

## Interleukin-18(IL-18) and Atherosclerosis

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### Abstract

**Background-** Atherosclerosis is a dynamic and progressive disease arising from the combination of endothelial dysfunction and inflammation. So, inflammatory mediators are important causative and progressive factors in atherosclerosis. Interleukin-18 (IL-18) is a pleiotropic cytokine which may have a key role in atherosclerosis, because the results of recent studies indicated that IL-18, either directly as a pro-inflammatory factor or indirectly as an interferon inducing factor, has central role in the inflammatory cascade. In present study, we evaluated the relation between blood concentration of IL-18 with atherosclerosis and its severity and also the relation of this cytokine with the other known risk factors of coronary artery disease (CAD).

**Methods-** 138 patients who underwent diagnostic cardiac catheterization and had the inclusion criteria for this study, were selected. In the beginning, a questionnaire containing personal information and medical history was completed for each of them and blood samples were collected 24 hours before catheterization. We measured blood level of IL-18 and CRP in all samples. The results of these tests and the angiography were entered in the questionnaire and then information was analyzed using regression analyzing, K2 and statistical T test. P values of <0.05 were considered statistically significant.

**Results-** By using the results of angiography, patients were divided in two groups. 1- Control group or normal coronary arteries (32 people) 2- Case group (106 people) or patients who were suffering from CAD. In second group, 6 patients (5.6%) had minimal CAD, 44 patients (41.5%) had single-vessel disease (SVD), 27 patients (25.4%) had 2VD and 29 patients (27.5%) had 3VD. In the laboratory tests, the mean IL-18 blood level in patients with CAD was 258.1 pgr/ml but in normal coronary arteries group it was 216.6 pgr/ml. The average blood level of IL-18 in patients who had minimal CAD was 235.5 pgr/ml, in SVD was 247.2 pgr/ml, in 2VD was 263.7 pgr/ml and in patients with 3VD was 274.1 pgr/ml. Also average CRP blood level in normal group was 8.9 mgr/L while in patients with CAD it was 20.4 mgr/L.

**Conclusion-** The results of this study indicated that there was a direct and significant correlation between blood IL-18 level with existence ( $P<0.001$ ) and severity of CAD( $P<0.01$ ) and IL-18 can be an independent predictor risk factor of atherosclerosis(*Iranian Heart Journal 2012; 13 (1):11 -16*).

**Keywords:** Inflammation■Atherosclerosis■Interleukin 18■CAD■Cytokine

Received Apr. 3, 2012; Accepted for publication May. 25, 2012

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ince atherosclerosis was known as an inflammatory disease, several researches has been done on blood markers of inflammation, including some cytokines.<sup>1-2</sup> Cytokines are soluble molecules that secrets by producing cells in certain circumstances and induce their biological effects on target cells after binding to their specific receptors. Interleukins are most important group of cytokines that are divided into several families. IL-18, a new member of IL-1 family, is a pleiotropic cytokine(can act on different cell types .this property allows a cytokine to mediate diverse biologic effects) that plays a central role in the inflammatory process and acts in both innate and acquired immunity<sup>3</sup>.

This cytokine promote the production of gamma interferon (IFN  $\gamma$ ) by T lymphocytes, natural killer cells (NK cells) and a group of macrophages. In addition, it acts directly as a pro inflammatory cytokine by inducing production of IL-1b, IL-8 and expression of adhesion molecules. IL-18 can stimulate the production of GM-CSF, TNF  $\alpha$  and inducible nitric oxide synthesize by mononuclear and mesenchymal cells. Due to many actions, IL-18 is essential in host defense, especially against intracellular bacterial, viral and fungal infections. Recent studies indicated that IL-18 as an inflammatory mediator, may have a significant role in pathogenesis of atherosclerosis.<sup>4-7</sup> In response to endothelial cells damages, or heart tissue damages, pro IL-18(inactive IL-18) secret by endothelial cells (ECs), infiltrated neutrophiles, resident macrophages and smooth muscle cells. Then, pro IL-18 is activated by caspase -1 (IL-1 beta converting enzyme). Activated IL-18 induce IFN  $\gamma$  secretion and progress of inflammatory process.<sup>8-9</sup> This Cytokine can promote different heart diseases especially CHD by another mechanisms.

IL-18 can cause apoptosis (programmed cell death) in human cardiac micro-vascular endothelial cells that can lead to thrombosis and tissue damages. Also it promotes migration and proliferation of smooth muscle

cells. In addition, it stimulates fibroblasts proliferation and causes fibrosis. These properties are specific, because studies have shown that we can prevent these effects by IL-18 neutralizing antibody.<sup>10-11</sup>

IL-18 can cause heart damage by modulation of mitogen activated protein kinas activity and also change the intracellular calcium.

This property is also specific.<sup>12-13</sup> In this study we measured blood concentration of IL-18 in patients with atherosclerosis and control group and evaluated the correlation between concentration of IL-18 with occurrence and severity of the disease and also with other known risk factors.

## Material and Methods

138 patients who were admitted for diagnostic coronary catheterization between May to November of 2010 in Shaheed Rajaie cardiovascular medical and research center and had the following inclusion criteria were selected:

- Age between 40 to 75 years.
- No history of coronary angiography, surgery, cardiac events, infectious and inflammatory diseases in the past three months.
- No history of cancer or autoimmune diseases.
- Not using anti-inflammatory drugs.
- Not using lipid-lowering and immune suppressive drugs in the past month.
- No history of fever for two weeks before admission.

At first, a questionnaire containing personal information, medical history and presence of known risk factors of atherosclerosis was completed for each of them. Blood samples were taken 24 hours before angiography and immediately separated samples serum and stored in freezer at -20° until testing time. Routine tests and also measurement of blood level of IL-18 and high sensitive quantitative CRP tests were done for each sample and results of laboratory tests and angiography of

each patient were performed in their questionnaire. Patients who suffered from other cardiovascular diseases in addition to CAD and cases who did not have inclusion criteria, were excluded. Measurement of IL-18 was done using ELISA (Enzyme -Linked Immuno Sorbent Assay) method with the Bendermed Company's kits. High sensitive quantitative CRP test was done by immunoturbidimetry method and using Pars Azmoon company's kits. To minimize errors in the above tests, all samples were done in duplicate. For record and analyzing the results, we used SPSS software.

The results were presented as mean or number and percentage. To compare the results, we used regression analyzing, K2 and statistical T test. P values < 0.05 were considered statistically significant.

**Results-** There were 58 females and 80 males among the 138 patients. Mean age was 58. 8 years. 32 patients had normal coronary artery (control group) and 106 patients had CAD (case group). Among the CAD group, 6 patients (5.6%) had minimal CAD, 44 patients (41.5%) had single vessel disease (SVD), 27 patients (25.4%) had two vessel disease (2VD) and 29 patients (27.5%) had three vessel disease (3VD). Mean serum level of the IL-18 in the patients with normal coronary arteries (control group) was 216.6 pgr/ml whereas in patient with CAD (case group) was 258.1 pgr/ml. Analysis of Results indicated that there was significant relation between this cytokine and atherosclerosis ( $P < 0.001$ ). The average level of IL-18 in patients with minimal CAD was 235.5 pgr/ml, in SVD was 247.2 pgr/ml, in 2VD was 263.7 pgr/ml and in 3VD was 274.1 pgr/ml which showed a direct significant relation between severity of the disease and concentration of IL-18 in blood ( $P < 0.01$ ).

We did not find any relation between the level of IL-18 and hypertension, smoking, family history and diabetes. Although analysis of laboratory tests' results showed that there was a direct relation between IL-18 and cholesterol and reverse relation between this

cytokine and HDL, but they were statistically insignificant. In addition there was a direct relation between serum level of CRP and IL-18 which was not statistically significant. More information about the results is shown in Tables I and II.

## Conclusion

The results of this study indicate that the IL-18 can be an independent risk factor of atherosclerosis.

The study of Stefan Blankenberg, et al. 2003,<sup>5</sup> which measured the level of IL-18 in 10,600 healthy European men between 50 to 59 years old and followed them up for 5 years indicated that the IL-18 can be an independent predictor factor for next coronary events in European men. Also previous review of these researchers (2002) showed that the IL-18 is a strong cardiovascular death predictor in stable and unstable angina.

Sims & Smiths 2010,<sup>14</sup> by reviewing the results of many studies about the relation between IL-18 and atherosclerosis, have announced that IL-18 and its receptor which expresses in atheroma and serum concentration of IL-18 is increased in patients with acute coronary events and IL-18 is an independent predictor factor for death in patients with CAD. Another study done by Tenger et. al ,2005,<sup>15</sup> on knockout mice showed that IL-18 can promote secretion of IFN  $\gamma$  by NK cells, macrophages and vascular cells and this stimulation is enough to development of atherosclerosis and this effect (pro-atherogenic) is T lymphocytes independent. In research done by Rafaelel Rosso, et al. 2005,<sup>16</sup> serum level of IL-18 in patients with stable and unstable angina were measured and compared with normal people, in both groups of patients, IL-18 were significantly higher than control group. Also, results of various studies about the relation between this cytokine and atherosclerosis in human and mouse, like Suchanek. H, et al. 2005,<sup>17</sup> and Martins.TB, et al. 2006, studies<sup>18</sup> and research done by Pan Yuna et

al. 2008,<sup>19</sup> indicated that there is direct relation between IL-18 and atherosclerosis. Also IL-18 is an independent predictor risk factor for CAD. In addition, in some studies such as Pan Yuna study similar to our study, a direct relation between IL-18 and CAD severity was observed. If the supplementary studies will have similar results, measurement of the IL-18 level can be used as an independent predictor risk factor of atherosclerosis.

The average blood level of the CRP mgr/lit	20.4	8.9
The average blood level of IL-18 pgr/ml	258.1	216.6

**Acknowledgement-** We express our appreciation to Sahand Afshan Teb Company for providing the all kits of this study.

**Table I. Known atherosclerosis risk factors and concentration of CRP and IL-18 in blood in both Case and Control group**

	Sample group (106 persons)	Control group (32 persons)
The age mean	56.7 ± 10.6	54.5 ± 9.7
Female	%40.6	%46.8
Male	%59.4	%53.2
Cholesterol above 200mgr/dl	%29.3	%21.4
DHL Less than 35 mgr/dl	%31.1	%23.3
Hypertension	%34.7	%22.9
Smoking	%28.2	%20.3
Diabetes	%19.4	%13.5
Family history	%19.8	%14.6

**Table II. The relation between IL-18 and severity of atherosclerosis**

CAD	Number of patients	Percentage of patients	Average of IL-18 pgr/ml
Minimal CAD	6	%5/6	235.5
SVD	44	%41.5	247.2
2VD	27	%25.4	263.7
3VD	29	%27.5	274.1

### References

- 1- Ross R. Atherosclerosis: An inflammatory disease. *N Engl J Med.* 1999; 340: 115-126.
- 2- Libby P, Ridker PM, Maseri A. inflammation and atherosclerosis. *Circulation.* 2002; 105: 1135-43
- 3- Abul k Abbas, Andrew H Lichtman, Shiv PillaiGth, Cellular and molecular immunology 6<sup>th</sup> edition. philadelphia: Saunders Elsevier; 2007: 267-301
- 4- Gracie JA, Robertson SE, McInnes IB. Interleukin-18. *J Leukoc Biol* 2003; 73: 213-24
- 5- Stefan Blomkenberg, Gerald Luc, Pierre Ducimetiere, Dominique Arveiler, Jean Ferrieres, Philippe Amouyel, et.al. IL-18 and the risk of coronary heart disease in European men. *Circulation* 2003; 108: 2453-59
- 6- Ziad Mallat MD.PhD, Anne Corbaz Bsc, Alexandra Scoazes Bsc.Sandrine Besnard PhD, Guy Leseche MD,Yolande Chvatchko PhD, et.al. Expression of IL-18 in human atherosclerosis plaques and relation to plaques instability. *Circulation* 2001; 104: 1598-1603.
- 7- HeikoMuhl, Josef Pfeilschifter. IL-18 Bioactivity: a novel target for immunopharmacological anti-inflammatory intervention. *European journal of pharmacology.* 2004; 500: 63-71
- 8- Charles A Dinarello. IL-18 and the pathogenesisof inflammatory disease. *Seminars in nephrology* 2007 ;Vol. 27; No 1: 98-114
- 9- Paul E Szmitko Bsc, Chao-Hung Wong MD, Richard D. Weisel,John R de Almeida Bsc, Todd J Anderson MD,Subodh Verma PhD. New markers of inflammation and endothelial cell activation. *Circulation.* 2003; 108: 1917-23
- 10- Bysani Chandrasekar, Anthony J Valent, Gregory L Freeman. Lenin Mahimainathan, Srinivas Mummidi. IL-18 induces human cardiac endothelial cell death. *Biochemical and biophysical research communications.* 2006;339:956-63
- 11- Joseph Hung, Brendan M Mcquellan,, Caroline M.L. Chapman, Peter L Thompson, John P Beilby. Elevated interleukin 18 levels are associated with the metabolic syndrome. *A artheroscler thromb vasc boil* 2005;25: 1268-73
- 12- Meijing Wong, Troy A Markel, Daniel R Meldrum Proinflammatory IL-18 in the heart. *Shock* 2008( Jul); 30(1): 3-10
- 13- Gardes, N., Sukhova, G.K., Libby, R., Young, J.L., Schonbeck, U.. Expression of interleukinh 18 and functional IL-18 receptor on human vascular endothelial cells, smooth muscle cells and macrophages: implications for atherogenesis. *J. Exp. Med.* 2002;195: 245-257
- 14- Johne. Sims, Dirke. Smiths. IL-1 family: regulators of immunity. *Nature reviews immunology.* Feb 2010; 10: 89- 102
- 15- Tenger C, Sundberger A, Jawienj, Zhoux. IL-18 accelarates atherosclerosis accompaind by elevation of IFN- $\gamma$  CXCL 16 expression independently of T cells,

atherioscler thromb Vas boil 2005; 25(4): 791-6

- 16- Rafael Ross, Arie Roth, Ilzhak Herz. Serum level of IL-18 in patients with stable and unstable angina pectoris. International journal of cardiology 2005; 98: 45-48
- 17- Suchanek H, Mysliwska J, Siebert J, Wieckiewicz J, Hak L, Szyndler K. High serum IL-18 concentrations in patients with CAD and type 2 diabetes mellitus. Euro cytokine Netw sep 2005; 16 (3): 174-85
- 18- Martins TB, Anderson JL, Muhlestein JB. Risk factor analysis of plasma cytokines in patients with CAD. Am J Clin Path. 2006 Jun; 125 (6):906-13
- 19- Pan Yuna, Tang Ren-Guanga, Huang Zhao-Heb. Clinical significance of serum level of IL-18 in patients with CAD. Journal of modern laboratory medicine 2008;03:39-49

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