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## РІВЕНЬ ІЛ-10 ЗНИЖЕНИЙ У ПАЦІЄНТІВ ІЗ СЕРЦЕВО-СУДИННИМИ ЗАХВОРЮВАННЯМИ, НЕЗАЛЕЖНО ВІД ПРОВЕДЕННЯ АНГІОГРАФІЧНИХ ДОСЛІДЖЕНЬ

Інтерлейкін-10 (ІЛ-10) та інтерферон-гамма (ІФН- $\gamma$ ) є важливими, відповідно, проти- та прозапальними цитокінами, які можуть брати участь у патогенезі серцево-судинних захворювань. Крім того, фактори навколишнього середовища – такі, як рентгенівське випромінювання – здатні модулювати експресію цитокінів. Через те, що рентгенівське випромінювання використовується при ангіографії, ця процедура, можливо, змінює експресію цитокінів.

**Мета.** Оцінити рівні ІЛ-10 та ІФН- $\gamma$  сироватки крові у пацієнтів із серцево-судинними захворюваннями (у яких наявний або ж відсутній стеноз судин) порівняно зі здоровими контролями, а також ефекти ангіографії щодо вмісту цитокінів у сироватці крові.

**Матеріали та методи.** Дослідження було проведено серед 80 учасників – по 20 випадків у кожній групі (здорові контролі та особи із серцево-судинними захворюваннями без стенозу судин, із стенозом 1 судини та стенозом більш ніж 1 судини) для оцінки рівнів ІЛ-10 та ІФН- $\gamma$  у сироватці крові за допомогою методу імуноферментного аналізу. Для з'ясування впливу процедури ангіографії на рівень ІЛ-10 та ІФН- $\gamma$  у сироватці крові, вміст обох цитокінів також порівнювали у групах 2, 3 та 4 до та після ангіографічного дослідження.

**Результати.** Рівень ІЛ-10, але не ІФН- $\gamma$ , у сироватці крові здорових осіб групи контролю був більшим, ніж у всіх пацієнтів із серцево-судинними захворюваннями. Рівень ІЛ-10 та ІФН- $\gamma$  у сироватці крові не змінювався після ангіографії, а також не відрізнявся у курців та осіб, які не курять, так само як і у споживачів опію та осіб, які опій не вживають.

**Висновки.** Завдяки результатам можна зробити висновок, що ІЛ-10 можна ймовірно вважати інгібітором серцево-судинних захворювань, незалежно від тривалості ангіографії та рентгенографії, в той час як ІФН- $\gamma$  не проявляє ефектів у іранських пацієнтів, які страждають на серцево-судинні захворювання.

**Ключові слова:** ангіографія, рентгенографія, ІЛ-10, ІФН- $\gamma$ .

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## IL-10 IS DOWN-REGULATED IN THE CARDIOVASCULAR DISEASES SUSPECTED PATIENTS, INDEPENDENT OF ANGIOGRAPHY

IL-10 and interferon-gamma (IFN- $\gamma$ ) are the important anti and pro-inflammatory cytokines, respectively, which may participate in the cardiovascular disease pathogenesis. Additionally, environmental factors, such as the X-ray, can modulate cytokine expression. Due to the fact that X-ray is used during angiography, hence, angiography may alter expression of the cytokines.

**Objective.** Accordingly, this project was aimed to assess IL-10 and IFN- $\gamma$  serum levels within cardiovascular patients (with and without vessel stenosis) versus healthy controls and also the effects of angiography on the serum levels of the cytokines.

**Material and methods.** This study was performed on the 80 participants, including twenty cases in each group (healthy controls and cardiovascular patients without vessel stenosis, stenosis of 1 vessel and stenosis of more than 1 vessel) to evaluate IL-10 and IFN- $\gamma$  serum levels using ELISA technique. The IL-10 and IFN- $\gamma$  serum levels also compared within group 2, 3 and 4 before and after angiography to explore the effects of the technique on the IL-10 and IFN- $\gamma$  serum levels.

**Results.** IL-10, but not IFN- $\gamma$ , serum levels were higher in the healthy controls than all cardiovascular patients. IL-10 and IFN- $\gamma$  serum levels were not altered after angiography and also were not differ in the smoker versus non-smoker and opium consuming versus non-opium consuming participants.

**Conclusion.** Due to the results it may be concluded that IL-10 can be considered as a plausible inhibitor of cardiovascular diseases independent of angiography duration and X-ray, however, IFN- $\gamma$  has no effects in the Iranian patients suffering from cardiovascular diseases.

**Key words:** angiography, X-ray, IL-10 and IFN- $\gamma$ .

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### INTRODUCTION

IL-10 and interferon-gamma (IFN- $\gamma$ ) are the anti and pro-inflammatory cytokines, respectively, which play several roles in the physiological, immunological and cardiovascular diseases [1, 2]. IL-10 is produced by several immune cells, including T regulatory cells, macrophages and Th2 cells [3], while, IFN- $\gamma$  is secreted by T helper 1 (Th1) and natural killer (NK) cells. IL-10 not only is an anti-inflammatory molecule, but also plays as an angiogenesis factor [4]. However, IFN- $\gamma$  has the contrast functions and is either a pro-inflammatory or angiostasis molecule [5]. Thus, their roles in the pathogenesis of acute coronary syndrome (ACS) are plausible.

Additionally, it has been reported that the environmental factors play critical roles in the regulation of immune cells functions, including cytokine production [6]. X-ray, as an environmental factor, is a main factor to alter immune responses [7]. Therefore, the techniques, that are based on the using of X-ray, such as angiography, may be associated with altered immune cell functions. Due to the fact that angiography is a main technique for diagnosis of vessel stenosis [8], hence, several patients who have ACS containing unstable angina may be at risk of immune cell altered functions after angiography.

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Thus, the main aims of this project were to explore the IL-10 and IFN- $\gamma$  serum levels in the healthy controls and compare it with the patients who had ACS and also evaluate the effects of angiography on the IL-10 and IFN- $\gamma$  serum levels in the patients.

## MATERIAL AND METHODS

### Subjects

IL-10 and IFN- $\gamma$  serum levels were evaluated in the 80 participants who were divided to the four groups (group 1: healthy controls; group 2, 3 and 4: patients with no, 1 and more than 1 vessel stenosis, respectively). The groups were similar regarding gender, age, diabetes, alcohol drinking, smoking, drugs, and opium. Accordingly, participants in the group 1, 2, 3 and 4 had ( $57.84 \pm 1.87$ ), ( $57.90 \pm 2.35$ ), ( $59.09 \pm 1.70$ ) and ( $60.52 \pm 2.31$ ) years old, respectively. The frequencies of male and female participated in this study were 60 and 20 cases, respectively. However, 14, 17, 13 and 16 in the groups 1, 2, 3 and 4 were male, while female were 6, 3, 7 and 4, respectively. However, the participants who had autoimmune, allergies, infectious and kidney diseases were excluded from the project.

The expert cardiologists entered the patients in the study based on the existence of ACS containing unstable angina, non-ST elevation myocardial infarction (NSTEMI), and ST elevation myocardial infarction (STEMI), and also patient who had typical chest pain with positive exercise stress test (EST). Accordingly, the patients, but not controls, who had the criteria for angiography were underwent angiography by the expert MD cardiologists. After local anesthesia, the selective coronary angiography was done from right femoral artery using 6 French sheaths percutaneously and Judkins left and right catheters. During angiography, the contrast media (visipaque) was injected into both left and right coronary arteries. The angiography was done at standard views. The number of stenotic vessels and severity of the stenosis was determined by the expert cardiologist based on the comparison with normal vessels.

The blood samples were collected in the non-pre-treated coagulant agent's tubes from the patients, before and 3 hours after angiography, to collect serums. Due to the fact that the controls had not angiography criteria, one sample was obtained from them, immediately after entrance to the project. Rafsanjan University of Medical Sciences Ethical Committee confirmed the investigation protocol (code: RUMS.REC.1397.123), after that the consent forms were filled out by the participants.

### Cytokine assay

IL-10 and IFN- $\gamma$  serum levels were checked using the appropriate kits from Karmania Pars Gene Company, Kerman, Iran, and based on the manufacture guidelines.

### Statistical analysis

The data normality distribution was checked by One-Sample Kolmogorov-Smirnov test under SPSS version 16 and accordingly, the non-parametric tests were used to analyze the raw data. Likely, IL-10 and IFN- $\gamma$  serum levels between the groups were compared using Kruskal-Wallis H and in the smoking and opium consuming versus non-smoking and opium non-consuming, respectively, were calculated using Mann-Whitney U tests. IL-10 and IFN- $\gamma$  serum levels correlations with other variables, such as X-ray doses, duration of angiography and age were evaluated by Spearman Correlation test. IL-10 and IFN- $\gamma$  serum levels before and after angiography was compared by Two-Related-Samples test. The  $p$  value  $\leq 0.05$  was considered significant.

## RESULTS

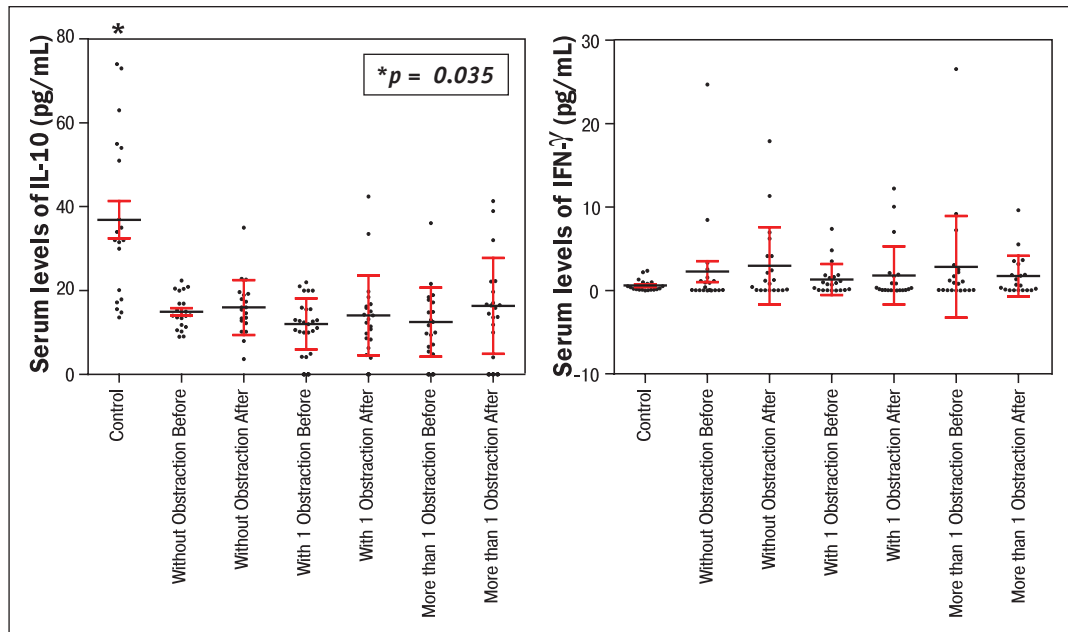
IL-10 serum levels in the groups 1, 2, 3 and 4 before angiography were 15.4100 (14.0000–17.1900), 14.5130 (11.4365–19.5200), 12.0000 (7.4675–14.2680) and 12.1285 (6.7643–17.6438), respectively. As it is illustrated in the figure 1, the IL-10 serum levels were significantly decreased ( $p = 0.035$ ) in all patients (group 2, 3 and 4) when compared to healthy controls (group 1). The figure also demonstrated that there were not significant differences between before and after angiography regarding IL-10 serum levels in the group 2 ( $p = 0.263$ ), 3 ( $p = 0.289$ ) and 4 ( $p = 0.313$ ).

Figure 1 also demonstrated that not only IFN- $\gamma$  serum levels were not changed among the groups ( $p = 0.907$ ), but also angiography had no significant effects on the IFN- $\gamma$  serum levels in the groups 2 ( $p = 0.089$ ), 3 ( $p = 0.999$ ) and 4 ( $p = 0.411$ ).

Data collections revealed that the Entrance Surface Dose to chest region and heart in the group 2, 3 and 4 were equally to ( $222.92 \pm 35.15$ ), ( $411.13 \pm 62.43$ ) and ( $678.10 \pm 150.51$ ) mGy, respectively.

In parallel with the results, Spearman correlation test showed that there were not significant differences between the IL-10 and IFN- $\gamma$  serum levels and the variables, including age, angiography duration, X-ray doses and also the percent of vessel stenosis. Table 1 illustrates the details of the analysis.

Figure 2 revealed that smoking did not change IL-10 and IFN- $\gamma$  serum levels in the group 1 ( $p = 0.352$  and  $p = 0.423$ , respectively), 2 ( $p = 0.263$  and  $p = 0.758$ ,



**Figure 1.** IL-10 and IFN- $\gamma$  serum levels before and after angiography and also among the groups, including controls

**Table 1**  
IL-10 and IFN- $\gamma$  serum level correlations with X-ray dose, age, angiography duration, and obstruct percent

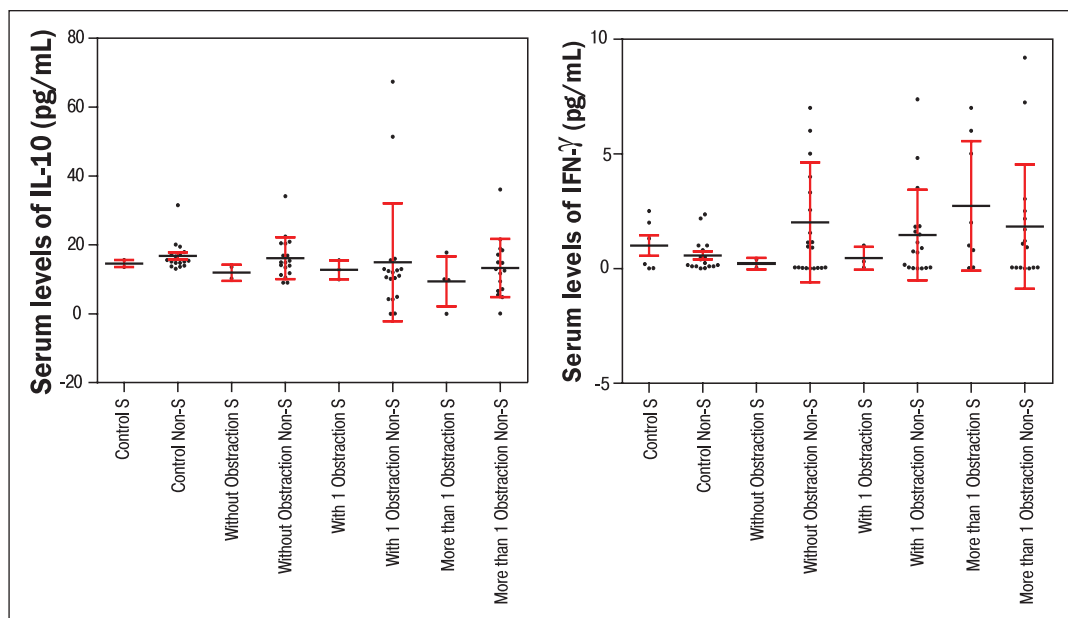
		IL-10	IFN- $\gamma$	IL-10	IFN- $\gamma$	IL-10	IFN- $\gamma$	IL-10	IFN- $\gamma$	
		Group 1	Group 1	Group 2	Group 2	Group 3	Group 3	Group 4	Group 4	
Spearman's rho	<b>Age</b>	Correlation Coefficient	-0.012	-0.185	0.198	0.285	0.293	0.321	-0.060	0.181
		P value	0.960	0.448	0.402	0.223	0.198	0.156	0.818	0.487
	<b>Angiography duration</b>	Correlation Coefficient	-	-	-0.177	0.269	-0.205	-0.024	0.328	-0.008
		P value	-	-	0.456	0.251	0.385	0.919	0.170	0.974
	<b>X-ray dose</b>	Correlation Coefficient	-	-	-0.066	0.067	0.178	-0.002	0.298	-0.358
		P value	-	-	0.782	0.779	0.440	0.993	0.215	0.132
	<b>Obstruct percent</b>	Correlation Coefficient	-	-	-	-	0.325	0.037	-0.233	0.224
		P value	-	-	-	-	0.237	0.896	0.384	0.405

respectively), 3 ( $p = 0.688$  and  $p = 0.546$ , respectively) and 4 ( $p = 0.494$  and  $p = 0.524$ , respectively). Opium addiction also did not change the serum levels of IL-10 and IFN- $\gamma$  in the groups (Fig. 3).

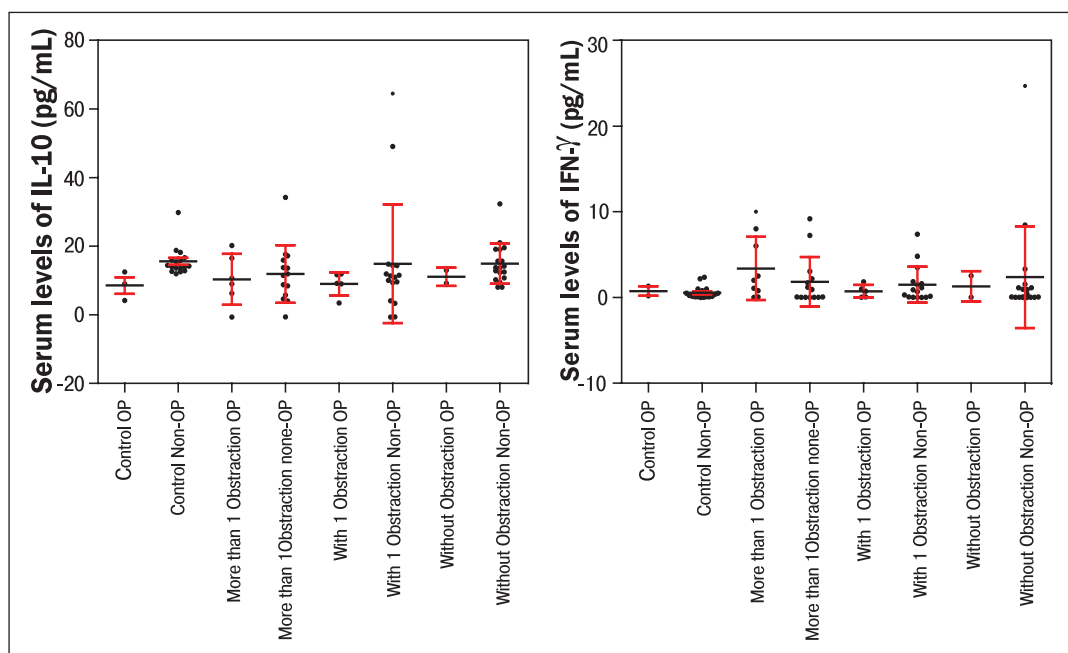
## DISCUSSION

Cardiovascular diseases are the main common prevalent disorders worldwide [9]. The main factors which are associated with increased risks of the diseases may be targeted as the future treatment strategies. This investigation revealed that IL-10 serum levels were significantly higher in the healthy controls compared to the patients, independent of the vessel stenosis, angiography duration and X-ray doses. IL-10 is an important anti-inflammatory molecule which plays several roles, including suppression of pro-inflammatory molecule expression, down-regulation of major histocompatibility complex (MHC) molecules and also inhibits angiogenesis [10]. The results of the current study showed that IL-10 serum levels were significantly decreased in the patients when compared to controls independent of vessels stenosis.

Due to the results, IL-10 may be considered as an important factor, which its production is decreased during cardiovascular diseases and it may be proposed that its concentration may be considered as a diagnostic criteria in predicting of the ACS. Additionally, the results demonstrated that angiography and its related variable factors such as X-ray had no effects on the IL-10 production and it may confirm the safety of the angiography protocol. In parallel with our results it has been reported that number and functions of T regulatory cells decreased in the atherosclerotic patients [11]. T regulatory cells are the main source of IL-10 [10], hence, due to our results, it appears that down-regulation of IL-10 may be related to the decreased number and functions of T regulatory cells in the patients. Additionally, the roles played by micro RNA (miR)-19a on the down-regulation of IL-10 has been reported by and colleagues [12]. Mir-19a is a main molecule to induce vascular inflammation and foam cell generation in the atherosclerosis [13]. Thus, it appears that epigenetic factors also are the main factor for down-regulation of IL-10 in the patients.



**Figure 2.** IL-10 and IFN- $\gamma$  serum levels in the smoking/non-smoking participants



**Figure 3.** IL-10 and IFN- $\gamma$  serum levels in the opium/non-opium consuming participants

It has been demonstrated that smoking and opium consuming are two important risk factors for the cardiovascular diseases [14]. Our results demonstrated that the risk factors are unable to change the IL-10 serum levels and it may be related to the low number of smoker and opium consumers in our evaluated patients and it needs to be explored using large sample size population.

Although several investigations have been reported that IFN- $\gamma$ , as Th1 marker, is a potential risk factor for atherosclerosis and other cardiovascular disease related complications [1, 2], our results demonstrated that not only it did not differ among the groups, but also angiography and its related variables such as X-ray doses did not change its serum levels. Interestingly, smoking and opium consuming also did not alter IFN- $\gamma$  serum levels among the participants. Thus, it seems that IFN- $\gamma$  may not participate in

the pathogenesis of cardiovascular diseases in the Iranian population. Interestingly, it has been reported that IL-10 has antagonist effects to IFN- $\gamma$ , and due to the results up-regulation of IL-10 in the controls were not associated with decreased serum levels of IFN- $\gamma$  in the controls. Therefore, it appears that other cytokines, including pro and anti-inflammatory cytokines, significantly participate in the pathogenesis of cardiovascular diseases and it relates to the network functions of the cytokines [15]. Thus, it is worthy to evaluate other pro and anti-inflammatory cytokines in the patients and the effects of the angiography on the expression of the molecules.

Collectively, it appears that IL-10, but not IFN- $\gamma$  can be considered as a factor involved in the pathogenesis of cardiovascular diseases and its declination can be associated with inflammatory responses in the diseases.

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## CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

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