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Angiography; an Inducer of TGF-B during Early Atherosclerosis

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ABSTRACT

Introduction: Angiography is an X-ray-based technique for the diagnosis of disorders of the arteries, veins, and heart chambers. X-ray is a potential factor for the modulation of immune responses, including cytokines, the key molecules that participate in cardiovascular disease pathogenesis. Due to the potential roles of interleukin-6 (IL-6) and transforming growth factor-beta (TGF-β) in cardiovascular disease, this study was designed to evaluate the IL-6 and TGF-β serum levels before and after angiography in atherosclerosis suspected patients.

Material and Methods: In this experimental study, the IL-6 and TGF-β serum levels were explored, using the enzyme linked immunosorbent assay (ELISA) technique, in the healthy controls as well as three groups, including atherosclerosis suspected patients without, obstruction of 1 and more than 1 vessel. The IL-6 and TGF-β serum levels also were evaluated before angiography and 3 hours after angiography in the last three groups. SPSS software was used for data analysis.

Results: The results showed that TGF-β serum levels were significantly higher in the controls in comparison of other groups (p> 0.001). Angiography and smoking increased TGF-β (p= 0.027) and IL-6 (p= 0.035) serum levels, respectively, in atherosclerotic patients with obstruction of 1 vessel. Age had a positive, moderate correlation with IL-6 in the non-atherosclerotic patients (rs: 0.479, p= 0.039).

Conclusion: Down-regulation of TGF-β may be associated with induction of inflammation in the patients. Angiography, via up-regulation of TGF-β, may reduce inflammation in the patients and smoking may increase the progression of atherosclerosis in atherosclerotic patients. Age may increase the risk of cardiovascular diseases via up-regulation of IL-6.

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Introduction

Cytokines are the important arms of immune responses which participate in the either physiological and or pathological functions of the immune system [1-3]. It has been demonstrated that the pro-inflammatory cytokines are the main parts of the cytokines which participate in human pro-inflammatory based diseases, including atherosclerosis [4, 5]. Accordingly, the roles played by interleukin-6 (IL-6) in the pathogenesis of atherosclerosis have been reported by previous investigations [6, 7]. For example, the roles played by IL-6 in the accelerating of atherosclerosis in animal models have been reported previously [7]. IL-6 also has been introduced as a risk factor for the development of atherosclerosis [8].

Additionally, it has been documented that transforming growth factor-beta (TGF-β) is a cytokine that has dual roles in the immune system, and inflammatory and pro-inflammatory functions [9]. TGF-β alone can increase the development of T regulatory lymphocytes, the anti-inflammatory roles, and in association with IL-6 induce the development of Th17 lineage, the most important lineage that participates in the pathogenesis of human pro-inflammatory based diseases [10]. TGF-β also is the main factor that is involved in the induction of fibrosis in the inflamed tissues, and its roles in the pathogenesis of atherosclerosis have been reported, as well [11]. Thus, the environmental factors which are associated with alteration in expression of the IL-6 and TGF-β serum levels may be considered a risk factors for induction and stimulation of atherosclerosis.

Physicians use angiography, which is known also as arteriography, to visualize the inside or lumen for the diagnosis of atherosclerosis. X-ray angiography is a kind of angiography to make the blood vessels visible using contrast agents [12]. X-ray is an

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environmental factor that can be associated with altered immune cell functions [13]. For example, X-ray can up-regulate the expression of cell surface innate immunity receptors entitled "toll-like receptors (TLRs)" [14]. Since the innate immune cell receptors' interactions with their ligands lead to induction of several intracellular pathways which are associated with up-regulation of cytokines [15, 16], X-ray may be considered as an environmental factor for alteration of the expression of the cytokines. Based on the roles of the cytokines in the atherosclerosis pathogenesis, hence, it may be hypothesized that angiography may be a risk factor for altered expression of the cytokines which participate in the induction and stimulation of atherosclerosis. Thus, the main aim of this study was to examine the effects of angiography on the serum levels of IL-6 and TGF-β.

Additionally, this study was also aimed to evaluate the effects of the angiography duration and X-ray doses on the IL-6 and TGF-β serum levels. Moreover, due to the potential correlations between atherosclerosis severity and cytokine serum levels, the effects of angiography-related factors on the expression of IL-6 and TGF-β, the participants were divided into three groups, without, with obstruction of 1 and with obstruction of more than 1 vessels.

Materials and Methods

Subjects

In this experimental study, the IL-6 and TGF-β serum levels were explored, using the ELISA technique, in the healthy controls as well as three groups, including atherosclerosis suspected patients without, obstruction of 1 and more than 1 vessel. Accordingly, 80 participants were divided into four groups (20 cases in each group), group 1: Non-suspected atherosclerosis controls, group 2: Atherosclerosis suspected patients without vessel obstruction, group 3: Atherosclerosis suspected patients who had one vessel obstruction, and group 4: Atherosclerosis suspected patients who had more than one vessel obstructions. The groups were matched regarding sex, age, alcohol drinking, opium, smoking, and diabetes. The participants were enrolled to angiography based on the expert cardiologists' examinations and showing the angiography criteria which was explained in our previous investigation [17]. The expert cardiologists have diagnosed atherosclerosis in suspected patients and also performed angiography based on the comparison of the damaged/normal vessels. The participants who had a history of autoimmune, kidney, and infectious diseases and also allergies were excluded from the project. Before and 3 hours after angiography 5 mL of the whole blood samples were collected in the non-pre-treated tubes to collect their serum. Rafsanjan University of Medical Sciences Ethical Committee by RUMS.REC.1396.159 code approved the project protocol. The participants also filled out the consent form before blood collection.

Cytokine assay

IL-6 and TGF-β serum levels were measured using commercial kits (Karmania Pars Gene Company, Kerman, Iran), and based on the manufacturer guidelines.

Statistical analysis

The distributions of raw data were not normal. Accordingly, One-Sample Kolmogorov-Smirnov and Wilcoxon signed-rank test under SPSS version 16 were used to evaluate the normality distribution of the data and the differences in IL-6 and TGF-β serum levels before and after angiography, respectively. Kruskal-Wallis H test was used to compare IL-6 and TGF-β serum levels among the groups before angiography. Non-smoking versus smoking and non-addicted versus opium-addicted IL-6 and TGF-β serum levels were compared using Mann-Whitney U tests. The correlations of IL-6 and TGF-β serum levels, X-ray doses, age, and the duration of angiography were explored using the Spearman Correlation test. The p-value ≤ 0.05 was considered significant.

Results

The results demonstrated that IL-6 Serum levels in the patients who had no vessel obstruction before angiography was 5.2355 (1.6993- 6.6150) and after angiography was 4.6590 (0.8788- 6.9768). The two-Related-Samples test revealed that the difference was not significant (p= 0.823). TGF-β serum levels also were not changed after angiography (1.7035 (0.0425- 11.5230)) in the patients who had no vessel`s obstruction when compared to before (2.6455 (0.0225- 13.5040)) angiography (p= 0.478).

IL-6 serum levels also were not changed after angiography in groups 3 (p= 0.848) and 4 (p= 0.9) when compared to before angiography. TGF-β serum levels were not also different in group 4 (p= 0.350) before and after angiography. However, angiography led to up-regulation of TGF-β serum levels in group 3 (p= 0.027). Figure 1 illustrates the effects of angiography on the serum levels of TGF-β.

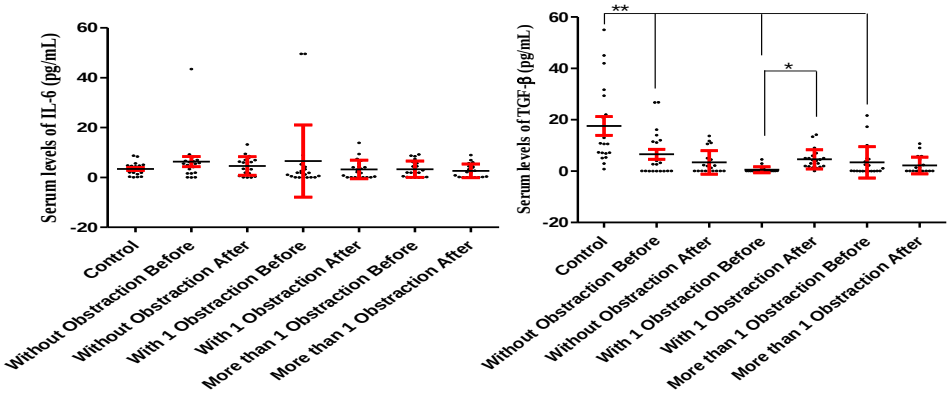

Figure 1. IL-6 and TGF-β serum levels comparison among the control, without vessel`s obstruction, with 1 vessel`s obstruction and with more than vessel`s obstruction groups, as well as before and after angiography in the group 2, 3 and 4. '*' means that the differences are significant.

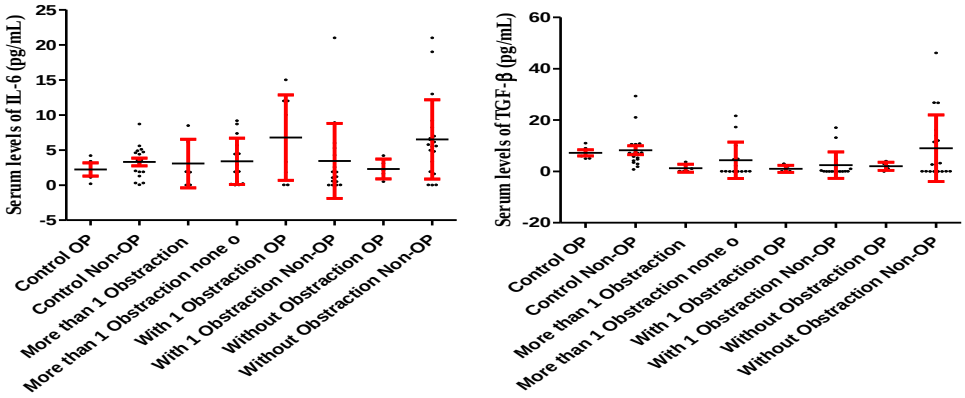

Figure 2. IL-6 and TGF-β serum levels in the smoking versus non-smoking participants. '*' means that the differences are significant.

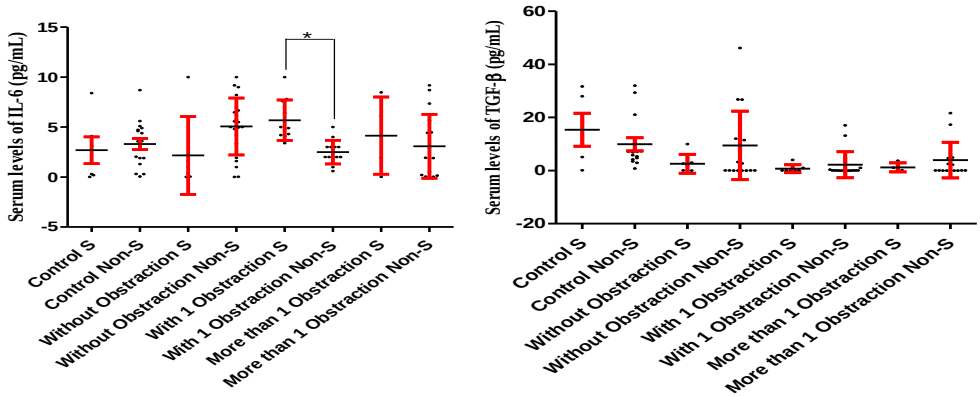

Figure 3. IL-6 and TGF-β serum levels among opium addicted versus non-addicted participants.

Although, serum levels of IL-6 were not different among the groups (p= 0.248), when compared to each other before angiography, TGF-β serum levels were higher in the controls when compared to other groups (p> 0.001).

Figure 2 shows that IL-6 (p= 0.035), but not TGF-β, serum levels significantly increased in the smoking participants in the group 3 when compared to non-smoking cases. However, smoking had no effects on the IL-6 and TGF-β serum levels in other groups, including group 1 (p= 1.0 for IL-6 and p= 0.491 for TGF-β), group 2 (p= 0.126 for IL-6 and p= 0.442 for TGF-β), group 3 (p= 0.262 for TGF-β) and group 4 (p= 0.682 for IL-17 and p= 0.892 for TGF-β).

Figure 3 demonstrated IL-6 and TGF-β serum levels did not alter in the opium addicted participants versus non-opium addicted cases in the group 1 (p= 1.0 for IL-6 and p= 0.491 for TGF-β), group 2 (p= 0.379 for IL-6 and p= 0.674 for TGF-β), group 3 (p= 0.905 for IL-6 and p= 0.719 for TGF-β) and group 4 (p= 0.659 for IL-6 and p= 0.968 for TGF-β).

Table 1. Correlations among IL-6 and TGF-β serum levels, age, angiography duration, X-ray dose, and obstruct percent in all groups.

			IL-6 Group 1	TGF-β Group 1	IL-6 Group 2	TGF-β Group 2	IL-6 Group 3	TGF-β Group 3	IL-6 Group 4	TGF-β Group 4
Spearman's rho	Age	Correlation Coefficient	0.054	0.111	0.479*	0.266	0.063	0.268	-0.111	0.150
		P value	0.826	0.652	0.039	0.257	0.787	0.240	0.671	0.565
	Angiography Duration	Correlation Coefficient	-	-	0.033	0.091	0.148	0.176	0.269	-0.289
		P value	-	-	0.889	0.702	0.535	0.457	0.265	0.230
	X-ray dose	Correlation Coefficient	-	-	0.020	-0.117	0.350	0.176	0.275	-0.379
		P value	-	-	0.935	0.623	0.120	0.445	0.255	0.109
	Obstruct Percent	Correlation Coefficient	-	-	-	-	0.495	0.016	0.082	0.482
		P value	-	-	-	-	0.061	0.954	0.762	0.059

*Age had a significant positive moderate correlation with IL-6 in the group 2.

The Spearman correlation test showed that age had a significant positive, moderate correlation with IL-6 in group 2 (rs: 0.479, p= 0.039). Table 1 shows the details of the correlation tests.

Discussion

It has been demonstrated that TGF-β plays dual roles in the immune system from the development of T regulatory lymphocytes, to regulating pro-inflammatory responses, to the progression of Th17 lymphocytes, the inflammatory cells. As mentioned early, TGF-β led to the development of T regulatory and Th17 lymphocytes in IL-6 independent and dependent, respectively, manner. Due to the results which revealed that IL-6 serum levels were not changed among the groups, while TGF-β serum levels were decreased among the patients when compared to controls, it appears that TGF-β down-regulation may be associated with decreased function of T regulatory lymphocytes. Since atherosclerosis is a pro-inflammatory-based disease and based on our results, it appears that TGF-β plays key role in the pathogenesis of atherosclerosis, maybe through decreased number and functions of T regulatory lymphocytes. In parallel with our results, previous investigations demonstrated that anti-atherosclerotic therapies are associated with up-regulation of TGF-β, as the anti-inflammatory cytokine [18]. However, the roles played by TGF-β in the induction of atherosclerosis have also been documented [19], but due to the fact that cytokines play their roles in a network manner [20, 21], for example, the pro-inflammatory functions of TGF-β in the presence of IL-6 [9], thus it is important to evaluate the cytokine serum levels simultaneously.

Interestingly, our results revealed that angiography is associated with up-regulation of TGF-β, but not IL-6, in the participants who suffered from the obstruction of 1 vessel (Group 3). Because IL-6 was not changed after angiography in the patients, hence, it may be hypothesized that angiography may reduce inflammation in atherosclerotic patients who have suffered from low levels of vessel obstruction, but not in patients with severe atherosclerosis. However, as mentioned in figure 2, smoking is a risk factor for up-regulation of IL-6 in group 3. Therefore, it may be proposed that angiography may be associated with an increased risk of development of Th17 lymphocytes, the risk factor for the development of atherosclerosis [22], in patients with smoking. However, the conclusion needs to be confirmed by evaluation of T regulatory and Th17 lymphocytes population in the patients.

Although previous investigations proved the roles played by opium on the cytokine levels in human [23], this study demonstrated that opium did not affect significantly IL-6 and TGF-β serum levels in the participants. Based on the fact that the sample size in the opium-addicted participants was low, hence, it seems that although there were differences between the groups, but it was no significant. Thus, it seems that more sample size needs to be evaluated to clear the roles played by opium on the IL-6 and TGF-β serum levels.

The results also showed that age has a moderate correlation with IL-6 serum levels in the suspected patients who have not suffered from atherosclerosis. Due to the fact that the patients suffered from some symptoms which are the primary risk factor for the development of atherosclerosis, thus, it seems that age-related up-regulation of IL-6 may be a risk factor for the development of cardiovascular disease.

Collectively, the main message of this study is that the angiography may affect immune cell functions via alteration in cytokine production and it may limit repeated using the technique in a short time.

Conclusion

Collectively, TGF-β down-regulation may be a reason for the induction of inflammation in the patients. Additionally, angiography may reduce inflammation in the patients via up-regulation of TGF-β, and smoking and age may increase the progression of atherosclerosis through up-regulation of IL-6.

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References

1. Weitzmann MN. The Role of Inflammatory Cytokines, the RANKL/OPG Axis, and the Immunoskeletal Interface in Physiological Bone Turnover and Osteoporosis. Scientifica (Cairo) 2013;2013(10):125705.
2. Tecchio C, Cassatella MA. Neutrophil-derived cytokines involved in physiological and pathological angiogenesis. Chem Immunol Allergy. 2014;99:123-37.
3. Dab H, Hachani R, Sakly M, Bricca G, Kacem K. Physiological regulation of pro-inflammatory cytokines expression in rat cardiovascular tissues by sympathetic nervous system and angiotensin II. Gen Physiol Biophys. 2013;32(4):569-75.
4. Correa CR, Dias-Melicio LA, Calvi SA, Lastoria S, Soares AM. Activation of monocytes and cytokine production in patients with peripheral atherosclerosis obliterans. J Inflamm (Lond). 2011;8(23):23.
5. Eastwood JA, Taylor DA, Johnson BD, Resende M, Sharaf BL, Ahmed B, et al. Premature atherosclerosis in premenopausal women: Does cytokine balance play a role? Med Hypotheses. 2017;109:38-41.
6. Lin B, Feng DG, Wang F, Wang JX, Xu CG, Zhao H, et al. MiR-365 participates in coronary atherosclerosis through regulating IL-6. Eur Rev Med Pharmacol Sci. 2016;20(24):5186-92.
7. Bernberg E, Ulleryd MA, Johansson ME, Bergstrom GM. Social disruption stress increases IL-6 levels and accelerates atherosclerosis in ApoE-/- mice. Atherosclerosis. 2012;221(2):359-65.
8. Haddy N, Sass C, Droesch S, Zaiou M, Siest G, Ponthieux A, et al. IL-6, TNF-alpha and atherosclerosis risk indicators in a healthy family population: the STANISLAS cohort. Atherosclerosis. 2003;170(2):277-83.
9. Karimi-Googheri M, Daneshvar H, Nosratabadi R, Zare-Bidaki M, Hassanshahi G, Ebrahim M, et al. Important roles played by TGF-beta in hepatitis B infection. J Med Virol. 2014;86(1):102-8.
10. Zare-Bidaki M, Assar S, Hakimi H, Abdollahi SH, Nosratabadi R, Kennedy D, et al. TGF-beta in Toxoplasmosis: Friend or foe? Cytokine. 2016;86:29-35.
11. Toma I, McCaffrey TA. Transforming growth factor-beta and atherosclerosis: interwoven atherogenic and atheroprotective aspects. Cell Tissue Res. 2012;347(1):155-75.
12. Cimen S, Gooya A, Grass M, Frangi AF. Reconstruction of coronary arteries from X-ray angiography: A review. Med Image Anal. 2016;32:46-68.
13. 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.
14. 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.
15. Bagheri V, Askari A, Arababadi MK, Kennedy D. Can Toll-Like Receptor (TLR) 2 be considered as a new target for immunotherapy against hepatitis B infection? Hum Immunol. 2014;75(6):549-54.
16. Karimi-Googheri M, Arababadi MK. TLR3 plays significant roles against hepatitis B virus. Mol Biol Rep. 2014;41(5):3279-86.
17. Razavi S, Ahmadi-Roknabadi F, Safarian M, Mehdipour A, Anbarian A, Mirzamohammadi M, et al. Il-10 Is down-Regulated in the Cardiovascular Diseases Suspected Patients, Independent of Angiography. Probl Radiac Med Radiobiol. 2019;24:449-54.
18. Pereira MM, Santos TP, Aras R, Couto RD, Atta ML, Atta AM. Serum levels of cytokines and chemokines associated with cardiovascular disease in Brazilian patients treated with statins for dyslipidemia. Int Immunopharmacol. 2014;18(1):66-70.
19. Tang T, Wilson PG, Thompson JC, Nelson C, Yoder MH, Tannock LR. Prevention of TGFbeta induction attenuates angII-stimulated vascular biglycan and atherosclerosis in Ldlr-/- mice. J Lipid Res. 2013;54(8):2255-64.
20. Wu T, Du R, Hong Y, Jia L, Zeng Q, Cheng B. IL-1 alpha regulates CXCL1, CXCL10 and ICAM1 in network form in oral keratinocytes. Clin Lab. 2013;59(9-10):1105-11.
21. Horst OV, Horst JA, Samudrala R, Dale BA. Caries induced cytokine network in the odontoblast layer of human teeth. BMC Immunol. 2011;12(9):1-13.
22. Taleb S, Tedgui A, Mallat Z. IL-17 and Th17 cells in atherosclerosis: subtle and contextual roles. Arterioscler Thromb Vasc Biol. 2015;35(2):258-64.
23. Asadikaram G, Igder S, Jamali Z, Shahrokhi N, Najafipour H, Shokoohi M, et al. Effects of Different Concentrations of Opium on the Secretion of Interleukin-6, Interferon-γ and Transforming Growth Factor Beta Cytokines from Jurkat Cells. Addict Health. 2015;7(1-2):47-53.