



The Role of TGF-beta1-a Treg Related Cytokine- in Atherosclerosis and Acute Coronary Syndrome

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Abstract

Background: The effect of transforming growth factor beta1 (TGF- 1) on the cardiovascular system is ambiguous. Many studies have shown that TGF- 1 may actually play a protective role in atherosclerosis. In other studies, TGF- 1 appears to be atherogenic. **Aim of study:** To detect the changes in transforming growth factor beta1 (TGF- 1) in patients with acute coronary syndrome in comparison to atherosclerotic patients with no previous vasculo-occlusive disorders and healthy controls. **Subjects and methods:** Three groups of patients were included: Twenty hypertensive atherosclerotic patients diagnosed as having acute coronary syndrome (ACS group). Twenty hypertensive atherosclerotic patients, with no previous vasculo-occlusive disorders (atherosclerotic hypertensive group). Ten healthy controls with no history of chronic illness or evidence of ongoing infection were included. Serum levels of TGF- 1 were measured in all subjects by ELISA. **Results:** The plasma level TGF- 1 was higher in the ACS group, in comparison to the atherosclerotic hypertensive group and was higher in the atherosclerotic hypertensive group, in comparison to the healthy controls (p value < 0.001). **Conclusion:** These findings support the suggested pro-atherosclerotic role of TGF- 1.

Keywords: Acute coronary syndrome, Atherosclerosis, TGF- 1, T regulatory cells.

Introduction

Acute coronary syndrome (ACS) is a spectrum of clinical presentations ranging from those with ST-segment elevation myocardial infarction (STEMI) to presentations of non-ST-segment elevation myocardial infarction (NSTEMI) or of unstable angina (Coven et al., 2011).

Atherosclerosis is a multifactorial disorder. Immune mechanisms appear to play an important role in atherosclerosis. Observing T cells and antibodies at different stages of advancing atheromatous plaques made this finding evident (De Boer et al., 1999).

T cell mediates inflammatory process which determines the growth and development of atherosclerotic lesions. Although the majority of the inflammatory cells in atherosclerotic lesions are macrophages, T lymphocytes represent up to 20 percent of the cells. Moreover, activation of T cells is

considered to play a vital role in destabilization of atherosclerotic plaque which may initiate plaque disruption and the onset of acute coronary syndrome (Mor et al., 2006).

In general, the regulatory T cells (Treg), a special subset of T cells, tightly control the effector functions of activated T cells. Treg play a central role in inducing and maintaining immunologic tolerance and the termination of immune responses. Lack or dysfunction of these cells lead to autoimmunity or aggravated pathogen-induced inflammation (De Boer et al., 1999).

In tissues these cells are best marked by their expression of transcription factor FOXP3 (Forkhead Box Protein P3), a member of the forkhead winged helix protein family of transcription factors (Mor et al., 2006).

Transforming growth factor 1 (TGF- 1) and IL-10 are known to be the Treg related cytokines (Cheng et al., 2008).

Transforming growth factor (TGF-) is a cytokine engaged in a wide range of different and often contradictory functions. Its effect on the cardiovascular system is also vague; on the one hand, there is a strong evidence for so-called 'protective cytokine hypothesis' considering TGF- to be an anti-atherogenic and plaque-stabilizing factor, but on the opposite side, TGF- has been proven to exert some proinflammatory effects (Dabek et al., 2006)a.

Aim of the Study

Our study aimed to detect the changes in transforming growth factor beta1 (TGF- 1) in patients with acute coronary syndrome in comparison to atherosclerotic patients with no previous vasculo-occlusive disorders and healthy controls.

Subjects and Methods

This study was performed as a case-control study during the period from January 2012 till June 2012. The study included 20 patients diagnosed with acute coronary (10 patients with myocardial infarction confirmed by significant rise of troponin I and creatine kinase MB levels and 10 patients with unstable angina, inclusion criteria: chest pain at rest with definite ischemic electrocardiographic changes: ST-segment changes and/or T-wave inversions), 20 hypertensive atherosclerotic patients, and 10 healthy individuals as the control group. Acute coronary syndrome patients were recruited from the coronary care unit (CCU) at Ain Shams University Hospital, whereas atherosclerotic patients were recruited from the outpatient clinics in Ain Shams University Hospital. Hypertension was defined by a previous medical diagnosis of hypertension or use of anti-hypertensive medications. Patients with any evidence of recent infection were excluded from the study, including those with urinary tract symptoms, productive cough, pleuritic pain, dyspnea, tachypnea, fever, or evidence of infection by urinalysis. Further exclusion criteria included patients with diabetes mellitus, body system failure (liver cell failure, renal failure, etc...), autoimmune diseases, or patients receiving steroids or cytotoxic drugs within the previous month before collecting samples.

Methods

Carotid duplex

Atherosclerosis was diagnosed by bilateral carotid duplex.

Transforming growth factor 1 levels in serum

Transforming growth factor 1 (TGF- 1) levels in serum from all patients and controls were measured as a marker for Treg cell activity. TGF- 1 was measured using an enzyme-linked immunosorbent assay. The blood samples were obtained from patients 5–24 h after the onset of chest pain in the acute coronary syndrome group. (Dabek et al., 2006)b. The procedures were done according to the manufacturer's instructions (RayBio®, USA).

Statistical analysis

Statistical analysis was done on a personal computer using the IBM® SPSS® Statistics version 20 (IBM® Corporation, Armonk, NY, USA).

Categorical data were presented as number (%). Association between ordinal and nominal variables was tested using the chi square test for linear by linear association. For comparison of nominal data among groups, the Pearson chi square test was used.

Non-normally distributed numerical data were presented as median (interquartile range) and intergroup differences were compared non-parametrically using the KruskalWallis test with application of the Mann-Whitney U-test whenever a statistically significant difference was detected with the Kruskal Wallis test.

Correlation between numerical variables was tested non-parametrically using Spearman's rank correlation. The correlation coefficient (rho) was interpreted as follows: rho < 0.2, no correlation; rho = 0.2 – 0.39, mild correlation; rho = 0.4 – 0.69, moderate correlation; and rho = 0.7 – 1.0, strong correlation. All reported P values are two tailed. P < 0.05 is considered statistically significant.

Results

This study was carried out on 40 hypertensive atherosclerotic patients (20 patients with acute coronary syndrome and 20 patients with no previous ischemic insult), and 10 totally healthy individuals as a

control group. Those patients were selected from Ain Shams University hospitals. Age and sex

characteristics of the patient groups are shown in table 1.

Table 1. Characteristics of the patient groups

Variable	ACS group (n=20)	Hypertension atherosclerotic group (n=20)	Control group (n=10)	P value
Age				0.688
40-44 yr	5 (25%)	3 (15%)	1 (10%)	
45-49 yr	0	1 (5%)	1 (10%)	
50-54 yr	3 (15%)	4 (20%)	2 (20%)	
55-60 yr	12 (60%)	12 (60%)	6 (60%)	
Gender				0.813
Male	13 (65%)	13 (65%)	7 (70%)	
Female	7 (35%)	7 (35%)	3 (30%)	

Transforming growth factor 1 levels among the three study groups:

Table 2. Comparison of TGF- 1 levels in the three study groups

TGF- 1 (ng/ml)	ACS group (n=20)	Hypertension atherosclerotic group (n=20)	Control group (n=10)	P value*
Median	29.8†	23.6†	3.7	< 0.001
Interquartile range	19.9 – 48.8	19.9 – 33.6	2.4 – 6.2	

*P value estimated with the Kruskal Wallis test.

†P < 0.001 versus control group (estimated with the Mann-Whitney U test).

There was a higher mean TGF- 1 among ACS patients compared to other groups. There was a higher mean TGF- 1 among hypertensive atherosclerotic patients compared to healthy individuals. The differences were statistically significant.

The correlation between lipids (triglycerides level and cholesterol level) and TGF- 1 level in the ACS group showed no statistical significant difference with

correlation coefficient (Spearman’s rho) -0.062 and 0.014 respectively.

The correlation between the TGF- 1 levels and the cardiac enzymes CK total and CK MB levels showed no statistical significant difference with correlation coefficient (Spearman’s rho) 0.190 and 0.200 respectively

Table 3. Relation between TGF- 1 levels and carotid duplex findings in the ACS and hypertension groups

ACS group (n=20)	TGF- 1 (ng/ml)	Carotid duplex (n=20)			P value
		A (n=5, 25%)	AP (n=11, 55%)	APS (n=4, 20%)	
	Median	33.8	44.9	22.2	0.120
	Interquartile range	16.5–61.8	26.5–52.0	4.8–25.8	
Hypertension atherosclerotic group (n=20)	TGF- 1 (ng/ml)	Carotid duplex (n=20)			P value
		A (n=4, 20%)	AP (n=11, 55%)	APS (n=5, 25%)	
		Median	20.6	22.5	
	Interquartile range	18.3–28.3	21–34	20.8–56.3	

A, atherosclerosis; AP, atherosclerosis plus plaque; APS, atherosclerosis plus plaque plus stenosis

There was a higher mean TGF- 1 level among the patients with atherosclerosis complicated by plaques among ACS group other than the remaining ACS patients with atherosclerosis only or associated with stenosis as demonstrated by carotid duplex.

There was a higher mean TGF- 1 level among the patients with atherosclerosis complicated by plaques and stenosis among hypertensive atherosclerotic group other than the remaining hypertensive atherosclerotic patients with atherosclerosis only or complicated by plaques as demonstrated by carotid duplex. The differences were not statistically significant.

Discussion

In Egypt, cardiovascular disease has emerged as the leading cause of death, and women are affected in large numbers. As was pointed by **Butala et al., 2011**, where females constitute 21% of patients with Acute myocardial Infarction (AMI) in Egypt which demonstrated lower percentage than reported in developed countries all over the world, but is comparable to the percentage of patients with AMI who were females in a study conducted in the Gulf region. Although women have a lower incidence of ACS and atherosclerosis than men, they have a relatively higher morbidity and mortality (**Thom et al., 2006**).

In our study, the groups were comparable as regards the gender with no statistically significant difference among acute coronary syndrome, hypertensive atherosclerotic patients and healthy individuals. The male patients were more prevalent than the female patients.

TGF- 1 level in serum, as one of the markers of T regulatory cells activity, was measured in all groups via ELISA technique representing one of the few studies on human subjects in contrast to the many studies performed on non-human subjects.

Our study revealed increased serum levels of TGF- 1 in the acute coronary syndrome and the atherosclerotic hypertensive groups compared to the control group.

On one hand, in consistent with our results, **Xu et al., 2010** pointed the up-regulation of TGF- 1 expression in plaque development and this appears to be atherogenic under a variety of circumstances, especially related to matrix remodeling.

Also consistent with our results, **Buday et al., 2010** demonstrated that elevated circulating TGF- 1 causes endothelial dysfunction through the nicotinamide adenine dinucleotide phosphate-oxidase NADPH oxidase system activation-induced oxidative stress, ameliorating atherosclerosis and hypertension in apolipoprotein E-deficient mice. These findings may explain accelerated atherosclerosis in patients with elevated plasma TGF- 1.

Anger et al., 2009 also showed increased TGF- 1 gene expression in severe calcified and stenotic human aortic valves, and **Piao and Tokunaga, 2006** observed an elevated expression of TGF- 1 in atherosclerotic lesions in human aorta autopsies, also, an especially strong over-expression and higher expression rate in the advanced lesions, indicating that TGF- 1 participates in the formation of early lesions and progression of the advanced lesions.

Lijnen et al., 2003 pointed that in essential hypertensive patients, TGF- 1 protein as well as TGF- 1 mRNA levels are hyperexpressed. This can be attributed to various factors such as elevated angiotensin II, increased systemic blood pressure per se, increased fluid shear stress and a differential expression of TGF- 1 linked to DNA polymorphism in the promoter. Hypertensive patients with microalbuminuria and left ventricle hypertrophy have a higher plasma TGF- 1 concentration.

On the other hand, many studies have shown a possible protective role played by TGF- 1 in atherosclerosis especially in stabilized plaques. Inhibition of TGF- 1 signaling using a neutralizing anti-TGF- 1, - 2, and - 3 antibody in ApoE-deficient mice increased the development of atherosclerotic lesions with increased inflammatory component and decreased collagen content (**Xu et al., 2010**).

Wang et al., 2015 stated in their recent study that nTreg have an atheroprotective effect in atherosclerosis. Therefore, expanding the population of nTregs in atherosclerotic or CHF patients might prove to be an effective therapeutic strategy.

Also, in contrast to our study, **Robertson et al., 2003**, in murine models of atherosclerosis; blocking TGF- 1 activation through expression of apolipoprotein, blocking systemic TGF- 1 signaling through expression of soluble T RII or neutralizing TGF- 1 antibodies, or decreasing circulating levels of TGF- 1 by targeted deletion of one allele is sufficient to enhance atherosclerosis.

Also, using Tamoxifen to increase TGF- 1 levels indirectly is sufficient to decrease atherosclerosis. TGF- 1 appears to stabilize atherosclerotic plaques by stimulating collagen secretion and promoting the formation of a collagen-rich fibrous cap (**Robertson et al., 2003**).

Hering et al., 2002 also showed that the serum concentration of active TGF- 1 is severely decreased in advanced atherosclerosis. The low levels of TGF- 1 are likely to lead to an exaggerated inflammatory response given its potent anti-inflammatory role, and **Stefoni et al., 2002** pointed that low blood level of active TGF- 1 associates with the severity of atherosclerosis, suggesting a protective role of TGF- 1 against atherosclerosis in humans.

Frutkin et al., 2009 pointed that TGF- 1 is an anti-atherogenic, vasculoprotective cytokine that limits atherosclerosis and prevents aortic dilation when it is over expressed in the heart and plasma.

Instead of either completely pro-atherogenic or antiatherogenic, TGF- 1 may have bifunctional roles in atherosclerosis. It is possible that TGF- 1 might play a protective role in earlier stages of atherosclerosis, but such an effect is lost in advanced stages of the lesion due to changed receptor expression (**Xu et al., 2010**).

Vascular smooth muscle cell (VSMCs) in early atherosclerotic lesions showed an increased expression of TGF- 1, TGF- 3, TGF- RI, and TGF- RII, the level of which was significantly diminished in advanced lesions. In fact, it was found that VSMCs from diseased vessels had less TGF- RII expression and therefore were less responsive to TGF- 1 and resistant to the antiproliferative effect of TGF- 1. The receptor variance might be one of the reasons behind the opposing effects of TGF- 1 on atherosclerosis (**Xu et al., 2010**).

The difference between our results and many studies which stated TGF- 1 protective role could be also attributed to other factors as our patients were hypertensive, hyperlipidaemic and many patients were on statins therapy at the time of the study.

Meng et al., 2012 showed that mRNA and protein levels of TGF- 1 and IL-10 were significantly increased in atherosclerotic plaques in ApoE mice after 6 wks of treatment with simvastatin. Therefore, the accumulation of Tregs by statins treatment in atherosclerotic plaques was correlated with the

increase of TGF- 1 and IL-10, and, decreased the levels of IL-1, IL-17 and IFN- in carotid plaques.

Simvastatin enhanced an accumulation of Tregs, subsequently decreased Th1 and Th17 response and modulated the Th1/Th2 balance toward a Th2 phenotype in the atherosclerotic plaques, and this result may be an additional mechanism of simvastatin in stabilizing vulnerable plaques (**Meng et al., 2012**).

Wang et al., 2015 concluded from their study that the inhibitory effects of atorvastatin on inflammation in ACS may be due to its positive effects on nTregs and restoration of immune homeostasis.

Our study showed that there was a higher mean TGF- 1 among ACS patients compared to other groups.

In consistent with our previous results, **Schaan et al., 2007** showed that TGF- 1 could promote atherogenesis by mediating excessive accumulation of extracellular matrix and by down-regulating thrombomodulin, promoting thrombogenesis at the sites of vessel wall injury, where it is released from platelets, smooth muscle cells and monocytes resulting in increased levels of active TGF- 1 levels in ACS.

Chen et al., 2014 stated in their study that serum TGF- 1, measured by ELISA, is closely associated with coronary artery disease (CAD). The underlying mechanism may be due to its regulatory effects on atherosclerosis and blood sugar. They showed that TGF- 1 is closely related to uric acid, which is considered to be marker of atherosclerosis, indicating that this cytokine may contribute to the establishment of CAD by regulating atherogenesis. They thought about TGF- 1 as a useful biomarker for diagnosis and risk stratification.

Favorably, **Kulach et al., 2010** showed high gene expression of TGF- 1 in all clinical forms of ACS and **Jin et al., 2011** showed that gene expression profile of TGF- 1 increased post-MI, peaked at day 2, and decreased to normal levels after day 7 in mice post-MI. Increased TGF- 1 levels at the early stage (day 3 post-MI) led to increased macrophage density and metalloproteinase-9 (MMP-9 levels), decreased fibroblast secretion of collagen and collagen deposition, and thereby, prolonged the progression of remodeling and also it has been shown that high TGF- 1 levels delays wound healing post-MI .

On the other hand, several studies demonstrated that patients with ACS have less peripheral Treg numbers, determined as either CD4+CD25+Foxp3+ or CD4+TGF- β 1+Th3-cells. In addition, functional properties of Treg appear to be compromised in ACS (Ji et al., 2009).

Zhu et al., 2014 found that serum TGF- β 1 levels were reduced in ACS patients compared with those of chronic stable angina (CSA) and chest pain syndrome (CPS) patients.

Ji et al., 2009 results revealed a significant decrease in peripheral Th3 number and levels of TGF- β 1 in patients with ACS including unstable angina and myocardial infarction as compared with those in patients with stable angina and chest pain syndrome, indicating that down regulation of Th3 cells in patients with ACS may play an important role in plaque destabilization and the occurrence of ACS.

Cheng et al., 2008 also demonstrated in the results of his study that patients with ACS revealed significant increase in number of peripheral Th17, and their related cytokines (IL-17, IL-6 and IL-23) and obvious decrease in Treg number, Treg related cytokines (IL-10 and TGF- β 1) and transcription factor (Foxp3) levels in comparison to patients with stable angina and normal coronary artery subjects. Results above indicate that Th17/Treg functional imbalance exists in patients with ACS, suggesting an important role for Th17/Treg imbalance in plaque destabilization and the onset of ACS.

In the present study, we detected no significant statistical correlation between hyperlipidemia (increased serum cholesterol and triglycerides) and TGF- β 1 level in both groups; acute coronary syndrome group and atherosclerotic hypertensive group, most probably due to the limitation of the number of studied subjects.

Favourably, Frutkin et al., 2009 used apolipoprotein E-null mice with transgenes that allow regulated over expression of active TGF- β 1 in their hearts, in comparison to littermate controls, these mice had elevated cardiac and plasma TGF- β 1, less aortic root atherosclerosis, fewer lesions in the thoracic and abdominal aortae, less aortic root dilation, and fewer pseudoaneurysms. This study revealed that TGF- β 1 overexpression has no effect on plasma lipids or cytokines, or on peripheral lymphoid organ cells.

But on the other hand, Zhou et al., 2009 showed that in mice fed with high cholesterol diet and in C57BL/6J mice treated with poloxamer P-407, an agent that elevates plasma cholesterol and triglycerides, severe hypercholesterolemia led to an increase in circulating TGF- β 1 levels, elevated TGF- β 1(+)CD4(+) Th3 cells in lesions and spleen, and increased Th3 dependent IgG2b antibodies to oxLDL. They noticed a positive correlation between plasma TGF- β 1 and cholesterol levels and between plasma TGF- β 1 and IgG2b anti-oxLDL. That elevation of TGF- β 1 may increase the stability of plaques through inhibiting T cell responses and macrophage activation and by stimulating collagen synthesis.

Also, Zhou et al., 2009 showed in the results of their study that severe hypercholesterolemia leads to a switch from Th1 to Th2/Th3 immune effector functions with an increase of TGF- β 1 in the circulation, the spleen and the lesions. Also the study revealed that the elevation of TGF- β 1 is followed by elevated Th3-dependent B cell responses to oxidized LDL (oxLDL), and also that the high plasma TGF- β 1 is proportionally correlated to the level of plasma cholesterol and to the titers of the IgG2b against oxLDL.

Chen et al., 2011 investigated the effects of a high fat diet on TGF- β 1 responsiveness in aortic endothelium and integration of cholesterol in tissues and showed that normal mice fed a high fat diet for 24 weeks exhibit atherosclerotic lesions and suppressed TGF- β 1 responsiveness in aortic endothelium. These mice exhibit greatly high integration of cholesterol into tissue plasma membranes. The study suggested that suppressed TGF- β 1 responsiveness in aortic endothelium has been shown to play a vital role in the pathogenesis of atherosclerosis in animals with hypercholesterolemia.

Chen et al., 2008 pointed that by suppressing TGF- β 1 responsiveness; high plasma cholesterol levels may take part in the pathogenesis of certain diseases (e.g., atherosclerosis).

As regards the role of TGF- β 1 as a therapeutic target, Lutgens et al., 2002 pointed that TGF- β 1 may be used as a therapeutic target. On one hand, TGF- β 1 would induce plaques characterized by both high extracellular matrix content and a low inflammatory cell content, which may help to stabilize the plaque. On the other hand, the possible disadvantages of

administration of TGF- 1 may be possible systemic fibrotic effects and an increase in plaque size. Targeted administration of TGF- 1 may overcome the first problem.

In conclusion, some studies emphasize TGF- anti-inflammatory and anti-atherogenic role. TGF- affects macrophage and T-cell activation, as well as proliferation of smooth muscle cells in the vessel wall. A diminished production or activity of this cytokine is believed to destabilize the plaque. These, as well as numerous experimental data constitute the basis of David Grainger's TGF- protective cytokine hypothesis (Dabek et al., 2006)a, but in contrast our findings supported the suggested role of TGF- 1 in enhancing atherogenesis.

This should encourage more studies to understand TGF- 1 controversial role in atherosclerosis and acute coronary syndrome on larger groups of patients. This controversial role of TGF- 1 needs also to be further investigated as a therapeutic target for its atherogenic versus anti-atherogenic role.

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