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Research Paper

Interleukin 4 Is an Important Factor to Overcome Cardiovascular Suspected Patients Independent of CC Chemokine Receptor 5

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ABSTRACT

Background and Aim: Interleukin 4 (IL-4) is the main Th2 cytokine, which confers several pleiotropism functions, including induction of fibrosis, and can be induced by CCR5-dependent signaling pathways. Thus, serum levels of IL-4 before and after angiography were explored in this project to clear the roles played by angiography indices, and its related X-ray on the cytokine and CC chemokine receptor 5 (CCR5) delta 32 mutation was explored to find its relation with IL-4 serum levels.

Materials and Methods: In this project, IL-4 serum levels and CCR5 delta 32 mutation were evaluated in the healthy controls, the Acute Coronary Syndrome (ACS) patients without vessel obstruction, those with obstruction of one vessel, and those with more than one vessel obstructions using ELISA and Gap-PCR techniques, respectively. The IL-4 serum levels were explored in the three groups before and after angiography.

Results: The results showed that IL-4 serum levels significantly decreased in the patients without, with one, and with more than one vessel obstructions in comparison to healthy controls. Angiography had no effects on the IL-4 serum levels in all groups when it was compared before and after angiography. IL-4 serum levels had a moderate positive correlation with angiography duration in patients who suffered from severe vessel obstruction. The CCR5 delta 32 mutation was not seen in the participants.

Conclusion: IL-4 can be considered an inhibitory factor for the generation of ACS. Due to the lack of CCR5 delta 32 mutation, it appears that the disease and serum levels of IL-4 were not affected by this mutation.

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1. Introduction

Interleukin 4 (IL-4) is a well-known Th2 lymphocyte secreted cytokine, which plays several pleiotropism roles in the immune responses from isotype switching into IgE to suppress Th1 responses [1]. It has been considered an anti-inflammatory cytokine because it can modulate cellular immunity as well [2]. IL-4 also is the main molecule, which participates in the induction/suppression of tissue fibrosis and also atherosclerosis plaque, either directly by interaction with endothelial cells or indirectly by affecting macrophages [3-5]. Additionally, several investigations have demonstrated that IL-4 serum levels were altered among patients who suffered from atherosclerosis and cardiovascular diseases [6, 7]. Therefore, the environmental factors, which alter the expression of IL-4, may be considered as research targets regarding their roles in the pathogenesis of atherosclerosis.

Human CC chemokine receptor 5 (CCR5) is the most important receptor for cysteine CCL3 (MIP-1α), CCL4 (MIP-1β), and CCL5 (RANTES), and has been categorized in the G-coupled receptors [8]. Due to the expression of the chemokine receptor on the immune cells, such as macrophages and T lymphocytes, the receptor plays a significant role in the induction of inflammation by recruitment of the immune cells to the inflamed tissues [9, 10]. Thus, it has been hypothesized that CCR5 may be considered a part of the inflammation puzzle in pro-inflammatory-based diseases. The interaction between CCR5 and IL-4 has been reported previously [11]. Additionally, a well-known 32 base pairs deletion (CCR5D32) within the unique exon of the CCR5 gene has been defined, which is prevalent among some populations as polymorphism and is associated with impaired functions of CCR5 [12]. Thus, it has been hypothesized that the CCR5 delta 32 mutation may be associated with some human pro-inflammatory based diseases.

It has been reported that angiography is a technique for atherosclerosis diagnosis and handling which uses x-ray [13]. X-ray is an environmental factor, which alters the expression of several molecules, including innate immunity receptors and cytokines [14, 15]. Thus, angiography may be considered an environmental factor to alter the expression of IL-4, as the well-known Th2 cytokine affects atherosclerosis. Therefore, in this project, IL-4 serum levels were evaluated in the four groups, including healthy controls (Group 1), the patients who were suspected to have atherosclerosis but did not suffer from vessels obstruction (Group 2), those suffering from obstruction of one vessel (Group 3), and those suffering from obstruction of more than one vessel (Group 4). Additionally, another aim of this study was to evaluate the CCR5 delta 32 mutation prevalence in the participants and its relation with IL-4 in the groups. This study aimed to evaluate the correlation between angiography indices and serum levels of IL-4 in the participants under angiography operation.

2. Material and Methods

Subjects

In this study, 80 participants were evaluated regarding the IL-4 serum levels and CCR5 delta 32 mutation and were divided into four groups, including healthy controls (Group 1, 20 cases), the patients with angiography criteria without vessel obstruction (Group 2, 20 cases), the patients with angiography criteria with one vessel obstruction (Group 3, 20 cases), and the patients with angiography criteria with more than one vessel obstruction (Group 4, 20 cases). The IL-4 serum levels were evaluated in the healthy controls once because they do not have angiography criteria and were not under angiography, while IL-4 serum levels were evaluated in groups 2, 3, and 4 before and 3 h after angiography by separating the serums from 5 mL whole blood samples. The participants in the groups were matched regarding age, sex, opium use, diabetes, smoking, drugs, and alcohol consumption. However, the participants with autoimmune, allergic, kidney, and infectious diseases were excluded from the project.

The angiography criteria were considered as the existence of acute coronary syndrome (ACS) containing unstable angina, typical chest pain, ST-elevation myocardial infarction (STEMI), positive exercise stress test (EST), and non-ST elevation myocardial infarction (NSTEMI) by the expert cardiologists due to the damaged to normal vessels comparison. Six French sheath and Judkins catheters (left and right catheters) were used to perform the selective coronary angiography following local anesthesia from the right femoral artery percutaneously. In the multiple projections manner, contrast media (Visipaque) was injected directly into both left and right coronary arteries.

Cytokine assay

IL-4 serum levels were explored using a commercial kit from Karmania Pars Gene Company, Kerman, Iran, and according to the manufacturer's guidelines. Briefly, 60 mL serum and the standard were added to the corresponding anti-IL-4 coated vials and incubated at 4

ºC overnight. Then, the vials were washed, and 60 mL Biotin-conjugated anti-IL-4 was added and incubated for 1 h at room temperature on the shaker and the vials were washed. After that, 60 mL HRP-Avidin was added and incubated for 1 h at room temperature on the shaker. After washing, 60 mL substrate was added and after 15 min, incubation was stopped using stopping solution and evaluated using ELISA reader, EMP Company, China, at 450 nm.

DNA extraction

DNA was purified from whole blood using a kit (Cat # KPG-DNK-Plus) from Karmania Pars Gene Company, Kerman, Iran, according to the manufacturer's guidelines. Briefly, 250 mL of lysis solution and 150 mL of blood were added to the sterile DNase-free tubes and after shaking, the tubes were incubated for 5 min at room temperature, and then 50 mL of solution A was added. Then, the tubes were centrifuged at 12000 RPM for 5 min and the watery supernatants were moved to new sterile DNase-free tubes. In this step, 250 mL of precipitation solution was added and after 5 min of incubation at -20 ºC, centrifugation was done at 12000 RPM for 5 min. The supernatant was removed and 250 mL of washing solution was added and centrifuged again. The supernatant was removed and after incubation at room temperature for 3 min up to drying, 30 mL of sterile DNase free water was added.

Assessment of CCR5D32 mutation

The CCR5 delta 32 mutation was evaluated using a commercial kit (Cat # KPG-CCR5D) from Karmania Pars Gene Company, Kerman, Iran, according to the manufacturer's guidelines. Briefly, to perform the Gap-PCR technique, 10 mL of master mix, 5 mL of activator, and 5 mL of sample DNA/negative and positive controls were added to the sterile DNase free tubes and the following program was executed: 95 ºC for 5 min and then, 40 cycles of 95 ºC for 30 s, 58 ºC for 20 s, and 72 ºC for 20 s. The protocol was extended by 5 min of incubation at 72 ºC. Primer sequences were 5`-CAAAAAGAAGGTCTTCATTACACC-3` for forward and 5`-CCTGTGCCTCTTCTTCTCATTTCG-3` for reverse. A 3% Agarose gel was used for electrophoresis of PCR products and accordingly, the PCR product size of 188 and 156 bp indicated wild and mutated types, respectively.

Statistical analysis

The one-sample Kolmogorov-Smirnov test using SPSS version 16 showed that the distributions of the raw data were not normal; hence, the non-parametric tests were applied to analyze the IL-4 serum levels data before and after angiography and also the groups before angiography were analyzed by two-sample paired and Kruskal-Wallis tests, respectively. IL-4 serum levels in the smoking/non-smoking, opium-addicted/non-addicted, and male/female patients were calculated using the Mann-Whitney U test. The correlations among the variables, including X-ray doses, duration of angiography, age, and IL-4 serum levels were evaluated by the Spearman correlation coefficient. A $P<0.05$ was considered significant.

3. Results

The results showed that although IL-4 serum levels were not changed before and after angiography in the group 2 (P=1.0), group 3 (P=0.272), and group 4 (P=0.334), it was significantly higher in the control group compared to other groups, either before or after angiography (P=0.002). Figure 1 illustrates the IL-4 serum levels in the groups.

The results also demonstrated that IL-4 serum levels were not altered between smoking and non-smoking participants in the groups 1 (P=0.947), 2 (P=0.450), 3 (P=0.219), and 4 (P=0.738). Figure 2 shows the results regarding IL-4 serum levels in the smoking and non-smoking participants.

As it is illustrated in Figure 3, IL-4 serum levels did not significantly differ between opium consuming participants in all groups, including group 1 (P=0.742), 2 (P=0.450), 3 (P=0.866), and 4 (P=0.452).

Table 1 shows the correlation between IL-4 serum levels and other variables, including age, angiography duration, X-ray doses, and the percentage of vessel obstruction. There was a moderate positive correlation between IL-4 serum levels and angiography duration in group 4 (r: 0.492, P=0.032). The results demonstrated that there was no CCR5 delta 32 mutation among the participants. In other words, the participants in groups 1, 2, 3, and 4 had a wild type of CCR5 gene.

4. Discussion

The results showed that IL-4 serum levels significantly decreased in the patients who had angiography criteria compared to healthy controls, the individuals without angiography criteria, independent of vessel obstruction or not. IL-4 may be considered a factor to suppress ACS symptoms, including unstable angina, typical chest pain, STEMI, positive EST, and NSTEMI. It has been

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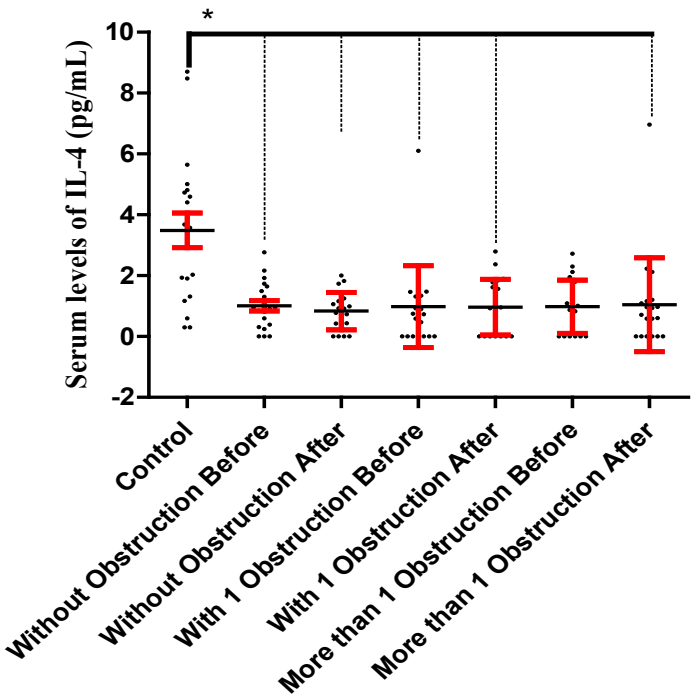

Figure 1. Comparison of Interleukin 4 (IL-4) serum levels among the groups and before and after angiography

The results showed that IL-4 serum levels were significantly decreased in patients with and without vessel obstruction compared to healthy controls. Angiography had no effects on the IL-4 serum levels in all the groups. * P=0.002.

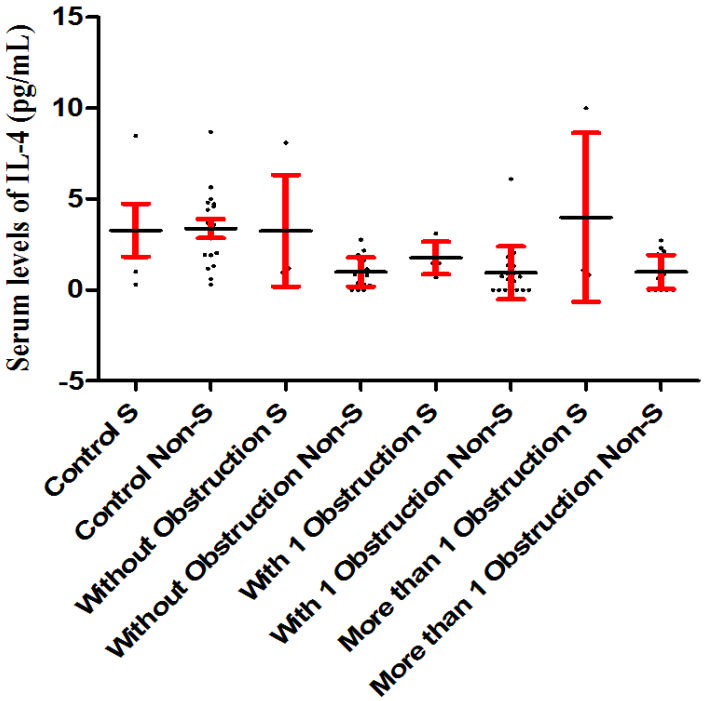

Figure 2. Interleukin 4 (IL-4) serum levels between the smoking and non-smoking participants before angiography

IL-4 serum levels were not significantly different in all groups when the smoking participants compared to non-smoking participants.

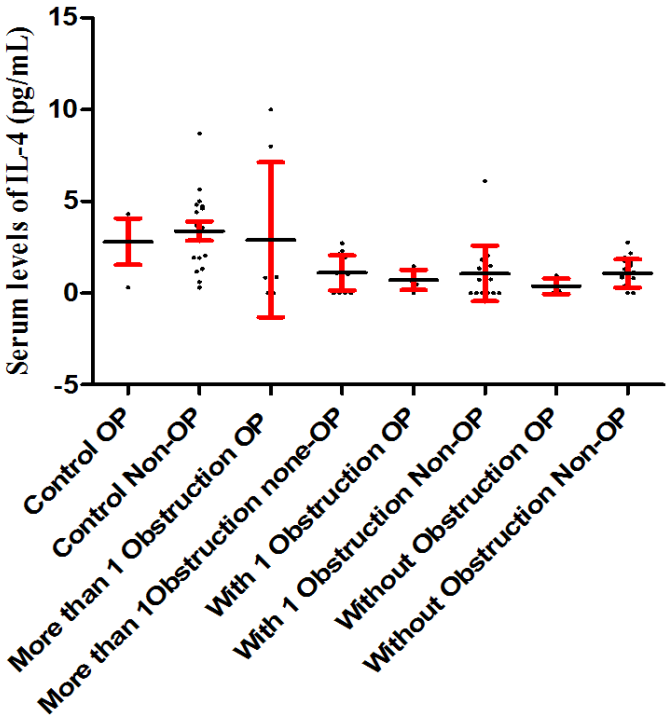

Figure 3. Interleukin 4 (IL-4) serum levels in the opium and non-opium addicted participants before angiography

IL-4 serum levels were not significantly different in the opium-addicted compared to non-opium-addicted participants in the groups separately.

reported that the patients with ACS suffer from increased pro-inflammatory cytokines and subsequently, the pro-inflammatory micro-environment of the heart and vessels [16]. Due to the fact that IL-4 acts as an anti-inflammatory cytokine and decreases several pro-inflammatory functions of the immune system, such as the production of pro-inflammatory cytokines, it can be hypothesized that down-regulation of IL-4 is the main reason for the induction of ACS symptoms. In other words, it may be hypothesized that IL-4 down-regulation in the patients

Table 1. Correlation between Interleukin 4 (IL-4) serum levels, angiography duration, X-ray doses, the percentage of obstruction, and age in the groups

Statistical Test	Characteristins	Data	IL-4			
			Group 1	Group 2	Group 3	Group 4
Spearman's rho	Age	Correlation Coefficient	-0.058	0.193	0.173	0.051
		P	0.815	0.415	0.453	0.844
	Angiography Duration	Correlation Coefficient	-	-0.359	0.053	*0.492
		P	-	0.121	0.826	0.032
	X-ray dose	Correlation Coefficient	-	0.003	0.331	0.350
		P-value	-	0.990	0.143	0.141
	Obstruct Percent	Correlation Coefficient	-	-	0.367	-0.201
		P	-	-	0.179	0.455

* There was a moderate positive correlation between IL-4 serum levels and angiography duration in group 4.

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with unstable angina, typical chest pain, STEMI, positive EST, and NSTEMI may be a reason for the up-regulation of pro-inflammatory Th1 cytokines. However, the hypothesis needs to be assessed via evaluation of other pro-inflammatory cytokines in the patients. In parallel with our results, Hilderman et al. revealed that serum levels of tumor necrosis factor-alpha (TNF-α), IL-1β, and IL-10 were not changed before and after angiography in patients that underwent elective coronary angiography [17]. Collectively, no alteration in IL-4 serum levels before and after angiography, which was revealed in this project, is the main advantage of the study results and demonstrates the safety of angiography.

Although there was no association between angiography and IL-4 serum levels among groups 1, 2, and 3, our results demonstrated that IL-4 serum levels had a moderate positive correlation with angiography duration in the ASC patients who had more than one vessel obstruction. It appears that it is a normal immune response to the environmental factors in the condition of severe ASC to overcome the pro-inflammatory conditions. Szkodzinski et al. revealed that angiography leads to up-regulation of IL-4 serum levels in ACS patients with severe left ventricular dysfunction [18].

Additionally, our results demonstrated that neither smoking nor opium use affected IL-4 serum levels in all groups. This is in contrast with previous investigations, which demonstrated that smoking and opium alter IL-4 serum levels both in vivo and in vitro conditions [19-23]. However, no effects of the smoking and opium use on the IL-4 serum levels may be related to the various ethnicities regarding our population. Because the sample size of our investigation was low, 20 cases in each group; hence, it appears that the effects of angiography need to be explored by other investigations with larger sample sizes.

Moreover, the results showed no prevalence of CCR5 delta 32 mutation among the participants. Thus, it can be concluded that not only do cardiovascular diseases have no correlation with the mutation in the Iranian population, but also they cannot affect IL-4 serum levels in patients. In other words, the patients had a complete form of CCR5 on their immune cells, because of no existence of CCR5 delta 32 mutation.

5. Conclusion

According to the results, serum levels of IL-4 did not change before and after angiography. Thus, the safety of angiography is confirmed. However, the positive correlation of angiography duration with IL-4 serum levels proposes caution when using prolonged angiography. No prevalence of CCR5 delta 32 mutation confirmed that vessel obstruction had no relation to the mutation in the Iranian population.

Ethical Considerations

Compliance with ethical guidelines

The Ethical Committee of Rafsanjan University of Medical Sciences approved the protocol of this study (Code: RUMS.REC.1396.214), and the participants filled out the consent forms before the investigations.

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Authors' contributions

Methodology: Soheila Yousefi; Vahid Mohammadi-Shahrokhi: Conceptualization, Data collection, study validation, supervision, and Writing – original draft; Ali Mehdipour, Mohammad Safarian; Funding acquisition and Resources: Mehdi Zeinali, Mojtaba Mirzamohammadi and Ali Anbarian; Writing – review & editing: Maral Mohammadi-Shahrokhi; Data analysis and Supervision: Mohammad Kazemi Arababadi.

Conflict of interest

The authors declared no conflict of interest.

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References

[1] Wang T, Secombes CJ. The evolution of IL-4 and IL-13 and their receptor subunits. Cytokine. 2015; 75(1):8-13. [DOI:10.1016/j.cyto.2015.04.012][PMID]

[2] Huang XL, Wang YJ, Yan JW, Wan YN, Chen B, Li BZ, et al. Role of anti-inflammatory cytokines IL-4 and IL-13 in systemic sclerosis. Inflamm Res. 2015; 64(3-4):151-9. [DOI:10.1007/s00011-015-0806-0][PMID]

[3] Zhao XN, Li YN, Wang YT. Interleukin-4 regulates macrophage polarization via the MAPK signaling pathway to

protect against atherosclerosis. Genet Mol Res. 2016; 15(1). [DOI:10.4238/gmr.15017348][PMID]

[4] Boss M, Kemmerer M, Brüne B, Namgaladze D. FABP4 inhibition suppresses PPARγ activity and VLDL-induced foam cell formation in IL-4-polarized human macrophages. Atherosclerosis. 2015; 240(2):424-30. [DOI:10.1016/j.atherosclerosis.2015.03.042][PMID]

[5] Hong HY, Lee HY, Kwak W, Yoo J, Na MH, So IS, et al. Phage display selection of peptides that home to atherosclerotic plaques: IL-4 receptor as a candidate target in atherosclerosis. J Cell Mol Med. 2008; 12(5B):2003-14. [DOI:10.1111/j.1582-4934.2008.00189.x][PMID] [PMCID]

[6] Zhao SL, Mo ZH, He HH, Zhao LL, Xie YH. Imbalance of T-helper 1/T-helper 2 cytokines and impaired glucose tolerance among patient with acute coronary syndrome. J Cancer Res Ther. 2018; 14(Supplement):S480-S5. [DOI:10.4103/0973-1482.194346] [PMID]

[7] Mirhafez SR, Zarifian A, Ebrahimi M, Ali RF, Avan A, Tajfard M, et al. [Relationship between serum cytokine and growth factor concentrations and coronary artery disease (Persian)]. Clin Biochem. 2015; 48(9):575-80. [DOI:10.1016/j.clinbiochem.2015.02.002][PMID]

[8] Jayasuriya H, Herath KB, Ondeyka JG, Polishook JD, Bills GF, Dombrowski AW, et al. Isolation and structure of antagonists of chemokine receptor (CCR5). J Nat Prod. 2004; 67(6):1036-8. [DOI:10.1021/np049974l][PMID]

[9] Tarancon-Diez L, De Pablo-Bernal RS, Álvarez-Rios AI, Rosado-Sánchez I, Dominguez-Molina B, Genebat M, et al. CCR5+ CD8 T-cell levels and monocyte activation precede the onset of acute coronary syndrome in HIV-infected patients on antiretroviral therapy. Thromb Haemost. 2017; 117(6):1141-9. [DOI:10.1160/TH16-11-0867][PMID]

[10] Costa C, Traves SL, Tudhope SJ, Fenwick PS, Belchamber KB, Russell RE, et al. Enhanced monocyte migration to CXCR3 and CCR5 chemokines in COPD. Eur Respir J. 2016; 47(4):1093-102. [DOI:10.1183/13993003.01642-2015][PMID]

[11] Jiang PJ, Zhao AM, Bao SM, Xiao SJ, Xiong M. Expression of chemokine receptors CCR3, CCR5 and CXCR3 on CD4(+) T cells in CBA/JxDBA/2 mouse model, selectively induced by IL-4 and IL-10, regulates the embryo resorption rate. Chin Med J (Engl). 2009; 122(16):1917-21. [PMID]

[12] Faulds D, Horuk R. Possible mechanism for the generation of the HIV-1-resistant form of the CCR5 delta32 mutant chemokine receptor. Curr Biol. 1997; 7(9):R529-30. [DOI:10.1016/s0960-9822(06)00272-7][PMID]

[13] Eckert J, Schmidt M, Magedanz A, Voigtländer T, Schmermund A. Coronary CT angiography in managing atherosclerosis. Int J Mol Sci. 2015; 16(2):3740-56. [DOI:10.3390/ijms16023740][PMID] [PMCID]

[14] Liu T, Pei H, Xu D, Zhang Y, Wan J, Wu X, et al. GANRA-5 protects mice from X-ray irradiation-induced dysfunction of the immune system. Free Radic Res. 2014; 48(8):875-82. [DOI:10.3109/10715762.2014.919389][PMID]

[15] Yoshino H, Kashiwakura I. Involvement of reactive oxygen species in ionizing radiation-induced upregulation of cell surface Toll-like receptor 2 and 4 expression in human monocytic cells. J Radiat Res. 2017; 58(5):626-35. [DOI:10.1093/jrr/rrx011] [PMID] [PMCID]

[16] Csiszar A, Ungvari Z. Synergistic effects of vascular IL-17 and TNFalpha may promote coronary artery disease. Med Hypotheses. 2004; 63(4):696-8. [DOI:10.1016/j.mehy.2004.03.009][PMID]

[17] Hilderman M, Qureshi AR, Abtahi F, Witt N, Jägren C, Olbers J, et al. The cholinergic anti-inflammatory pathway in resistant hypertension treated with renal denervation. Mol Med. 2019; 25(1):39. [DOI:10.1186/s10020-019-0097-y][PMID] [PMCID]

[18] Szkodzinski J, Hudzik B, Osuch M, Romanowski W, Szygula-Jurkiewicz B, Polonski L, et al. Serum concentrations of interleukin-4 and interferon-gamma in relation to severe left ventricular dysfunction in patients with acute myocardial infarction undergoing percutaneous coronary intervention. Heart Vessels. 2011; 26(4):399-407. [DOI:10.1007/s00380-010-0076-2] [PMID]

[19] Rodríguez-Rabassa M, López P, Rodríguez-Santiago RE, Cases A, Felici M, Sánchez R, et al. Cigarette smoking modulation of saliva microbial composition and cytokine levels. Int J Environ Res Public Health. 2018; 15(11):2479. [DOI:10.3390/ijerph15112479] [PMID] [PMCID]

[20] Mitra A, Vishweswaraiah S, Thimraj TA, Maheswarappa M, Krishnarao CS, Sundararaja Lokesh K, et al. Association of elevated serum GM-CSF, IFN-γ, IL-4, and TNF-α concentration with tobacco smoke induced chronic obstructive pulmonary disease in a south indian population. Int J Inflam. 2018; 2018:2027856. [DOI:10.1155/2018/2027856] [PMID] [PMCID]

[21] Miranda TS, Heluy SL, Cruz DF, da Silva HD, Feres M, Figueiredo LC, et al. The ratios of pro-inflammatory to anti-inflammatory cytokines in the serum of chronic periodontitis patients with and without type 2 diabetes and/or smoking habit. Clin Oral Invest. 2019; 23(2):641–50. [DOI:10.1007/s00784-018-2471-5]

[22] Ayatollahi-Mousavi SA, Asadikaram G, Nakhaee N, Izadi A, Keikha N. [The effects of opium addiction on the immune system function in patients with fungal infection (Persian)]. Addict Health. 2016; 8(4):218-26. [PMID] [PMCID]

[23] Ghazavi A, Solhi H, Moazzeni SM, Rafiei M, Mosayebi G. [Cytokine profiles in long-term smokers of opium (Taryak) (Persian)]. J Addict Med. 2013; 7(3):200-3. [DOI:10.1097/ADM.0b013e31828baede][PMID]

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