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Effect of smoking on cyanide, IL-2 and IFN-γ levels in saliva of smokers and nonsmokers

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ABSTRACT

Introduction: Tobacco use is widely spread throughout the world. Smoking has several adverse effects on human health ranging from minor health conditions to death.

Aim: This study aimed to investigate effect of smoking on level of saliva cyanide, interleukin-2 (IL-2) and interferon-gamma (IFN-γ) among smokers compared to nonsmokers in the city of Ilam, Iran.

Material and methods: This study was carried out among two equal groups of smokers as cases and nonsmokers as controls ($N = 76$) which were matched in terms of their age range. Dental roll and direct saliva method were used to collect samples. The saliva sample was stored at –18°C. The level of salivary cyanide was measured using the spectrophotometric method. IL-2 and IFN-γ were measured by ELISA.

Results and discussion: We found level of cyanide in the saliva of smokers was higher than that in nonsmokers. In addition, level of cyanide in the smokers' saliva increased (164.21 ± 18.54 μg/mL) significantly compared to nonsmokers (42.63 ± 24.01 μg/mL). A significant increase was found in the level of IFN-γ and IL-2 among smokers compared to nonsmokers. However, there was a significant decrease in the level of IFN-γ and IL-2 with increased intensity of smoking.

Conclusions: Heavy smoking was associated with an increased level of salivary cyanide and a decreased level of sera IFN-γ. Recognizing immunosuppression mechanisms produced by cigarette-smoking is a platform for identifying the best therapeutic and management approaches in smoke-induced diseases.

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1. INTRODUCTION

Cigarette smoking is the main risk factor for the accelerated decline in lung function and development of chronic obstructive pulmonary disease. Smoking is one of the most frequent addictions. Smoking cigarettes decreases blood flow to the extremities due to the increased peripheral vasoconstriction, especially relating to digital and forearm hemodynamics.[1] Moreover, cigarette smoking generates many toxic and carcinogenic compounds harmful to the health, such as nicotine, nitrogen oxides, carbon monoxide, hydrogen cyanide and free radicals.[2,3] Smoking affects a wide range of immunological functions in humans and experimental animals, including both the humoral and cell-mediated immune responses.

Immunosuppression effects of smoking depend on the amount and duration of smoke exposure, ethnic background, and gender. It is postulated that this increased susceptibility reflects cigarette smoke-induced impairment of the immune system.[4] It has been postulated that smoke may impair host defense by exhausting the signaling pathways upon which lymphocyte antigen receptors rely.

Cyanide ion is naturally present in saliva, urine, and blood serum and its concentration varies according to diet, among other factors.[4] Cyanide is considered to be a biomarker in distinguishing smokers from nonsmokers. Cyanide is a potent toxic agent which inhibits the activity of cytochrome oxidase.[5,6]

There is no evidence on the exact amount of cyanide and thiocyanate in the human's saliva and no reference value has been reported for salivary cyanide. A previous study[7] indicated that denaturation of oxyhemoglobin produces cyanide from thiocyanate and that active oxygen scavenge reagents suppressed cyanide production. Chronic inhalation of cyanide alters a wide range of immunological functions, including innate and adaptive immune responses. It has been speculated that several health consequences of chronic cyanide inhalation might be due to adverse effects on the immune system.[8–10] Late 1960s was the first time that possible relationship was found between elevated occurrence of diseases related to cyanide and smoke-induced changes in the immune and inflammatory processes.[11] Cytokines are kind of proteins that are secreted by different cells and perform paracrine, endocrine and even autocrine functions. Cytokines can relatively penetrate into blood-brain barrier and bind to receptors of neurons and glial cells. Furthermore, cytokines have the capacity to be produced inside the central nervous system.

Smoking suppresses alveolar macrophage and T cells function.[11,12] Immune cells communicate with each other via cell–cell interactions and production of cytokines which act through specific receptors expressed on the target cell membrane.[11] It has been shown that cigarette smoking decreases cytokines levels, except IgE which is significantly elevated in smokers.[13] Interferon-gamma (IFN-γ) dimerized soluble cytokine which is the sole member of type II IFNs.[14] IFN-γ is produced predominantly by natural killer cells as a part of the innate immune response.[15] Interleukin-2 (IL-2) is a type of cytokine signaling molecule in the immune system. Additionally, IL-2 promotes the differentiation of T cells into effector T cells. It has also been shown that the production of IL-1β, IL-2, IFN-γ, and tumor necrosis factor-α (TNF-α) in human peripheral blood is suppressed by cigarette smoke extracts.[16] Previous studies demonstrated that cigarette smoking suppresses the production of IL-1β, IL-6, and TNF-α by alveolar macrophages obtained from the bronchoalveolar lavage fluids of smokers.[17,18] In the current study, authors investigated the effect of smoking on level of saliva cyanide, IL-2 and IFN-γ among smokers compared to nonsmokers.

2. AIM

This study aimed to investigate effect of smoking on level of saliva cyanide, IL-2 and IFN-γ among smokers compared to nonsmokers in the city of Ilam, Iran.

3. MATERIAL AND METHODS

In this case-control study, cases ($n = 38$) were male smokers and controls ($n = 38$) were aged matched nonsmokers. Out of 38 controls 21 were female while, 17 were male. This study was approved by ethics committee of Ilam University of Medical Science. Participants signed a written consent form prior to data collection. Subjects included into this study did not suffer from any serious health condition, had no history of drug usage and had no history of blood donation as well as transfusion in the past 6 months. The clinical data, medical history and other relevant information were collected from subjects by face to face interview. Samples were prepared by two methods: dental roll and direct saliva method. The saliva sample were stored at –18°C.

3.1. Determination of salivary cyanide

Sample sera in an amount of 0.1 mL of was added to 0.1 mL of kit standardized solution, incubated for 90 minutes at 37°C. Plates were washed 3 times in TBS. 0.1 mL of biotinylated antihuman antibody was added and incubated for 60 minutes at 37°C. Plates were washed 3 times in TBS followed by adding 0.1 mL of ABP solution incubating for 30 minutes. After 5 times washing, 0.9 mL of ABP solution was added incubating for 30 minutes at 37°C. Stop solution in an amount of 0.1 mL was added to each sample and sample OD was measured using ELISA reader. The levels of salivary cyanide were measured using spectrophotometric method.

3.2. Cytokine measurements

Salivary concentrations of IL-2 and IFN-γ in all samples were measured by commercial ELISA kit (Booster Company) in accordance with the manufacturer's instructions. Salivary IL-2 and IFN-γ levels (μg/mL) in each sample were calculated using a standard curve obtained from calibrators in the kit.

4. RESULTS

Mean age of smokers were 32.71 ± 7.52 years old and mean age of nonsmokers were 29.79 ± 9.72 years old. The level of cyanide in the salvia of smokers was significantly higher (164.21 ± 18.54 μg/mL) than that in nonsmokers (42.63 ± 24.01 μg/mL) (Table). A significant increase of total IL-2 level was recorded in smokers (31.34 ± 76.75 μg/mL) when compared to controls (8 ± 0.46 μg/mL) (Table). The intensity of smoking was further categorized into light, moderate and heavy smoker. It was shown that level of total IL-2 among light smokers (mean 30.75, SD 15.32) was higher than that among heavy smokers (mean 10.57, SD 4.4). Our findings also showed that level of IFN-γ was significantly higher among smoker (13.16 ± 12 μg/mL) compared to nonsmokers (2.62 ± 1.21 μg/mL) (Table). It was shown that level of total IL-2 among light smoker (mean 16.6, SD 14.73) was higher than that among heavy smokers (mean 8.05, SD 7.92).

Table. Mean (± SE) of effect of cigarette smoke on IFN-γ, cyanide and IL-2 in saliva of smokers and nonsmokers.

P value	Smoker	Control	Parameters
0.000	13.16 ± 12	2.62 ± 1.21	IFN-γ, μg/mL
0.003	31.34 ± 76.75	8 ± 0.46	IL-2, μg/mL
0.002	164.21 ± 18.54	42.63 ± 24.01	Cyanide, μg/mL

5. DISCUSSION

This study revealed a significant increase in salivary cyanide and decrease in IFN-γ and IL-2 among smokers. Smoking is associated with both release and inhibition of pro-inflammatory and anti-inflammatory mediators. A large network of pulmonary and systemic cytokines is involved in chronic inflammation of smokers. Cigarette smoke induces the release of TNF-α, TNF-α receptors, IL-1, IL-6, IL-8 and granulocyte-macrophage colony-stimulating factor (GM-CSF). On the other hand, smoking has also been associated with decreased IL-6 production through toll-like receptors (TLR)-2 and, decreased IL-10 production via TLR-2 activation and also decreased IL-1b, IL-2, TNF-a, and IFN-g production by mononuclear cells. The inhibitory effects of cigarette smoking have been attributed to nicotine, hydroquinone and to carbon monoxide in the smoke. Klein et al.[19] found that exposure to cigarette caused 70% loss of enzyme activity and increased the salivary cyanide concentration. Understanding the possible effect of cigarette smoking on variety of immune responses would be beneficial to evaluate the interactions between these factors.

Cytokines are known to play an important role in the immune response to infections. Alveolar macrophages in smokers, discharge significantly lower amount of IL-1, IL-6, and TNF-α compared to nonsmokers.[20] Cigarette smoke may affect immune responses by altering the Th1 and Th2 ratio. In fact, cigarette smoke-associated airway hyperactivity is believed to be a Th2-driven disorder. Cytokines such as IFN-γ and IL-2 are produced by Th1 cells, the presence of IFN-γ and IL-2 in the smokers is indicative of cell mediated immune response. In our study, we found that smoke exposure can have an impact on immune responses. In addition, our data showed that, in smokers, the concentration of saliva cyanide is affected by the amount and period of smoking. Cigarette smoking has been shown to suppress the mitogenic responses and the production of IL-1β, IL-6 and TNF-α by alveolar macrophages.[21,22] However, little is known about the nature of the immunosuppressive compounds in the cigarette smoke. Decreased serum IL-1, IL-2, and TNF-α have been observed in patients with lung cancer, and decreased levels of IFN-γ are associated with shorter survival.[23] In addition, IL-2 alone or in combination with other therapies can enhance antitumor immunity.[24,25] Transfection of tumor cells with the genes encoding IL-2 and IFN-γ induces a marked increase in antitumor cytotoxic T-cell activity and tumor destruction. These studies provide strong evidence that the production of IL-1, TNF-α, IL-2, and IFN-γ are critical for antitumor immunity, and suppression of these cytokines by components of cigarette smoke may increase the risk of infection, lung cancer, and other malignancies. Some studies suggested that smoking might decrease IFN-γ and IL-2.[26,27] In contrast, another study revealed that exposure to smoke also appears to increase both IFN-γ and IL-2 levels.[28,29]

It was also shown that Th1 has decreased production of IFN-γ in smokers compared to nonsmoker.[30–32] Thus, smoking can alter the balance of cytokines produced by T helper cells. These studies have provided some strong evidence that the production of IL-2 and IFN-γ are critical for immune responses and suppression of these cytokines by components of cigarette smoke (cyanide) may increase the risk of infection and lung cancer. It has been shown that exposure to cigarette smoke inhibits the function of circulatory dendritic cells which specifically inhibit key Th1 cytokine production and triggers development of Th2 responses.

6. CONCLUSIONS

Findings of the current study showed that cyanide level in saliva and IL-2 and IFN-γ levels in the sera of smokers were significantly higher compared to nonsmokers. Furthermore, it was revealed that a heavy smoking led to an increased level of salivary cyanide and a decreased level of sera IFN-γ. Such changes in cytokine levels can be attributed to different components of cigarette such as cyanide and its derivatives. As cyanide is a toxic agent and its impact on immune system is confirmed according to these results, further studies are needed to evaluate the exact mechanism behind this interference of cigarette and its derivatives such as cyanide and the immune system response.

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