study was that fungal infection and HIV, alone and together, could seriously alter immune system function as assessed by measuring the levels of the plasma cytokines. Therefore, these results provide important new information relative to the putative immune-based factors associated with resistance and/or susceptibility to OC in HIV-positive persons. However, more research on this topic needs to be undertaken before the association between the immune system response and opportunistic fungal infections in HIV-positive patients is understood.

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Footnotes

Authors' Contribution: Seyyed Amin Ayatollahi Mousavi developed the original idea and the protocol; Gholamreza Asadikaram contributed to the development of the protocol, and developed and prepared the manuscript; Nouzar Nakhaee contributed to the development of the protocol and analyzed the data; Alireza Izadi was the guarantor, abstracted the data, designed the curve, prepared and wrote the manuscript.

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