American Journal of Men's Health
2017, Vol. 11(4) 1169–1173

DOI: 10.1177/1557988315601724
journals.sagepub.com/home/ajmh
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Impact of Cigarette Smoking on Serum Pro- and Anti-Inflammatory Cytokines and Growth Factors

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Abstract

Inflammation plays a key role in the initiation, progression, and clinical manifestation of atherosclerosis. Cigarette smoking is a risk factor for atherosclerosis and cardiovascular disease. The aim of the current study was to investigate the serum concentrations of 12 cytokines and growth factors (EGF, INF-γ, IL-1α/-1β/-2/-4/-6/-8/-10, MCP-1, TNF-α, and VEGF) in an Iranian population, including 192 smokers, comparing these values with concentrations in nonsmokers. One hundred and ninety-two cases were enrolled from the Mashhad University of Medical Sciences. Of these cases, 82 were cigarette smokers and 110 were nonsmokers. Sex and age were matched for the two groups. The serum concentration of 12 cytokines and growth factors were determined using EV-3513-cytokine-biochip arrays, by competitive chemiluminescence immunoassays. The level of serum MCP-1 was significantly ($p < .001$) lower in the female group of cigarette smokers (mean = 88.1 dL/ng), compared with nonsmokers (mean = 155.6 dL/ng). There were no significant differences for the other cytokines and growth factors between the groups. Our finding demonstrate the association of MCP-1 with cigarette smoking, supporting further studies in larger population on evaluating the role of cigarette smoking on pro-/anti-inflammatory cytokines.

Keywords

inflammation, cytokines, growth factors, cigarette smoking

Introduction

Smoking causes 140,000 premature deaths from cardiovascular disease (CVD) annually in the United States, representing about 30% of all smoking-related death (Paffenbarger, Hyde, Wing, & Hsieh, 1986). Smoking is an important risk factor for atherosclerosis and CVD (Asthana et al., 2010). The World Health Organization proposes smoking as a preventable risk factor for CVD (Lao et al., 2009). Approximately 36% of the population-attributable risk for myocardial infarction is because of smoking (Asthana et al., 2010).

It has been documented that inflammation may be one mechanism by which cigarette smoking affects CVD (Asthana et al., 2010; Krintus, Kozinski, Kubica, & Sypniewska, 2014). It has been reported that inflammation plays a key role in the initiation and promotion of atherosclerosis (Asthana et al., 2010; Krintus et al., 2014). The possible mechanism by which cigarette smoking may initiate or accelerate atherosclerosis is smoking-induced endothelial injury, and also activation of free radicals, such as nitric oxide radicals, singlet oxygen, and hydrogen peroxide. Oxidative stress promotes a systemic

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acute phase response possibly by activation of NF-κB, other cytokines, and adhesion molecules (Fröhlich et al., 2003; Mizia-Stec, Zahorska-Markiewicz, & Gasior, 2004). Several studies have shown the effect of smoking on the serum concentrations of some inflammatory markers, fibrinogen, plasma viscosity, and high-sensitivity C-reactive protein, compared with never smokers, and slightly increased for ex-smokers. Duration of smoking is positively associated with markers of inflammation. Conversely, duration of abstinence from smoking is documented to be inversely related (Fröhlich et al., 2003). Smoking can also affect the cardiovascular inflammatory response to secondhand smoke exposure among nonsmokers, such as a significant increase of high-sensitivity C-reactive protein, s-ICAM (soluble intercellular adhesion molecule-1), and s-VCAM (soluble vascular cell adhesion molecule-1) levels (Garza et al., 2014).

Acute effect of smoking has been shown to increase the levels of interleukin-8 (IL-8), lymphocytes, and neutrophils (Van der Vaart et al., 2005). Acute cigarette smoking induces a wide range of pro-inflammatory responses. IL-6 plays a key role in innate and adaptive immunity (Rodrigues et al., 2014; Van der Vaart, Postma, Timens, & ten Hacken, 2004). Previous studies suggested that serum IL-6 concentrations were decreased after in vitro exposure of smoke and IL-6 degradation was increased in bronchoalveolar lavage fluid of rats.

There is a growing body of evidence investigating the relationship between individual inflammatory markers and smoking status. Therefore, in the current study, the impact of cigarette smoking on 12 markers of inflammation, IL1α, IL1β, IL2, IL4, IL6, IL8, IL10, interferon-γ (INF-γ), tumor necrosis factor-α (TNF-α), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and monocyte chemoattractant protein-1 (MCP-1), was investigated in serum of current smokers compared with nonsmokers.

Materials and Method

Population

In the current cross-sectional study, 192 healthy cases including 82 smokers (smoking at least 5 cigarettes per day for more than 6 months) and 110 nonsmokers, matched for age and sex, were recruited from Mashhad University of Medical Sciences.

Exclusion criteria were patients with past and/or current diagnosis for malignancies such as stroke, myocardial infarction, diabetes mellitus, chronic liver and/or renal diseases; pregnant women; and alcohol consumption based on a questionnaire; taking any drugs including dietary supplements, anti-inflammatory drugs including aspirin and nonsteroidal anti-inflammatory drugs; or malignancy in a close family member. All cases were negative for viral markers of hepatitis and anti-HIV antibody. All participants completed a standardized questionnaire including the following: sex, ethnicity, age, dietary habits, family history of myocardial infarction, stroke, diabetes mellitus, and past medical history.

Informed written consent was obtained from all individuals using approved protocols by the Research Ethics Committee of Mashhad University of Medical Sciences.

Anthropometric Measurements

Anthropometric parameters, including height, body weight, and waist and hip circumference (HC), were measured in all the cases, while systolic and diastolic blood pressures were measured by sphygmomanometers (Mirhafez et al., 2014; Oladi et al., 2015; Emamian et al., 2015).

Lipid Profile of Population

Lipid profile levels, including total cholesterol (TC), fasting blood glucose, triglyceride, low-density lipoprotein (LDL), and high-density lipoprotein cholesterol (HDL-C) were measured using standard procedure as described earlier (Zomorrodian et al.,2015; Mirhafez et al., 2014).

Serum Cytokines and Growth Factor Levels

Blood samples were centrifuged at 1,500*g* for 20 minutes at room temperature to separate the serum. The levels of IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, IFN-γ, MCP-1, EGF, and VEGF were determined using an EV 3513 cytokine biochip array (Randox Laboratories, Crumlin, UK) and competitive chemiluminescence immunoassays (Mirhafez et al., 2015; Mirhafez et al., 2014).

Statistical Analysis

Data were analyzed using SPSS, Version 16. The normality of distribution was assessed using the Kolmogorov–Smirnov test. Descriptive statistics including mean ± standard deviation was considered for normally distributed variables or median ± interquartile range for variables that were not normally distributed. Student's *t* test was used to compare the clinical characteristics and baseline demographics between the groups for normally distributed variables. Mann–Whitney *U* test was used for continuous variables and nonnormally distributed variables. A Bonferonni correction was used for multiple comparisons. Chi-square or Fisher exact tests were used for categorical variables. The correlations between the cytokines and growth factors were assessed using Spearman's correlation analysis. A p value of less than .05 was considered statistically significant.

Table 1. Anthropometric and Biochemical Characteristics of the Study Population.

	Smokers (n = 82)			Nonsmokers (n = 110)		
	Male (56.1%)	Female (43.9%)	p Value	Male (50%)	Female (50%)	p Value
Age (years)	54.3 ± 11.7	52.5 ± 11.9	.482	54.8 ± 8.1	50.9 ± 10.2	.029
Height (m)	1.6 ± 0.1	1.5 ± 0.1	.001	1.6 ± 0.1	1.5 ± 0.1	.001
WC (cm)	96.0 ± 12.7	101.2 ± 13.2	.079	96.8 ± 10.2	94.3 ± 8.9	.175
HC (cm)	101.5 ± 8.2	107.6 ± 10.2	.003	101.9 ± 6.7	105.3 ± 9.6	.037
Weight (kg)	77.1 ± 17.2	72.3 ± 12.3	.170	74.6 ± 12.6	68.6 ± 12.9	.015
SBP (mmHg)	127.8 ± 18.0	128.6 ± 16.4	.836	131.8 ± 18.3	124.9 ± 16.1	.040
DBP (mmHg)	84.8 ± 9.8	83.7 ± 11.4	.643	85.7 ± 8.7	83.9 ± 9.5	.308
TC (mg/dL)	189.7 ± 43.9	213.2 ± 32.74	.009	182.6 ± 34.3	199.0 ± 39.1	.022
TG (mg/dL)	147.5 ± 12.8	151 ± 11.0	.528	138 ± 9.4	131 ± 9.2	.907
HDL (mg/dL)	40.1 ± 8.7	45.1 ± 8.8	.012	38.0 ± 6.7	46.5 ± 9.5	.001
LDL (mg/dL)	121.1 ± 37.3	132.6 ± 31.2	.153	118.5 ± 30.4	117.1 ± 30.7	.820
FBG (mg/dL)	82.4 ± 12.0	85.3 ± 13.7	.316	82.3 ± 12.0	84.9 ± 7.0	.164

Note. WC = waist circumference; HC = hip circumference; SBP = systolic blood pressure; DBP = diastolic blood pressure; TC = total cholesterol; TG = triglyceride; HDL = high-density lipoprotein; LDL = low-density lipoprotein; FBG = fasting blood glucose. Values are expressed as mean ± *SD*.

Results

Characteristics of the Population

The clinical and baseline characteristics of the population are summarized in Table 1. As reported in Table 1, 82 cases (43%) were smokers and 110 cases (57%) were nonsmokers. Smokers were divided into two subgroups: 46 (56.1%) men and 36 (36.9%) women, and nonsmokers were also divided in two subgroups: 55 (50%) men and 55 (50%) women (Table1). As reported in Table 1, females in the smokers group had had a significantly higher HC, HDL-C, and TC, compared with men. Moreover, nonsmokers had significantly different means in height, weight, systolic blood pressure, TC, and HDL, while no differences were observed for other clinical and baseline characteristics of the population, including waist circumference, fasting blood glucose, triglyceride, standing diastolic blood pressure, and LDL-C (Table 1).

Level of Cytokines and Growth Factors in the Study Population

Serum concentration of 12 cytokines/growth factors, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, MCP-1, IFN-γ, EGF, and VEGF, were assessed using EV 3513 cytokine biochip array in all the subjects. As reported in Table 2, the level of MCP-1 was significantly decreased only in females from 155.6 ± 87.8 to 88.1 ± 84.5 in smokers versus nonsmokers, respectively ($p < .05$). Similar results were also observed for IL-2, while no statistically significant differences were detected for other cytokines (Table 2).

Discussion

This is the first study evaluating the expression of 12 cytokines and growth factors (IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, INF-γ, TNF-α, VEGF, EGF, and MCP-1) in relation to cigarette smoking status in an Iranian population. Our findings demonstrate a significantly lower serum MCP-1 in smokers compared with nonsmokers in the female group. Consistent with our findings, Ouyang et al. (2000) identified that cigarette smoke contains potent inhibitors like nicotine, catechol, and hydroquinone for cytokine production such as IL-1β, IL-2, IFN-γ, and TNF-α in humans, which might explain at least in part the lower level of MCP-1 in smokers, compared with nonsmokers.

Several studies have illustrated a significant difference in the level of IL-6 in smokers and nonsmokers in relation to markers of systemic vascular inflammation and smoking in women (Bermudez, Rifai, Buring, Manson, & Ridker, 2002; Sunyer et al., 2009). However, no relationship was observed between smoking status and serum levels of IL-6 and IL-8. Moreover, some studies have observed that the number of IFN-γ-secreting cells was extremely reduced in smokers and cigarette smoking depleted Th(1) cytokine-secreting cells (Hagiwara et al., 2001; Sunyer et al., 2009). Similarly, some studies have observed that aged chronic cigarette smoke–exposed rats had a markedly higher serum levels of TNF-α, IL-6, and IL-10 (Bermudez et al., 2002; Hao et al., 2014; Sunyer et al., 2009), although no differences were detected in our population.

Smoking-induced endothelial dysfunction may lead to the activation of inflammatory markers within a vascular

Table 2. Inflammatory, Anti-Inflammatory, and Growth Factor Markers.

	Nonsmokers			Smokers			Male	Female
	Male	Female	*p* Value	Male	Female	*p* Value	*p* Value (smokers vs. nonsmokers)	*p* Value (smokers vs. nonsmokers)
CRP (dL/ng)	1.4 ± 2.7	4.1 ± 5.2	.002	1.3 ± 2.2	1.9 ± 2.5	.161	.780	.17
IL-1α (dL/ng)	0.5 ± 0.1	0.6 ± 0.1	.930	0.5 ± 0.1	0.6 ± 0.1	.717	.731	.482
IL-1β (dL/ng)	0.6 ± 0.3	0.6 ± 0.3	.641	0.6 ± 0.3	0.6 ± 0.2	.779	.745	.682
IL-2 (dL/ng)	2.8 ± 0.6	3.4 ± 1.6	.017	2.8 ± 0.5	2.7 ± 0.4	.310	.780	.002
IL-4 (dL/ng)	2.2 ± 0.6	1.9 ± 0.5	.020	2.7 ± 3.5	2.1 ± 0.5	.283	.339	.407
IL-6 (dL/ng)	1.5 ± 1.2	1.4 ± 1.9	.732	2.3 ± 7.4	1.2 ± 0.9	.397	.444	.615
IL-8 (dL/ng)	19.8 ± 45.9	11.6 ± 20.0	.236	27.2 ± 69.4	10.8 ± 13.6	.133	.538	.849
IL-10 (dL/ng)	1.3 ± 1.3	0.9 ± 0.7	.081	1.2 ± 1.2	1.0 ± 0.5	.284	.848	.488
MCP-1 (dL/ng)	110.1 ± 77.7	155.6 ± 87.8	.006	92.6 ± 80.2	88.1 ± 84.5	.814	.279	.001
IFN-γ (dL/ng)	0.8 ± 0.5	1.1 ± 2.8	.439	0.9 ± 2.5	0.6 ± 0.4	.530	.762	.330
TNF-α (dL/ng)	1.8 ± 0.9	1.8 ± 0.9	.722	2.7 ± 3.4	1.7 ± 0.7	.104	.104	.751
EGF (dL/ng)	85.6 ± 88.6	87.6 ± 106.4	.916	95.3 ± 121.4	53.3 ± 104.9	.105	.646	.134
VEGF (dL/ng)	78.2 ± 56.9	102.1 ± 74.7	.064	106.5 ± 118.1	114.2 ± 107.5	.762	.119	.560

Note. CRP = C-reactive protein; IL-1α = interleukin-1α; IL-1β = interleukin-1β; IL-2 = interleukin-2; IL-4 = interleukin-4; IL-6 = interleukin-6; IL-8 = interleukin-8; IL-10 = interleukin-10; MCP-1 = monocyte chemoattractant protein-1; INF-γ = interferon-γ; TNF-α = tumor necrosis factor-α; EGF = epidermal growth factor; VEGF = vascular endothelial growth factor. Data are expressed as mean ± *SD*.

wall (Fröhlich et al., 2003; Mizia-Stec et al., 2004). In particular, Conklin, Zhao, Zhong, and Chen (2002) demonstrated a significant increased level of VEGF mRNA and protein due to nicotine and cotinine. In addition, Konturek, Bielanski, Konturek, Bogdal, and Oleksy (1989) reported that cigarette smoking could cause a marked reduction in basal salivary and duodenal EGF. Conversely no differences were detected in the level of VEGF and EGF between the groups. Traves, Culpitt, Russell, Barnes, and Donnelly (2002) observed that MCP-1 level was increased in sputum samples of chronic obstructive pulmonary disease patients compared with nonsmokers and healthy smokers. Some studies measured growth-related oncogene-α, IL-8, TNF-α, matrix metalloproteinase-9, and MCP-1 in patients with stable chronic obstructive pulmonary disease who were all heavy smokers and with no underlying medical diseases. They identified MCP-1 as a marker that differed between patients and controls (Czyzewski, 2009; Ouyang et al., 2000). Other studies revealed that MCP-1 level was decreased in retinal pigment epithelium, in vitro and in vivo, as well as in smoker patients with respect to the control group (Pons & Marin-Castaño, 2011). In agreement with this observation, our results reported a significant reduction of MCP-1 level in our population in smokers versus nonsmokers.

A strength of the current study is that a large number of cytokines were determined in a well-characterized cohort of individuals. The main limitations were the cross-sectional study design and modest sample size. It is possible that other lifestyle features such as diet and physical activity of the participants may have an important effect on the cytokine profile. Therefore, further studies are needed to explore the role of these factors with respect to the inflammatory cytokines.

In conclusion, our findings demonstrated that cigarette smoking can alter circulating levels of pro- and anti-inflammatory cytokines and growth factors, supporting the need for further studies to unravel the role and association of emerging inflammatory markers with cigarette smoking.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was support by grant from the Mashhad University of Medical Sciences. The results obtained in this study are part of Dr. Elahe Kavousi1's thesis in MUMS.

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