Angiography significantly decreased serum levels of IL-8 independent of artery stenosis

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Research article

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Abstract

Background

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

Methods

Fifty-five 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 enzyme linked immunosorbent assay (ELISA) technique.

Results

Serum levels of IL-8, but not TNF-α, were significantly decreased following angiography. X-ray doses had moderate positive correlation with serum levels of TNF-α in the patients with more than one artery stenosis. Serum levels of IL-8 and TNF-α were not different among male and female participants in all groups.

Discussion

Angiography may be a protective factor for inflammation in IL-8 dependent manner. Using angiography in the patients with more than one artery stenosis needs to be executed cautiously.

Background

Cytokines are the important molecules that participate in the immune cell migration, activation and homeostasis [1]. Innate immune cells are the main producers and targets of the cytokines that affect several aspects of immune system outputs [2]. The roles played by the molecules in human diseases including infectious and non-infectious diseases have been reported completely [3]. Accordingly, it has been reported that interleukin-8 (IL-8), which is an innate immune cytokine, significantly affects neutrophil migration and functions [4]. Thus, this cytokine significantly participates in several human inflammatory related 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 and significantly participates in either defense against infectious agents or pro-inflammatory related diseases [5].
Artery stenosis is a pro-inflammatory related disease wherein immune cells damage artery endothelial cells and lead to the disorder, a common cardiovascular disease [6]. Due to the roles played by IL-8 and TNF-α in the pathogenesis of artery stenosis [7, 8], the environment factors that change the expression of the cytokines may be considered as protective or risk factor for artery stenosis.

Angiography, as a useful technique to recognize artery stenosis uses X-ray to detect some aspects of artery stenosis. Since X-rays can modulate immune cells to change cytokine productions, it may be hypothesized that the angiography, as an environmental factor, may be associated with altered expression of pro-inflammatory cytokines [9] and then pathogenesis of artery stenosis.

Based on the fact that there is not enough information regarding the effects of the angiography on the cytokine secretions by immune cells in the in vivo conditions, hence, the main aim of this project was to investigate the effects of the angiography on the serum levels of IL-8 and TNF-α among the patients with various status of artery stenosis.

**Material And Methods**

**Subjects**

In this project, serum levels of IL-8 and TNF-a were explored in 55 participants. According to the angiography criteria, the participants with the same status of age, sex, drugs, diabetes, smoking, opium and alcohol drinking were divided into three groups, including without (19 cases), with one (18 cases) and with more than one artery stenosis (18 cases). The patients that were suffering from immune related diseases, such as kidney diseases, autoimmune, allergies and infections were excluded from the project.

Participants who were associated with the existence of 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) were entered to the project because they had the essential criteria to undergo angiography by an expert MD cardiologist. Angiography was done according to the comparison of the damage to normal vessels using the contrast media (Visipaque) by injection into the left and right coronary arteries directly, in several projections. It was performed using 6 French sheath and Judkins catheters after local anesthesia (left and right catheters).

Peripheral blood samples were collected in the non-pretreated coagulant agent’s tubes to collate serum, before and 3 hours after angiography.

**Evaluation of IL-8 and TNF-a serum levels**

Serum levels of IL-8 (CN# KPG-HI8) and TNF-a (CN# KPG-HTNF) were evaluated using commercial kits from Karmania Pars Gene Company, Kerman, Iran, and according to the company instructions.

**Statistical analysis**
SPSS software version 20 was used to analyze the raw data and accordingly, data distribution was calculated by One-Sample Kolmogorov-Smirnov test. Based on the normal data distribution, Paired-Samples t test was used to compare serum levels of IL-8 and TNF-α before and after angiography in the groups, separately. Student t test was used to analyze serum levels of IL-8 and TNF-α in the males versus females in each group before angiography. The correlations between x-doses, age and serum levels of IL-8 and TNF-α were analyzed using Pearson correlation test.

Results

Statistical analysis showed that angiography significantly decreased the serum levels of IL-8 in all the participants. Accordingly, angiography led to decreased IL-8 serum levels from 153.04 ± 44.43 pg/mL to 29.08 ± 12.45 pg/mL (p = 0.014) in the participants without artery stenosis, 142.15 ± 41.60 pg/mL to 33.43 ± 16.97 pg/mL (p = 0.028) in the participants with one and 110.93 ± 27.99 pg/mL to 46.67 ± 18.04 pg/mL (p = 0.021) in the participants with more than one artery stenosis. Results demonstrated that the TNF-α serum levels were not significantly 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. Figure 1 illustrates the raw data regarding serum levels of IL-8 and TNF-α before and after angiography.

As it is shown in the Table 1, serum levels of IL-8 and TNF-α before angiography were not different in the males when compared to females in all groups (Table 1).

| Target | Gender | Without stenosis | P value | With one artery stenosis | P value | More than one artery stenosis | P value |
|--------|--------|------------------|---------|--------------------------|---------|-----------------------------|---------|
| IL-8   | Female | 133.90 ± 44.97   | 0.571   | 29.50 ± 25.11            | 0.280   | 314.08 ± 177.72             | 0.278   |
|        | Male   | 188.58 ± 100.11  |         | 160.92 ± 47.10           |         | 96.37 ± 26.97               |         |
| TNF-α  | Female | 6.10 ± 1.26      | 0.505   | 5.10 ± 2.64              | 0.613   | 6.28 ± 1.85                 | 0.556   |
|        | Male   | 7.35 ± 0.78      |         | 27.53 ± 17.44            |         | 12.84 ± 7.00                |         |

Data analysis revealed that serum levels of IL-8 and TNF-α before angiography were not different between males and females in the groups separately.

Pearson correlation analysis demonstrated that X-ray doses had positive moderate correlation with serum levels of TNF-α in the patients with more than one artery stenosis (r = 0.495, p = 0.037). Table 2 presents the results regarding the Pearson correlation analysis.
Table 2
Correlation among serum levels of IL-8 and TNF-α with X-ray doses and age in the groups.

|                         | Without artery stenosis | With one artery stenosis | More than one artery stenosis |
|-------------------------|-------------------------|--------------------------|------------------------------|
|                         | Age | X-ray dose | Age | X-ray dose | Age | X-ray dose |
| Before angiography      |     |            |     |            |     |            |
| IL-8 Pearson Correlation| -0.250 | -         | -0.301 | -         | 0.404 | -0.116     |
| P value                 | 0.287 |            | 0.184 |            | 0.107 | 0.638      |
| TNF-α Pearson Correlation| 0.143 | -         | 0.077 | -         | 0.263 | -0.083     |
| P value                 | 0.549 |            | 0.739 |            | 0.309 | 0.734      |
| After angiography       |     |            |     |            |     |            |
| IL-8 Pearson Correlation| 0.281 | 0.078     | -0.121 | -0.046     | -0.286 | 0.095     |
| P value                 | 0.230 | 0.744     | 0.600 | 0.482      | 0.265 | 0.709      |
| TNF-α Pearson Correlation| 0.179 | -0.231    | 0.269 | -0.117     | -0.340 | 0.495     |
| P value                 | 0.450 | 0.326     | 0.239 | 0.615      | 0.182 | 0.037      |

The results demonstrated that X-ray doses had a positive moderate correlation with serum levels of TNF-α in the patients with more than one artery stenosis.

Discussion

It has been reported that IL-8 and TNF-α play key roles in the pathogenesis of artery stenosis and cardiovascular diseases [10]. IL-8 can deteriorate the pathogenesis of artery stenosis by activation of neutrophils [7]. Cavusoglu and colleagues also reported that increased serum levels of IL-8 can be considered as a risk factor for the long-term acute coronary syndrome [8]. Therefore, the factor that decreased expression of IL-8 and TNF-α can be considered as the protective factors against artery stenosis. The results demonstrated that angiography significantly decreased serum levels of IL-8 in all groups, independent of artery stenosis and gender. Due to the aforementioned data, it may be hypothesized that angiography can be a protective factor to modulate 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 under angiography operation. Although angiography was unable to change serum levels of TNF-α, X-ray doses had a moderate positive correlation with serum levels of TNF-α in the patients with more than one artery stenosis. Thus, it appears that angiography may be an inducer of TNF-α secretion when it is used long term, which is associated with higher exposure to X-ray in the 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. However, it did affect the serum levels of TNF-α in other groups, which had less damaged arteries. Collectively, based on the results it may
be concluded that routine angiography can modulate the immune responses in IL-8 dependent manner. However, based on the fact that cytokines play their roles in a network manner, serum levels of other pro and anti-inflammatory cytokines need to be explored following angiography. Our previous investigation on the patients under angiography operation showed that angiography was unable to change serum levels of IL-10, as the potential anti-inflammatory cytokine, and interferon-gamma (IFN-γ), as the important T helper 1 pro-inflammatory cytokine [6]. Although several investigations proved the roles played by the cytokines in the pathogenesis of artery stenosis [11, 12], to the best of our knowledge there were no investigations regarding the effects of angiography on the serum levels of IL-8 and TNF-α. Thus, it seems that additional investigations need to be performed to clear the mechanisms used by angiography to reduce serum levels of IL-8.

There were no significant differences in serum levels of IL-8 and TNF-α between males and females in all groups. The results demonstrated that gender had no effects on the serum levels of both IL-8 and TNF-α. However, based on the fact that the number of females was lower than males in this project, it may affect our results. So, the roles played by gender on the serum levels of IL-8 and TNF-α need to be explored by further studies.

**Conclusion**

Angiography in routine time may be considered as a protective factor for inflammation in IL-8 dependent and TNF-α, artery stenosis and gender independent manner. Using angiography in the patients with more than one artery stenosis needs executed with caution.

**Abbreviations**

**IL-8**  
Interleukin-8  
**TNF-α**  
Tumor necrosis factor-alpha  
**IFN-γ**  
Interferon-gamma  
**ELISA**  
Enzyme linked immunosorbent assay  
**EST**  
Exercise stress test  
**ACS**  
Acute coronary syndrome  
**STEMI**  
ST elevation myocardial infarction  
**NSTEMI**  
non-ST elevation myocardial infarction
Declarations

- Ethics approval and consent to participate

The personal consent forms were filled out and signed by the patients, and the Islamic Azad University Sciences and Research Unit Ethical Committee confirmed the protocol of the current project by IR.IAU.SRB.1398.168 code.

- Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

- Competing interests

Authors have no conflict of interest to declare.

- Funding

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- Authors' Contributions

L.Z has performed the laboratory tests and writes a manuscript draft.

A.E and MKA have designed the project, analyzed data and write the manuscript.

M.S has performed the angiography.

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References

1. Sepehri Z, Masoumi M, Ebrahimi N, Kiani Z, Nasiri AA, Kohan F, Sheikh Fathollahi M, Kazemi Arababadi M, Asadikaram G: Atorvastatin, Losartan and Captopril Lead to Upregulation of TGF-β, and Downregulation of IL-6 in Coronary Artery Disease and Hypertension. PLoS One 2016, 11(12):e0168312.

2. Saïd-Sadier N, Ojcius DM: Alarmins, inflammasomes and immunity. Biomed J 2012, 35(6):437-449.

3. Vezzani A, Fujinami RS, White HS, Preux PM, Blümcke I, Sander JW, Löscher W: Infections, inflammation and epilepsy. Acta Neuropathol 2016, 131(2):211-234.

4. Kim KW, Kim BM, Lee KA, Kim HS, Lee SH, Kim HR: Reciprocal interaction between macrophage migration inhibitory factor and interleukin-8 in gout. Clin Exp Rheumatol 2019, 37(2):270-278.
5. Gao H, Liu L, Zhao Y, Hara H, Chen P, Xu J, Tang J, Wei L, Li Z, Cooper DKC et al: Human IL-6, IL-17, IL-1β, and TNF-α differently regulate the expression of pro-inflammatory related genes, tissue factor, and swine leukocyte antigen class I in porcine aortic endothelial cells. *Xenotransplantation* 2017, 24(2).

6. Razavi S, Ahmadi-Roknabadi F, Safarian M, Mehdipour A, Anbarian A, Mirzamohammadi M, Zeinali M, Kazemi Arababadi M: IL-10 is down-Regulated in the Cardiovascular Diseases Suspected Patients, Independent of Angiography. *Probl Radiac Med Radiobiol* 2019, 24:449-454.

7. Marino F, Tozzi M, Schembri L, Ferraro S, Tarallo A, Scanzano A, Legnaro M, Castelli P, Cosentino M: Production of IL-8, VEGF and Elastase by Circulating and Intraplaque Neutrophils in Patients with Carotid Atherosclerosis. *PLoS One* 2015, 10(4):e0124565.

8. Cavusoglu E, Marmur JD, Yanamadala S, Chopra V, Hegde S, Nazli A, Singh KP, Zhang M, Eng C: Elevated baseline plasma IL-8 levels are an independent predictor of long-term all-cause mortality in patients with acute coronary syndrome. *Atherosclerosis* 2015, 242(2):589-594.

9. Kulpe S, Dierolf M, Braig E, Günther B, Achterhold K, Gleich B, Herzen J, Rummeny E, Pfeiffer F, Pfeiffer D: K-edge subtraction imaging for coronary angiography with a compact synchrotron X-ray source. *PLoS One* 2018, 13(12):e0208446.

10. Cimini FA, Barchetta I, Porzia A, Mainiero F, Costantino C, Bertoccini L, Ceccarelli V, Morini S, Baroni MG, Lenzi A et al: Circulating IL-8 levels are increased in patients with type 2 diabetes and associated with worse inflammatory and cardiometabolic profile. *Acta Diabetol* 2017, 54(10):961-967.

11. Min X, Lu M, Tu S, Wang X, Zhou C, Wang S, Pang S, Qian J, Ge Y, Guo Y et al: Serum Cytokine Profile in Relation to the Severity of Coronary Artery Disease. *Biomed Res Int* 2017, 2017:4013685.

12. Omer W, Naveed AK, Khan OJ, Khan DA: Role of Cytokine Gene Score in Risk Prediction of Premature Coronary Artery Disease. *Genet Test Mol Biomarkers* 2016, 20(11):685-691.