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Evaluation of the Serum Levels of TNF-α and IL-8 in the Patients Undergoing Angiography

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Abstract

Introduction: Angiography is a safe cardiovascular technique for the diagnosis and treatment of cardiovascular disorders. The potential effects of angiography on the levels of cytokines are yet to be clarified completely. Interleukin-8 (IL-8) and tumor necrosis factor-alpha (TNF-α) are important pro-inflammatory cytokines that participate in the pathogenesis of artery stenosis. The aim of this study was to investigate the effects of angiography on the serum levels of IL-8 and TNF-α.

Methods: Sixty participants in three groups, without, with one, and with more than one artery stenosis, were explored in this project. Serum levels of IL-8 and TNF-α were measured in the participants before and after angiography using the enzyme linked immunosorbent assay (ELISA) technique.

Results: Serum levels of IL-8, but not TNF-α, significantly decreased following angiography from 61.45 (9.80-266.72) to 7.00 (3.82-17.85) pg/mL ($P=0.008$) in participants without artery stenosis, from 79.40 (7.20-197.40) to 6.80 (3.85-29.50) pg/mL ($P=0.002$) in participants with one artery stenosis, and from 91.25 (12.80-145.97) to 10.75 (6.97-49.82) pg/mL ($P=0.004$) in participants with more than one artery stenosis. The X-ray dose had a moderate positive correlation with serum levels of TNF-α in patients with more than one artery stenosis ($r=0.584$, $P=0.009$). Serum levels of IL-8 and TNF-α were not different between male and female participants in none of the groups.

Conclusion: Angiography may be a protective factor against inflammation by suppressing IL-8.

Keywords: Artery stenosis, Angiography, X-ray, Cytokine, IL-8, TNF-α

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Introduction

Cytokines are important molecules that participate in immune cell migration, activation and homeostasis.[1] Innate immune cells are the main producers and targets of cytokines, which affect several aspects of the immune system.[2] The roles played by these molecules in human diseases, including infectious and non-infectious diseases, have been under investigation.[3] Accordingly, it has been reported that interleukin-8 (IL-8), which is an innate immune cytokine, significantly affects neutrophil migration and function[4] and participates in the pathogenesis of several human inflammatory diseases that are affected by neutrophils. In addition, it has been demonstrated that tumor necrosis factor-alpha (TNF-α) is a main innate immune cytokine that participates in defense against infectious agents and in the development of pro-inflammatory diseases.[5]

Artery stenosis, a common cardiovascular disease, is a pro-inflammatory disease wherein immune cells damage artery endothelial cells, leading to the disorder's development.[6] Due to the roles played by IL-8 and TNF-α in the pathogenesis of artery stenosis,[7,8] the environmental factors that change the expression of these cytokines may be considered as either protective or contributor for artery stenosis. Accordingly, it has been documented that IL-8 and TNF-α, by inducing inflammation and recruiting immune cells such as neutrophils, stimulate plaque formation during atherosclerosis.[7,8] Angiography,

as a useful technique to recognize artery stenosis, uses X-ray to detect some aspects of artery stenosis. Since X-ray can modulate the production of cytokines by immune cells, it may be hypothesized that angiography, as an environmental factor, may be associated with altered expression of pro-inflammatory cytokines,[9] sharing a role in the pathogenesis of artery stenosis. Based on the fact that there is inadequate information regarding the effects of angiography on cytokine secretion by immune cells *in vivo*, the main aim of this study was to investigate the effects of angiography on the serum levels of IL-8 and TNF-α in patients with artery stenosis.

Material and Methods

Subjects

This project was performed in Ali-Ebn Abitaleb hospital affiliated with Rafsanjan University of Medical Sciences, during April 2020 to January 2021. The serum levels of IL-8 and TNF-α were explored in 60 participants. The sample size was considered as our previous investigation on the same patients under angiography.[6,10] According to angiography criteria, participants with the same age range, sex, drug use history, diabetes, smoking, opium use, and alcohol drinking were divided into three groups, including without (20 cases), with one (20 cases), and with more than one artery stenosis (20 cases).

Peripheral blood samples were collected in tubes containing no anti-coagulants to collate serum before and three hours after angiography. So, serum levels of IL-8 and TNF-α were determined both before and after angiography.

The patients suffering from immune-related diseases, such as kidney diseases, autoimmune conditions, allergies, and infections were excluded from the study. The patients presenting with essential criteria for undergoing angiography (i.e., typical chest pain, positive exercise stress test [EST], acute coronary syndrome [ACS] containing unstable angina, ST elevation myocardial infarction [STEMI], and non-ST elevation myocardial infarction [NSTEMI], as detected by an experienced MD cardiologist) entered the study. Angiography was done according to the comparison of the damage to normal vessels using the contrast media (Visipaque) injected into the left and right coronary arteries directly in several projections. It was performed using six French sheath and Judkins catheters after local anesthesia (left and right catheters).[6] The artery stenosis percentage was calculated by the same MD cardiologist via observation.

Evaluation of IL-8 and TNF- Serum Levels

The serum levels of IL-8 (CN# KPG-HI8) and TNF-α (CN# KPG-HTNF) were determined using enzyme linked immunosorbent assay (ELISA) commercial kits from Karmania Pars Gene Company, Kerman, Iran, according to the company's instructions. Briefly, 50 L serum and 50 µL standards were added to ELISA plates and after one hour of incubation, washed by the washing buffer. After that, 50 µL detection antibody was added and incubated for one hour and then washed three times. In the next stage, horseradish peroxidase-avidin was added and incubated for 30 minutes. After five times of washing, 50 µL substrate (3, 3', 5, 5'-tetramethylbenzidine + H_2O_2) was added and incubated after 15 minutes. Then the reaction was stopped by adding the stopping solution. The yellow color was measured at 450 nm by MBG ELISA reader (China). [1]

Statistical Analysis

SPSS software version 20 was used to analyze the data, and accordingly, data distribution was calculated by one-sample Kolmogorov-Smirnov test in each group separately. Based on the abnormal data distribution, Wilcoxon signed-rank test was used to compare the serum levels of IL-8 and TNF-α before and after angiography in the study groups. Mann-Whitney U test was used to compare serum levels of IL-8 and TNF-α in males versus females in each group before angiography. Data were reported as median (25^{th} percentiles-75^{th} percentiles). The correlation between X-ray doses, age, and serum levels of IL-8 and TNF-α were analyzed using the Spearman correlation test. Age had a normal distribution, so it was analyzed using one-way ANOVA, and gender was analyzed using the chi-square test.

Results

Data analysis revealed that the groups were not different regarding age and gender. The demographic data of the participants are presented in Table 1.

Statistical analysis showed that angiography significantly decreased the serum levels of IL-8 in all the participants. Accordingly, angiography led to a decrease in IL-8 serum level from 61.45 (9.80-266.72) to 7.00 (3.82-17.85) pg/mL ($P=0.008$) in participants without artery stenosis, from 79.40 (7.20-197.40) to 6.80 (3.85-29.50) pg/mL ($P=0.002$) in participants with one artery stenosis, and from 91.25 (12.80-145.97) to 10.75 (6.97-49.82) pg/mL ($P=0.004$) in participants with more than one artery stenosis.

The results demonstrated that TNF- serum levels did not significantly change after angiography in the participants without ($P=0.507$), with one artery stenosis ($P=0.313$), and with more than one artery stenosis ($P=0.722$).

As shown in Table 2, before angiography, the serum

Table 1. Demographic Data of the Participants

Variables		Without Stenosis (n = 20)	With One Artery Stenosis (n = 20)	More Than One Artery Stenosis (n = 20)	*P* Value
Gender	Female	11	8	9	0.626
	Male	9	12	11	
Age (y)		57.90 ± 2.35	59.09 ± 1.70	60.52 ± 2.31	0.696

Table 2. Serum Levels of IL-8 and TNF-α in Female Versus Male Participants Before Angiography

Cytokine	Gender	Without Stenosis	*P* Value	With One Artery Stenosis	*P* Value	More Than One Artery Stenosis	*P* Value
IL-8 (pg/mL)	Female	10.35*	0.874	5.67	0.108	12.50	0.322
	Male	10.79		11.89		9.64	
TNF-α (pg/mL)	Female	9.08	0.142	9	0.546	9.92	0.773
	Male	13.14		11.33		10.75	

Data are reported as mean rank.

levels of IL-8 and TNF-α were not different between males and females in the study groups.

The results revealed that the artery stenosis percentage in the groups with one and more than one artery stenosis were 71.66 ± 6.46 and 91.68 ± 0.92, respectively. Figure 1 shows the serum levels of IL-8 and TNF-α in patients without or with artery stenosis.

Spearman correlation demonstrated that X-ray dose had a positive moderate correlation with the serum levels of TNF-α in patients with more than one artery stenosis ($r = 0.584$, $P = 0.009$). Age and artery stenosis percentage did not have significant correlations with IL-8 and TNF-α serum levels in the participants. Table 3 presents the results regarding Spearman correlation analysis.

Discussion

It has been reported that IL-8 and TNF-α play key roles in the pathogenesis of artery stenosis and cardiovascular diseases.[11] IL-8 can deteriorate the pathogenesis of artery stenosis by activating neutrophils.[7] Cavusoglu and colleagues also reported that increased serum levels of IL-8 could be considered as a risk factor for long-term ACS.[8] Therefore, any factor that would decrease the expression of IL-8 and TNF-α, or other inflammatory mediators, can be considered protective against artery stenosis. Our results demonstrated that angiography significantly decreased the serum levels of IL-8 in all study groups, independent of artery stenosis condition and gender. Due to the aforementioned data, it may be hypothesized that angiography can modulate the expression and secretion of IL-8 by innate immune cells. Based on the potential roles played by IL-8 against artery stenosis, it appears that angiography can modulate pro-inflammatory responses in the participants undergoing angiography.

However, the serum levels of TNF-α were not changed after angiography. Thus, it seems that angiography may not change TNF-α release from vessels or its production by immune cells. Although angiography was unable to change the serum levels of TNF-α, X-ray dose had a moderate positive correlation with the serum levels of TNF-α in patients with more than one artery stenosis. Thus, it appears that angiography may be an inducer of TNF-α secretion when it is used in long-term, which is associated with higher exposure to X-ray in patients with more than one artery stenosis. So, its use in the patients with severe artery stenosis needs to be reduced as much as possible. On the other hand, angiography did affect the serum levels of TNF-α in other groups (i.e., patients with had fewer damaged arteries). Collectively, based on the results, it can be concluded that routine angiography can modulate immune responses in an IL-8 dependent manner. However, based on the fact that cytokines play their roles in the context of a network, the serum levels of other pro- and anti-inflammatory cytokines need to be explored following angiography. Our previous investigations on the patients undergoing angiography showed that angiography was unable to change the serum levels of IL-10, an anti-inflammatory cytokine, and interferon-gamma (IFN-γ), an important T helper 1 pro-inflammatory cytokine.[6] Although several studies have proved roles for cytokines in the pathogenesis of artery stenosis,[12,13] to the best of our knowledge, there were no

Table 3. The Correlation of Serum Levels of IL-8 and TNF-α with the X-ray dose, Age, and Artery Stenosis Percentage in the Study Groups

Time	Cytokine		Without Artery Stenosis		With One Artery Stenosis			More Than One Artery Stenosis		
			Age	X-Ray Dose	Age	X-Ray Dose	Artery Stenosis Percentage	Age	X-Ray Dose	Artery Stenosis Percentage
Before angiography	IL-8	Correlation Coefficient	-0.351	-	-0.123	-	0.044	0.259	-	0.093
		P value	0.130	-	0.595	-	0.875	0.315	-	0.732
	TNF-α	Correlation Coefficient	0.251	-	0.306	-	0.474	0.163	-	-0.179
		P value	0.286	-	0.177	-	0.074	0.531	-	0.508
After angiography	IL-8	Correlation Coefficient	-0.122	-0.239	0.014	-0.091	-0.122	0.269	-0.244	0.169
		P value	0.608	0.310	0.953	0.965	0.465	0.296	0.314	0.533
	TNF-α	Correlation Coefficient	0.023	-0.196	0.371	-0.058	0.185	0.163	**0.584**	-0.270
		P value	0.925	0.409	0.098	0.803	0.510	0.531	**0.009**	0.311

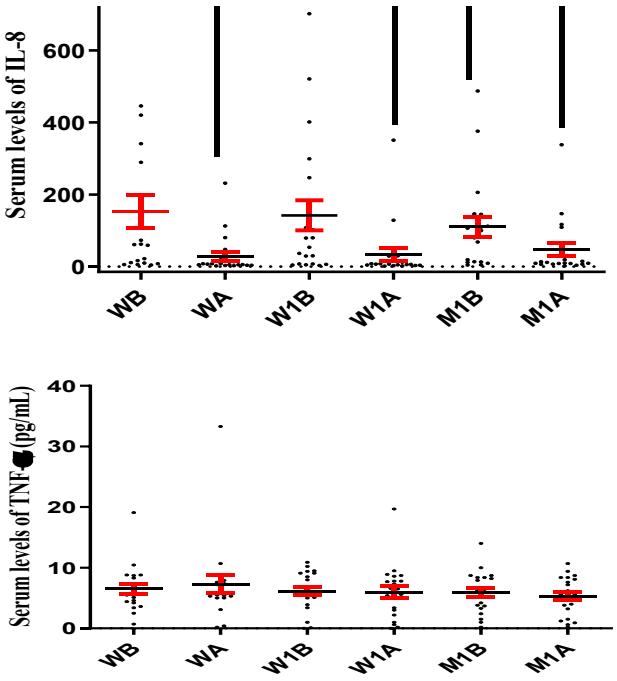

Figure 1. Serum levels of IL-8 and TNF-α Among the Participants. Data analysis showed that angiography significantly decreased the serum levels of IL-8 in all the participants, including the patients without (*P=0.014), with one (**P=0.028) and with more than one (***P=0.021) artery stenosis. Results showed that the serum levels of TNF-α were not changed after angiography in the participants without (P=0.456), with one (P=0.868) and with more than one (P=0.371) artery stenosis. WB: Without artery stenosis before angiography, WA: Without artery stenosis after angiography, W1B: With one artery stenosis before angiography, W1A: With one artery stenosis after angiography, W1B: With more than one artery stenosis before angiography and W1A: With more than one artery stenosis after angiography.

investigations regarding the effects of angiography on the serum levels of IL-8 and TNF-α. Thus, it seems that additional research is needed to clear the mechanisms through which angiography reduces the serum level of IL-8.

There were no significant differences in the serum levels of IL-8 and TNF-α between males and females in none of the groups. The results demonstrated that gender had no effects on the serum levels of IL-8 and TNF-α. However, based on the fact that the number of females was lower than males in this study, this notion may need further clarification and exploration in future studies.

Conclusion

Routine angiography may be considered as a protective factor against inflammation in an IL-8- and TNF-α-dependent manner, but independent of artery stenosis percentage and gender. Using angiography in patients with more than one artery stenosis needs to be with caution to avoid inflammation exaggeration by long-term X-ray exposure.

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Authors' Contributions

All authors have read and approved the manuscript. LZ performed laboratory tests and wrote the manuscript. AE and MKA designed the project, analyzed data and edited the manuscript. MS performed angiography.

Ethical Approval

Unit Ethical Committee confirmed the protocol of the current project by IR.IAU.SRB.1398.168 code. Consent forms were filled out and signed by the patients.

Competing Interests

Authors have no conflict of interest to declare.

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