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Serum Level of Interleukin-18 to Interleukin-10 Ratio after Percutaneous Coronary Intervention: A New Predictor of In-Stent Restenosis

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ABSTRACT

Despite advanced techniques of stent placement which cause fewer traumas to the vessel walls; as well as introduction of drug-eluting stents which result in the least induction of immune response, in-stent restenosis (ISR) is still one of the common and severe complications after PCI and stent placement. Intimal hyperplasia following immuno- inflammatory response of the arterial wall to balloon injury has been proposed as main mechanism of ISR. In a prospective study, we assessed the predictive role of Interleukin (IL)-18 and tumor necrosis factor alpha (TNF-α) as pro-inflammatory cytokines and IL-10 as anti-inflammatory cytokine and high sensitive C-reactive protein (hs-CRP) for ISR. 128 patients (mean age=59±10.2, femal e/male: 41/87)who underwent percutaneous coronary intervention (PCI) and stent implantation. Venous blood samples were obtained before and 24 hours after PCI. IL-18, IL-10, TNF-αand hs-CRP levels were determined. We followed the patients for24months and measured the incidence of ISR via angiography. Results were compared between ISR and non-ISR patients. 20 patients (15.6%) developed ISR. Serum level of IL-18, TNF-α and hs-CRP have been increased in all patients 24 hours after PCI. Serum level of IL-18 at 24-hours was not different between ISR and non-ISR patients (p=0.239), while serum level of IL-10 was significantly higher in non-ISR group (p<0.001). IL-18/IL-10 was significantly higher in ISR patients than in non-ISR patients (p<0.001). IL-18/IL-10 can be applied as predictive factors for ISR.

Key words: Stents, Percutaneous Coronary Intervention, Cytokines, Thrombosis

INTRODUCTION

Despite advanced techniques of stent placement which cause fewer vessel walls injury as well as introduction of drug-eluting stents which result in the least induction of immune response, in-stent restenosis (ISR) is still one of the most common and severe complications after PCI and stent placement[1-3].Detailed molecular mechanism of ISR is

not completely understood. However, intimal hyperplasia following Immuno-inflammatory reactions, due to trauma or stimulation of vessel walls, has been proposed as main mechanism of ISR.

Several factors might play role in this process. Chemo-attractant components such as C_3a and C_5a (which product following complement cascade activation) and also tumor necrosis factor (TNF)-α, C-reactive protein (CRP) and pro-inflammatory cytokines are among these factors [4-6].

Interleukin (IL)-18 is pleotropic cytokine from IL-1 family which is also known as interferon (IFN)-γ inducing factor. It is involved in inflammatory processes in several ways. Induction of IFNα production by T lymphocytes, natural killer cells and macrophages, as a pro-inflammatory cytokine directly leads to production of IL-1b, IL-8, GM-CSF, TNFα and expression of adhesion molecules and also inducible nitric oxide synthesize by mononuclear and mesenchyme cells.As a result, IL-18 may have a fundamental role in ISR.In response to endothelial cells damage, pro IL-18 (inactive IL-18) is released from these cells as well as from the infiltrated neutrophils and macrophages and smooth muscle cells at the site of injury. The IL-18 is activated by caspase-1 enzyme (IL-1b converting enzyme) and then activated IL-8 leads to progression of inflammatory process [7-9].

IL-10 is an anti-inflammatory cytokine which can inhibit inflammatory process in several ways. One of the most important effects of this cytokine is inhibition of IL-12 production via macrophages. IL-12 leads to secretion of IFN-γ. The other effect of IL-10 is prevention of expression of MHC II molecules on the surface of macrophages that results in prevention of cellular immune system activation and inflammatory process [10, 11].

The TNF-α acts on several cells and induce programmed cell death (apoptosis). This cytokine activates T lymphocytes. Also it promotes migration of the neutrophils and macrophages to the site of the inflammation. In addition induces endothelial cells and macrophages to release chemo-attractants factors [12, 13]. Via the prevention of the lipoprotein lipase activity, this cytokine results in increased systematic levels of VLDL and triglycerides.

CRP is an acute phase reactant protein which due to its ability to bind to the C-protein of pneumococcal capsule is named CRP. This protein can be found in serum of healthy individuals in small amounts. However, during activation of inflammatory processes, it can be produced up to hundreds times and as an opsonin increases phagocytic activity. On the other hand, through activation of complement cascade, CRP leads to release of several components of this system which help in the progression of inflammatory process and also leads to aggregation of leukocytes in the vascular walls [14, 15].

In general, due to molecular activity of these factors it would be possible to have an important role in the process of ISR.

Objectives

In the present study we aimed to investigate the probable role of the above mentioned factors in development of ISR in group of patients undergoing percutaneous coronary intervention (PCI) and stent placement.

MATERIALS AND METHODS

Study Population

In a prospective study from January to October 2013, 128 patients who underwent percutaneous coronary intervention (PCI) and stent implantation in the catheterization laboratory of Rajaie Cardiovascular Medical and Research Center, Tehran, Iran participated. All the patients were between 30-70 years old and excluded if had a history of angiography, surgery, cardiac events, infectious or inflammatory diseases in the past three months before admission, history of cancer or auto immune diseases, previous use of anti-inflammatory, immunosuppressive or lipid lowering drugs during recent 2 weeks and history of fever in the past week. Study protocol was approved by the research committee of Rajaie Cardiovascular Medical and Research Center.

Blood sampling

Venous blood samples were obtained before PCI and stent placement and also 24 hours after PCI. Serum samples were frozen at 70 °c until analysis.

All samples were done in duplicated. IL-18, IL-10 and TNF-α were measured by ELISA (Enzyme-Linked Immunosorbent Assay) method (with the kits produced by the R & D Co.). The hs-CRP was measured by Immunoturbidimetric assay (with the kits produced by the Pars Azmoon Co.)

Statistical Analysis

Fitness of interval variables to normal distribution was assessed via one-sample Kolmogorov-Smirnov test. Data presented as mean ± standard deviation for the interval and count (percent) for the categorical variables. Associations between ISR and immunological or patients' characteristics were investigated by Student's t or Pearson's chi square tests. Statistical analysis was performed by using IBM SPSS Statistics 19 for Windows (IBM Inc., Armonk, NY, USA). P values ≤0.05 was considered as statistically significant.

RESULTS

Baseline characteristics

One hundred twenty-eight patients (mean age = 59±10.2, female/male: 42/86) participated. In-stent restenosis was happened in 20 patients (15.6%).Patients' characteristics were presented and compared between two groups of participants with and without ISR in table 1. No significant differenced were observed between the groups about the mentioned characteristics.

Table 1.Comparison of baseline characteristics between in-stent restenosis patients and control group*

	ISR (n=20)	Non-ISR (n=108)	P value
Age (years)	59±10.1	59.1±10.2	0.968
Gender (F/M)	7/13	40/68	0.562
Smoke	8(40%)	41(38%)	0.738
Family History	4(20%)	23(21.3%)	0.917
Hypertension	9(45%)	46(42.6%)	0.654
Dyslipidemia	9(45%)	49(45.4%)	0.821
Diabetes	7(35%)	25(23.1%)	0.821

** Data presented as mean ± SD or count (%)*
Abbreviation: ISR: in-stent restenosis

Associations between ISR and Serum IL-18, TNF α, IL-10 andhs-CRP

Mean serum level of IL-18, TNF α, IL-10, hs-CRP and the ratio of IL-18/ IL-10 was determined before and after PCI in all the patients. These levels and the mean changes of the factors were compared between two groups and the results presented in Table 2.

It was observed that the mean serum levels of IL-18, TNF-α and hs-CRP and also their changes through the time were not significantly different between the groups.

Table 2.Characteristics of lesion , PCI and stent in study participants

	ISR (n=20)	Non ISR (n=108)	P value
Number of lesions undergone PCI	29	149	-
Diameter of stenosis (%)	86±6.02	85±5.40	0.625
ACC/AHA Lesion Type B2/C	88%	87%	0.817
Length of Lesions (mm)	27.78±6.22	26.42±5.64	0.322
Diameter of Reference vessel (mm)	2.69±0.29	2.94±0.32	0.481
Stent length (mm)	29.91±7.07	28.66±6.85	0.963
PCI on LAD	55%	53%	0.213
PCI on RCA	25%	27%	0.142
PCI on LCX	15%	17%	0.681
PCI on diagonal	5%	3%	0.294
SVD	45%	48%	0.582
2VD	40%	40%	0.736
3VD	15%	12%	0.412

**Data presented as mean ± SD or count (%)*
Abbreviation: ISR: in-stent restenosis

In ISR group, mean serum level of IL-10 was significantly lower than non-ISR patients after PCI (2.0 ± 1.1 vs. 2.9 ± 1.6 ng/L, p = 0.002). An increase in serum level of IL-10 was also observed through 24 hours in control group (p<0.001). Finally, ratio of IL-18 to IL-10 was significantly higher in patients in the ISR group comparing to patients in the non-ISR groups (149.8±37.5 vs. 96.5±24.1; p <0.001). The increase in this index was dramatically greater in ISR patients compared to control group during 24 hours after PCI (56.1 ± 3.6 vs. 1.3 ± 1.2; p <0.001).

Table 2. Mean serum level of the cytokines before and after PCI in patients with and without in-stent restenosis*

	Before PCI			24 hours after PCI			Within Group Difference †		
	ISR Group (n=20)	Non-ISR Group (n=108)	P Value	ISR Group (n=20)	Non-ISR Group (n=108)	P Value	ISR Group (n=20)	Non-ISR Group (n=108)	P Value
IL-18 (ng/L)	243.6 ± 53.6	238 ± 47.6	0667	299.6± 73.5	279.8 ± 67.9	0.239	56 ± 10.2	41.8 ± 8.2	0.935
TNFα (ng/L)	6.3 ± 3.1	6.2 ± 3.2	0.613	7.5± 3.9	6.9 ± 3.8	0.733	1.2 ± 0.5	0.7 ± 0.5	0.999
hs-CRP (mg/L)	17.3 ± 9.1	18.1 ± 9.5	0.359	45.8± 19.7	45.4 ± 19.6	0.533	28.1 ± 11.6	27.3 ± 9.2	0.614
IL-10 (ng/L)	2.3 ± 1.4	2.5 ± 1.5	0.613	2.0 ± 1.1	2.9 ± 1.8	0.002	-0.6 ± 0.3	0.4 ± 0.3	<0.001
IL-18/IL-10	103.7± 30.2	95.2 ± 23.8	0.416	149.8± 37.5	96.5± 24.1	<0.001	56.1 ± 3.6	1.3 ± 1.2	<0.001

** Data presented as mean ± standard deviation*
† Within Group Difference = Factor's serum level 24 hours after PCI - Factor's serum level before PCI
Abbreviations: PCI: underwent percutaneous coronary intervention, IL: interleukin, TNF: tumor necrosis factor alpha, hs-CRP: high sensitive C-reactive protein

DISCUSSION

Pro-inflammatory cytokines and their role in the development and progression of atherosclerosis have been studies since several years ago. At first, their role was assessed in development of atherosclerosis. Several studies [16, 17] including the previous study by the authors [10] showed that there is a significant association between serum levels of some of these cytokines and the development and the severity of the coronary artery diseases. Recently, the association between serum level of cytokines and the development of ISR in patients undergoing PCI and stent placement has been come into attention and several of these cytokines have been introduced as predictive markers of ISR development.

Reem et al found no significant differences in the serum level of IL-8 before and after PTCA [18]. Results of the study by Schulze et al on patients who underwent PCI and stent placement showed that serum level of IL-1b (which also belongs to the IL-18 family) was significantly higher in patients with ISR than in non-ISR patients [19]. Moreover, several days after PCI serum level of IL-1b has been significantly decreased and this decline was higher in patients with non-ISR comparing to those with ISR. They introduced IL-1b as a predictive of ISR. In accordance to our findings, they also found no significant differences in the serum level of TNF-α.In contrast to our findings, some of the previous studies have found significant differences in IL-18 between ISR and non-ISR groups [20-22].
IL-10 is known as an anti-inflammatory cytokine. Feldman et al and Laurent et al. measured this cytokine in patients undergoing PCI and assessed the differences between ISR and non-ISR groups [23, 24]. Their results showed that IL-10 can be used as a predictive factor of ISR.

In the present study serum level of hs-CRP has been increased in all patients 24 hours after PCI compared to before PCI values. This finding is in agreement with most of the previous works in the literature. As the differences were not statistically significant between ISR and non-ISR groups; the presence of several factors which can increase CRP level, its measurement as a predictive marker of ISR is not recommended. However measurement of CRP has been introduced beneficial in addition to measurement of the other factors affecting the inflammatory process.

Ratio of IL-18 to IL-10 has been less studied. Our results showed that the ratio of IL-18 to IL-10 was significantly higher in patients with ISR as compared to those in the non-ISR groups. These results show that assessing the ratio of these two cytokines is accurate predictive marker of ISR that measurement of any of these two cytokines alone. However, to better elucidate the role of this ration in the prediction of ISR more investigations with larger sample sizes are suggested.

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