The Effects of Opium Addiction on the Immune System Function in Patients with Fungal Infection

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Abstract

Background: The use of narcotics such as opium exposes addicts as susceptible targets of different diseases so that they might easily be exposed to different diseases such as fungal infections. The present study aimed to investigate the effects of addiction to opium and fungal infection on plasma levels of certain cytokines including interleukin-4 (IL-4), IL-6, IL-17, Interferon gamma (IFN-γ) and transforming growth factor-β (TGF-β).

Methods: Present study included 72 individuals who were divided into 4 groups: 1) opium-addicted with fungal infection; 2) opium-addicted without fungal infection; 3) non-opium-addicted with fungal infection; and 4) normal individuals (non-opium-addicted and non-fungal infection). The fungal samples, after being detected and confirmed by a physician, were prepared based on clinical symptoms and then analyzed by direct smear and culture method. The measurement of the plasma level of cytokines was done by enzyme-linked immunosorbent assay (ELISA) method.

Findings: The comparison of the mean of the plasma level of cytokines showed that addiction to opium and fungal infection had significant effect on the plasma levels of IL-17, IFN-γ, TGF-β cytokines in all studied groups. The interaction of addiction to opium and fungal infection was only significant in the case of plasma level of IL-6.

Conclusion: Addiction to opium and fungal infection, either separately or simultaneously, poses significant effect on the immune system and causes disorders in the cytokine network and the immune system and also provides a suitable environment for fungal infection.

Keywords: Opium; Addiction; Fungal infection; Cytokine

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Introduction

Opium as a narcotic drug is more prevalent in the northern and southern parts of Iran. In a study conducted in Kerman, Iran, it was shown that the urine test of 15% of the subjects who were referred to one of the major medical diagnostic laboratories was positive. 

Opium is made of 5%-20% water, 20% different carbohydrates, 10%-20% alkaloids and some amounts of organic acids. Of significant alkaloids of opium, one could point to morphine (8%-17%), codeine (0.7%-5.0%), thebaine (0.1%-2.5%), papaverine (0.5%-1.5%), and noscapine (1%-10%).

The effects of opioids are realized by their connection to the opioid receptors that exist in different areas of the nervous system and other tissues. These receptors are also expressed in macrophages and lymphocytes. The endogenous and exogenous opioid materials are attached to these areas to different degrees and they have different effects.

In lower dosages, morphine has inflammatory effects while in higher dosages, it generates anti-inflammatory effects along with reduction of expression of interleukin-6 (IL-6), IL-1 and tumor necrosis factor alpha (TNF-α) through μ receptors. In case of chronic use of morphine, the replication of thymocytes and T lymphocytes will reduce. The Th1 CD4+ cells change into Th2 CD4+ through the cAMP-dependent pathway which finally blocks the production of Th1 cytokines (IL-2 and IFN-γ) and increases the production of Th2 cytokines (IL-4, and IL-5). The exogenous opioids directly simulate lymphocytes which affects the secretion and release of TGF-β.

Morphine induces the apoptosis of lymphocytes and macrophages. This is done by increasing the expression of Fas cell death receptor and stimulating Caspase-dependent external cell death pathway. The apoptotic cell death of lymphocytes is also influential upon the pathogenesis of the infectious agents and their sensitivity to infection. In addition, morphine plays a role in the inhibition of the B lymphocyte differentiation into the plasma cells, the reduction of the expression of major histocompatibility complex (MHC II) and the defection of the presentation of antigens via the μ receptor.

The usage of different narcotic drugs such as opium exposes the addicts to different kinds of diseases and makes them susceptible to numerous diseases such as fungal infection. The opportunistic fungi rarely lead to serious disease in their natural host but they create life-threatening infections in individuals with immune system disorders. The fungal infections are frequently observed among patients addicted to narcotics. These infections range from asymptomatic mucosal candidiasis to spreading infections and lethal meningitis.

In patients with fungal infections, the condition of the host’s immune system is highly significant. Almost all of those who have normal immune systems might get fungal infections in a latent manner and without representing explicit clinical symptoms. The studies done on rats clearly show the role of different subsets of T-cell and cytokines in controlling fungal infections so that type-1 immunity is induced by IL-12 and manifested through the secretion of interferon gamma (IFN-γ) and TNF-α by Th1 cells. These cells play a protective role against the fungal infections developed by candida, aspergillus and histoplasma. Type-1 immunity responses are essential for activating macrophages and neutrophils in phagocytosis of invading fungi. In contrast, type-2 immunity developed through high levels of IL-4 and IL-5 is manifested through Th2 cells, which is non-protective and in some cases, it intensifies the infection or symptoms of a disease.

IFN-γ is a significant regulator of acquired Th1 response against fungal infections. These immigrant cytokines stimulate the adhesion and anti-fungal activity of neutrophils and/or macrophages against a wide range of pathogenic fungi.

Studies have shown that the exposure of macrophages of human blood to morphine significantly reduces the expression of different interferons and their coding genes.

IL-4 is Th2 cytokine and it is mostly produced by TCD4+ cells. Still, other cells of the immune system such as NK cells, mast cells and basophiles can also produce these cytokines. The chronic treatment of rat with morphine leads to the differentiation of T-helper cells to Th2 and increase in the expression of IL-4. The direction toward Th2 response is often associated with more intense cases of fungal infection.

The transforming growth factor-β (TGF-β) is among the potent immunosuppressive cytokines...
which has disruptive effects on the host’s response to fungi. Considering the anti-inflammatory role of this cytokine in the inhibition of the secretion of inflammatory cytokines, it neutralizes the effective antifungal functions through phagocytes. Different studies have shown that narcotics can induce the in-vitro secretion of TGF-β.

The effective role of mucosal T-cells in health has been studied in different studies and they have shown that the new subsets of T CD4+ cells (i.e. TH17 Cells) are abundant in the digestive system. These cells produce IL-17 which is significant in the acquired immunity against exogenous fungi and bacteria. The inflammatory cytokines such as IL-6 regulate the leukocyte trafficking, proliferation and activation of the oxidative and non-oxidative antifungal responses by these cells. In another study, the significant increase in IL-6 concentration in opium-addicted individuals compared to the control group was mentioned. The rats with IL-6 defects showed disorders in the activation of antifungal responses of Th1 cells.

Considering the increase of the antibiotics-resistant fungal species and the frequent reports on fungal infections in individuals with immune system disorders and narcotic addiction, effective treatments against such infections and the control of fungal pathogens are significantly required. To understand this, extensive studies regarding the prevalence of fungal infections and the changes in the immune system of addicted individuals are being done. It is evident in order to attain this objective, the analysis of the immune system function of such individuals is required, which can also be evaluated through the measurement of cytokines embedded in the blood. Therefore, the present study was designed to measure and compare the plasma levels of IL-4, IL-6, IL-17, ILFN-y and TGF-β cytokines so as to highlight the effects of opium addiction and fungal infection on their levels.

Methods

The present cross-sectional study was conducted at the Department of Mycology and Parasitology of Kerman University of Medical Sciences. Present study included 72 individuals divided into four groups: 1) those addicted to opium with fungal infection, FI+/OA+; 2) those addicted to opium without fungal infection, FI-/OA+; 3) non-addicts with fungal infection, OA-/FI+; and 4) normal individuals, FI-/OA-. Each group had 18 individuals. All individuals were male and older than 18 years of age. These individuals were selected from the attendees of the Medical Mycology Laboratory of Afzalipour Medical School, Center for Behavioral Medicine, Afzalipour Hospital, Shafa Hospital and Bahonar Hospital of Kerman City. The exclusion criteria of the present study were: being female, being younger than 18 years old or having more than 60 years, and taking immunosuppressive drugs.

All patients received sufficient explanation regarding the steps and underlying method of the study design. After receiving signed approval form, demographic and clinical information of surveyed individuals including their age, sex, medical history, smoking, narcotic drug use, history of infectious disease, and medicine use was recorded in questionnaire form. This study was approved by the Ethics Committee of Kerman University of Medical Sciences.

All fungal samples including the sputum samples, skin, bronchoalveolar lavage (BAL), and mouth samples were prepared after the diagnoses and confirmation of a physician. The prepared samples were transferred to the Medical Mycology Laboratory of Kerman University of Medical Sciences. A direct slide was prepared from the transferred samples to the Medical Mycology Laboratory and then, they were analyzed under a microscope. One of the most significant diagnostic methods of fungal diseases is the preparation of a direct slide by potassium hydroxide solution (KOH) which is used for skin samples as well as other tissue samples such as sputum, pus, secretions, biopsy secretions, urine and fecal matters. For analysis, the samples of sputum, BAL and oral swabs were placed on glass slides, stained by Giemsa stain method and studied under a microscope. In addition, a culture of prepared samples was developed in Sabouraud’s dextrose agar with and without chloramphenicol. The cultures were incubated at 32 °C for three weeks in an incubator. The cultures were daily examined for the growth of their daily growth.

A 5 ml blood sample was taken from all participants. After centrifugation at 2500 rpm for 15 minutes, the plasma sample was obtained and
maintained at -20 °C freezer. The plasma level of IL-4, IL-6, IL-17, IFN-γ and TGF-β cytokines were measured by enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, USA) according to the manufacturer’s guidelines.

The raw data that was collected after receiving the demographic questionnaire and performing the tests for the measurement of the cytokines levels was analyzed in a descriptive and analytical manner by SPSS software (version 16, SPSS Inc. Chicago, IL, USA). To examine the effects of opium addiction and getting fungal infection on the plasma level of selected cytokines, two-way analysis of variance (ANOVA) was used and P < 0.05 was considered as statistical significance.

**Results**

The mean age of the 72 sampled individuals in this study was 35.43 ± 9.9. The mean age of the individuals in the OA+/FI+, OA+/FI−, OA−/FI+ and OA−/FI− groups were 40.36 ± 9.41, 31.52 ± 5.53, 40.44 ± 12.75 and 36.12 ± 4.91 years respectively. Among the 36 opium addicts, 28 individuals smoked opium and 8 participants had used an edible form of opium for more than 3 years.

In the OA−/FI+ group, candidiasis was observed among 15 individuals while aspergillosis was detected among 3 members of the groups. In the OA+/FI+ group, the diagnostically verified cases of candidiasis and aspergillosis were 16 and 2 individuals respectively.

The two-way ANOVA analysis showed that opium addiction had significant influence upon the plasma levels of IL-17, TGF-β and IFN-γ while its effect upon the plasma level of IL-4 and IL-6 was insignificant. In addition, the fungal infection had significant influence upon the plasma levels of TGF-β and IFN-γ while its effect upon the plasma levels of IL-4, IL-6 and IL-17 was insignificant. The mutual effect of opium addiction and fungal infection on the plasma level of IL-6 was significant but this was not the case for the plasma levels of the other cytokines studied here.

The comparison of the mean of the plasma levels of the cytokines showed that IFN-γ in OA+ individuals had a significant reduction in comparison with the OA- ones. In addition, FI+ individuals showed a significant increase in their plasma level of IFN-γ in comparison with the FI- individuals (P < 0.001). The TGF-β plasma level in the OA+ individuals in comparison with the OA- ones had a significant rise, while in the FI+ individuals, the TGF-β plasma level showed a significant reduction in comparison with the FI- individuals (P < 0.001). In OA+ individuals, IL-17 plasma level manifested a significant increase in comparison with the OA- ones (P < 0.001) but the fungal infection had an insignificant effect upon the level of cytokine (P > 0.05). With regard to IL-4 and IL-6, there was no significant difference between the OA+ and OA- individuals as well as between the FI- and FI+ individuals (P > 0.05) (Table 1).

| Cytokine | Opium addiction+ | Opium addiction- | FI+ | FI- |
|----------|------------------|------------------|-----|-----|
| IFN-γ (pg/ml) (mean ± SD) | 106.64 ± 10.62 | 75.56 ± 37.23 | 118.77 ± 30.11 | 88.74 ± 20.11 |
| TGF-β (pg/ml) (mean ± SD) | 450.65 ± 148.52 | 731.05 ± 259.8 | 450.21 ± 65.59 | 683.88 ± 94.76 |
| IL-4 (pg/ml) (mean ± SD) | 310.54 ± 10.32 | 308.59 ± 5.33 | 313.8 ± 18.43 | 310.87 ± 13.8 |
| IL-17 (pg/ml) (mean ± SD) | 165.33 ± 17.49 | 159.10 ± 47.45 | 130.91 ± 8.35 | 121.17 ± 26.62 |
| IL-6 (pg/ml) (mean ± SD) | 24.23 ± 6.03 | 27.21 ± 10.15 | 30.61 ± 10.55 | 26.40 ± 7.66 |

IFN: Interferon; TGF: Transforming growth factor; IL: Interleukin; FI: Fungal infection; SD: Standard deviation

P < 0.05 was considered significant
Discussion

The study showed that in all studied groups, the opium addiction had a significant influence on the plasma levels of IL-17, IFN-γ, and TGF-β, while it had no effect on the plasma levels of IL-4 and IL-6. In addition, all of the tested groups revealed the significant effect of fungal infection on IFN-γ and TGF-β while it had no effect on IL-4, IL-6 and IL-17. The mutual effect of addiction on opium and fungal infection was only significant upon the plasma level of IL-6 and it lacked significant effect on the level of the other analyzed cytokines in the present study. The main limitation of the study is not including cigarette smoking as a factor affecting the immune system.

Based on these results, the mean plasma level of IFN-γ in OA+ individuals in comparison with the OA- ones manifested a significant reduction and the same was observed for FI+ individuals in comparison with the FI- ones.

Similar results were found in a study by Shellito et al. who analyzed the production of cytokines by T CD4+ cells removed from lymph nodes and lung tissues of rats exposed to pneumocystis for 4 weeks. However, there are other studies with different results.

The studies have shown that the exposure of macrophages of human blood tissue to morphine significantly reduces the expression of different interferons (IFN-α, IFN-β and IFN-γ) and their coding genes. Regarding the critical role of IFN-γ in the immunity of Th1 cells against fungal infection, it seems that the long-term usage of narcotic drugs is associated with different types of fungal diseases in the addicts. Different studies in the past have also suggested that opium and morphine (an opioid derivative) reduce the secretion of IFN-γ. The results of the present study have shown that the mean level of IFN-γ cytokine in opium-addicted individuals compared with the non-addicts showed a significant decrease. This verifies that the use of opium has significant effects upon secretion of IFN-γ in the addicts. Therefore, it seems that addicts usually have Th1 and Th2 disorders and lack the ability to develop sufficient amounts of critical cytokines such as IFN-γ.

In the present study, the mean plasma level of IL-4 among the OA+ and OA- individuals as well as the FI+ and FI- ones did not show any significant change. As a result, the fungal infection and opium addiction lack significant interferential effects upon the level of this cytokine.

The results of the different studies on the effects of fungal infection upon the IL-4 level are paradoxical. In a study, the mean of type-2 cytokines such as IL-4 among patients with superficial fungal infection of psoriasis was significantly lower in comparison with the normal individuals of the control group. In another study, the level of IL-4 cytokine in the brain of rats infected with cryptococcosis was reported to be significantly higher than that of the control group. In contrast, the results of the present study did not show any significant change in the level of this cytokine among the individuals with fungal infection.

In a previous study, we showed that the level of IL-4 in opium addicts was significantly lower than that of the control group. Considering the key role of IL-4 in defending infection, production of Immunoglobulin E (IgE), and also blood safety, it seems that the immune system of blood is influenced in those individuals who are addicted to narcotics. In contrast, the results of the present study showed that opium addiction lacks significant effects upon the plasma level of IL-4. This difference might be due to the difference in the type of the analyzed narcotic drugs; accordingly, a study reported that chronic exposure of rats to morphine leads to the differentiation of T-helper and Th2 cells and increase in the expression of IL-4.

With regard to the results of the present study, the mean level of TGF-β among the OA+ versus the OA- individuals showed significant increase while its level was significantly lower in the FI+ patients in comparison with the FI- ones. Based on the related literature, few studies have investigated the effect of fungal infection on the level of this cytokine. A study showed that in rats with candidiasis, the regular cells CD4+ and CD25+ produce IL-10 and TGF-β and cause an increase in the level of this cytokine in these rats which prevents the total removal of the yeasts from their digestive system. In contrast, the results of our study showed that the mean plasma level of TGF-β in the rats in in-vivo conditions does not influence the primary results of the medicinal treatment against the pathogenic yeast but it plays an effective role in delaying resistance in the rats against this pathogenic yeast.
the role of this cytokine is not clear but it has been verified that treatment with recombinant TGF-β in rats delays the development of fungal diseases in them.\textsuperscript{31} Our results showed a significant increase in the plasma level of TGF-β in opium addicts. TGF-β is secreted by regulator T-cells and acts as an anti-inflammatory agent. Therefore, one might conclude that the regulator T-cells increase the level of this cytokine in order to control the inflammatory conditions in addicts (i.e. increase in the IL-6 level). It is noteworthy that TGF-β level in the culture of the lymphocytes belonging to opium addicts was less than that of the control group. It seems that the lack of inflammatory conditions in the culture is the main reason behind it.\textsuperscript{32} The high levels of TGF-β in opium addicts (due to its inhibitory effects on the function of immune cells) might justify the higher prevalence of infectious and malignant diseases among the addicts.

Considering the results of this study, opium addiction has significant effects on the plasma level of IL-17 while fungal infection lacks meaningful effects upon the plasma level of this cytokine.

Another study showed that neutralization of IL-17 during pulmonary aspergillosis and oral candidiasis leads to a reduction in the infiltration of neutrophils, increases the fungal load and lowers the level of the chemokines.\textsuperscript{33} This shows the significant role of chemokine in fungal infection and also battling against these infections.

Based on the review of the literature, there was no study on the effect of narcotic drugs on the level of IL-17. However, the results of the present study showed that addiction might significantly increase the level of this cytokine.

Our results also showed that fungal infection and opium addiction lacked significant effects upon the plasma level of IL-6. In addition, the analysis showed that there was no interaction between the two variables of the fungal infection and the opium addiction. This means that while the plasma level of IL-6 in OA-/FI- had increased in comparison with the OA-/FI+, the level of these cytokine in the OA+/FI- had decreased in comparison with the OA+/FI+. In other words, the change in the diagnostic results of the FI from negative to positive leads to an increase in this cytokine in non-addicted individuals while in the addicted individuals, its level decreased showing a mutual effect between FI and OA with regard to this cytokine.

A review by Lilly et al. showed out that the cases with oropharyngeal infections have increased levels of IL-6 in comparison with cases of asymptomatic colonization of Candida in the oral mucosa.\textsuperscript{34} In addition, Schaller et al. found that the epithelial cells infected with Candida albicans produce high levels of IL-6. This concurrency in the production of neutrophil-activator cytokines such as IL-6 and IL-8 in mucosal epithelium is associated with the immigration of neutrophils in such infections.\textsuperscript{35}

In another study, the authors found a significant increase in the IL-6 concentration within the plasma of opium-addicted individuals in comparison with the control group.\textsuperscript{25} The increase in the secretion of IL-6 plays a significant role in the inflammatory responses. Therefore, it seems that the addicted individuals suffer from a type of chronic inflammation. Some researchers reported that morphine in lower doses (morphine has pro-inflammatory effects in higher doses) exert anti-inflammatory effects along with reduction in the IL-6 secretion. Therefore, one could state that the long-term use of low-doses of opium by addicts simulates the production and secretion of higher levels of IL-6.\textsuperscript{22} In contrast to the results of previous studies, the results of present study did not reveal any significant change in the level of the IL-6 cytokine due to opium addiction and fungal infections.

**Conclusion**

Each one of the fungal infection and opium addiction cases, either separately or in combination with each other, pose significant effects on the immune system which leads to cytokines network disorder and impaired immune system cells. It seems that opium addicts suffer from a type of chronic inflammation, and in these individuals Th1 and Th2 systems are suppressed. The ultimate influence of these effects is their interference with immune system, which prepares a suitable condition for development of fungal infection.

**Conflict of Interests**

The Authors have no conflict of interest.

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تأثیر اعتیاد به تریاک بر عملکرد سیستم ایمنی افراد مبتلا به عفونت قارچی

درکت سید امین آیت‌اللهی، دکتر غلامرضا اسدی کرم، دکتر نوذر نخعی، علیرضا ایزدی، ناصر کیخا

چکیده

مقدمه:

مصرف مواد مخدر مثل تریاک، معتادان را در برابر انواع بیماری‌ها آسیب می‌پذیرد و آنان را در معرض ابتلا به بسیاری از بیماری‌ها از جمله عفونت قارچی قرار می‌دهد. تحقیق حاضر با هدف بررسی اثرات اعتیاد به تریاک و عفونت قارچی بر سطح سرمی سیتوکین‌های (Interferon gamma) IFN-γ، (Interleukin) IL-6، IL-17 و (Transforming growth factor-β) TGF-β1 انجام گردید.

روش‌ها:

جامعه مورد مطالعه را 72 نفر تشکیل دادند که در چهار گروه (مبتلا به تریاک و عفونت قارچی، غیر مبتلا به تریاک و عفونت قارچی) وارده شدند. نمونه‌های قارچی بر اساس علایم بالینی و پس از تشخیص و تایید توسط پزشک به روش ELISA (Enzyme-linked immunosorbent assay) بررسی گردید. سطح سرمی سیتوکین‌ها با استفاده از روش مورد سنجش قرار گرفت.

یافته‌ها:

مقایسه میانگین سطح سرمی سیتوکین‌های 17-IL، IFN-γ و TGF-β1 نشان داد که اثرات اعتیاد به تریاک و عفونت قارچی به ترتیب تأثیر معنی‌داری بر سطح سرمی سیتوکین‌های IFN-γ و TGF-β1 داشت. اثرات اعتیاد به تریاک و عفونت قارچی بر سطح سرمی IFN-γ و TGF-β1 در همه گروه‌های مورد بررسی داشت. اثرات متقابل اعتیاد به تریاک و عفونت قارچی تنها بر سطح سرمی IL-6 معنی‌دار بود.

نتیجه‌گیری:

اثرات اعتیاد به تریاک و عفونت قارچی به تنهایی و در تداخل یکدیگر، اثرات معنی‌داری بر سیستم ایمنی داشتند که موجب اختلال در شبکه سیتوکین‌ها و سیستم ایمنی می‌گردید و راه‌های پیش‌رفت عفونت قارچی فراهم می‌نماید.

واژگان کلیدی: تریاک، اعتیاد، عفونت قارچی، سیتوکین

ارجاع:

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