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Original Research Article

A Case Control Study on the Association of Transforming Growth Factor-β with Atherosclerosis

Dr. Veena Juliette A[1], Dr. Ananthan V[2]
[1]Assistant Professor of Biochemistry, Madras Medical College, Chennai- 600 003
[2]Associate Professor of Biochemistry, Govt Theni Medical College, Theni- 625 512

***Corresponding author**
Dr. Ananthan V
Email: rhymesanand@gmail.com

Abstract: Transforming Growth factor (TGF)-β is a multifunctional cytokine which plays a pivotal role in the genesis and progression of atherosclerotic plaques, particularly influencing the differentiation and proliferation of vascular smooth muscle cells that help maintain the normal blood vessel wall architecture. This major immune modulator has been shown to be both atherogenic and atheroprotective in various studies and hence the present study aims to observe the association of TGF-β with atherosclerotic disorder in a representative south Indian population attending a tertiary care hospital. 50 cases of atherosclerotic peripheral vascular disease without any other apparent inflammatory disorder were selected and their blood levels of TGF-β and other lipid profile parameters were compared statistically with 50 age and sex matched healthy individuals using student's t test and Pearson's correlation analysis. Mean serum TGF-β was significantly lower ($p<0.01$) in cases than controls and had a strong positive correlation with serum HDL and negative correlation with serum Triglyceride. Thus it can be inferred from this study that TGF-β has a protective role in atherosclerosis and its level is affected by other pro and anti atherogenic factors.
Keywords: Transforming Growth factor –β, Atherosclerosis, Peripheral Vascular Disease

INTRODUCTION

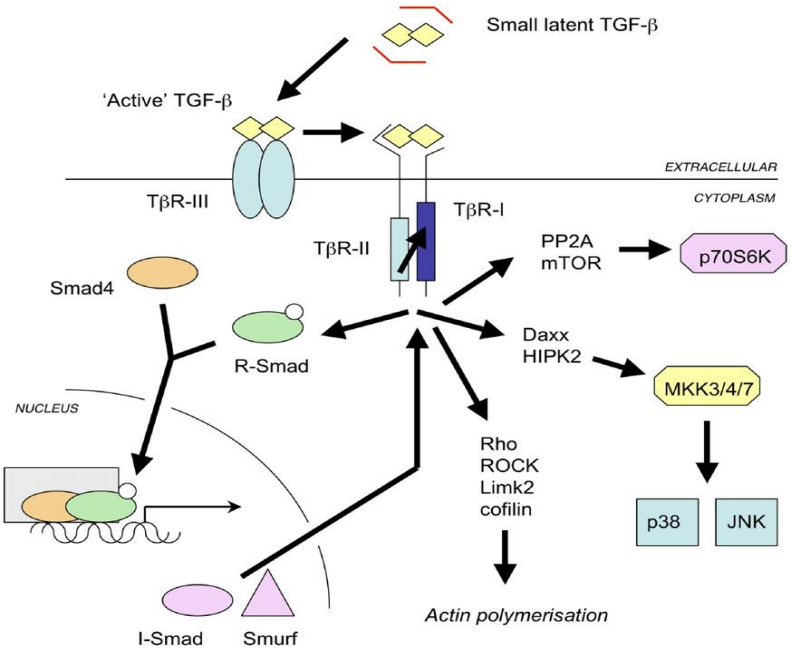
Transforming Growth factor type-β (TGF-β) is a superfamily of ligands, receptors, binding proteins and ligand traps that together play a key role in maintaining the normal vessel wall structure [1]. It consists of a large number of structurally related cytokines that help regulate a wide range of biological processes in humans from tissue differentiation in early development to innumerable immune functions in the adult organism. Members of the TGF-β superfamily are typically produced as inactive dimeric latent precursors and subsequently activated in the extracellular environment to release the receptor-binding ligands that share a common structural motif known as the cysteine knot [2]. It exists in three known subtypes in humans TGF-β1, TGF-β2 and TGF-β3. TGF-β1 is present in endothelial cells, vascular smooth muscle cells (VSMCs), myofibroblasts, macrophages, and other hematopoietic cells. It is recognized as the most pivotal TGF-β isoform for the cardiovascular system [3].

TGF-β is found to play a crucial role in the development and/or regression of malignant tumors, autoimmune diseases, organ fibrotic changes, kidney diseases and cardiovascular diseases, hypertension, inflammation, connective tissue diseases like Marfan syndrome, etc. Its vital functions include the regulation of cell cycle at G1 phase, regulation of the immune system through the Foxp3+ regulatory T cells, inhibition of the activation of lymphocytes and monocyte derived phagocytes [4, 5]. TGF-β1- deficient mice die in utero or in the perinatal period because of widespread uncontrolled inflammation [6]. Although it uses multiple pathways, the general signaling of the TGF-β1 ligand is through the SMAD pathway (fig.1); it also induces apoptosis through the DAXX pathway. The TGF-β receptors are single pass serine/threonine kinase receptors that exist in

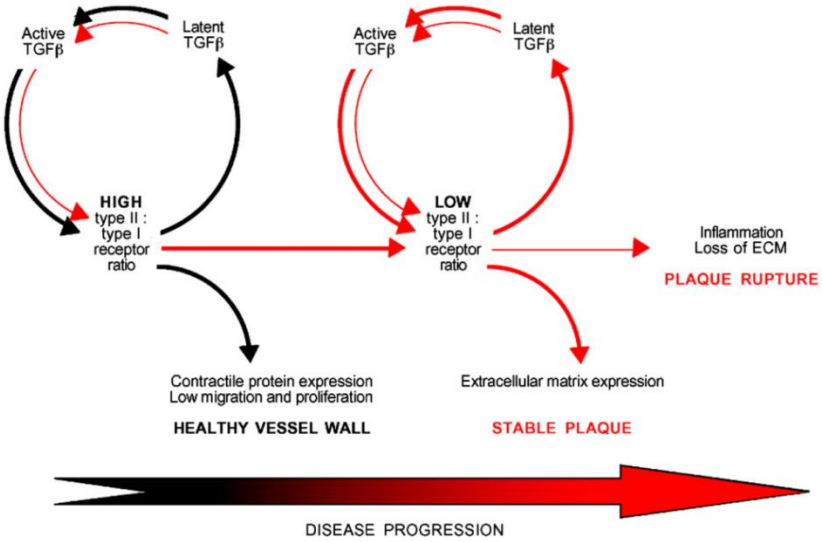
several different isoforms and can be homo or heteromeric [7].

Atherosclerosis is a chronic disease of the arterial wall where both innate and adaptive immunoinflammatory mechanisms are involved. It is a multifactorial disease, the cornerstone being dyslipidemia and oxidative stress. Inflammation is central to all stages of atherosclerosis. TGF-β is known to control cell proliferation, cell migration, matrix synthesis, wound contraction, calcification and the immune response, all being major components of the atherosclerotic process [8]. It is a major orchestrator of the fibroproliferative response to tissue damage. In the early stages of repair, TGF-β is released from platelets and activated from matrix reservoirs; it then stimulates the chemotaxis of repair cells, modulates immunity and inflammation and induces matrix production. At later stages, it negatively regulates fibrosis through its strong antiproliferative and apoptotic effects on fibrotic cells. In advanced lesions, TGF-β might be important in arterial calcification [9]. Selective defects in TGF-β signaling can disrupt otherwise coordinated pathways of tissue regeneration. A model for the action of TGF-β during atherogenesis is shown in fig.2 [2]. Several factors are known to affect the activation of TGF-β and hence its effects (fig.3).

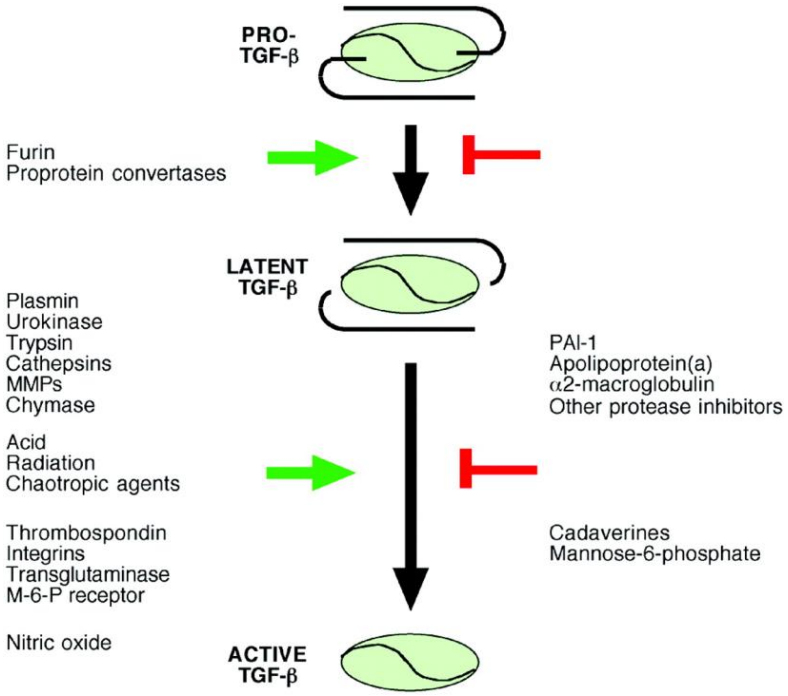
Though some studies in the past have shown TGF-β to be an atherogenic factor [10], suggesting that it could promote smooth muscle proliferation [11,12], cell culture studies showed that under majority of conditions it inhibits the same, hence giving rise to the protective cytokine hypothesis [13]. Extensive research work, including genetic work-up, has been done in various parts of the world to evaluate the role of TGF-β in atherosclerosis of the cardiovascular system, since it is a major health problem worldwide [14-17]. However, atherosclerosis of the peripheral vascular system contributes significantly to morbidity and mortality and yet remains an under diagnosed and under treated disease [18]. Risk factors for PVD include the traditional atherosclerotic risk factors and there is a considerable overlap between coronary and cerebro vascular diseases and PVD [19]. The association of TGF-β with peripheral vascular disease has not been proved decisively yet. Powerful molecule as it is, the presence or absence of it could be central to the evolution of the atherosclerotic plaque in the extremities. Hence this project was undertaken to study the association of TGF-β with atherosclerotic peripheral vascular disease.



Fig–1: Major TGF-β signaling pathways



Fig−2: Action of TGF-β on Vascular Smooth Muscle Cells during Atherogenesis



Fig−3: Factors affecting the activation of TGF-β1

MATERIALS AND METHODS

The study was conducted after obtaining approval from the Institutional Ethics Committee

STUDY POPULATION

CASES

50 patients of Peripheral Vascular Disease were recruited for the study. Diagnosis was made by measuring the ABPI- Ankle Brachial Pressure Index (cut-off value <0.9). Some of them had concomitant Coronary Artery Disease (CAD) and/or Cerebro Vascular disease (CVD). All patients with systemic hypertension, acute infection and inflammatory disorders, liver or renal disease, thrombotic tendencies and metastatic conditions were excluded. Peripheral vascular disease other than that due to atherosclerosis

was also excluded (eg. Thromboangitis obliterans, autoimmune diseases).

CONTROLS

50 apparently healthy individuals matched for age and sex were included. All of them were free from symptoms and signs of any kind of atherosclerotic vascular disease.

Sample collection and Processing

3 mL of blood was collected into a plain tube by Venipuncture after overnight fasting. The blood was allowed to clot and Serum was separated by centrifugation. 0.5 mL of serum was stored in eppendorf tubes at -20^{o} C for analysis of TGF β. The levels of total cholesterol, triglycerides and HDL cholesterol were measured in XL 300 fully automated analyzer by colorimetric methods using commercially available kits within 6 hrs of blood collection. LDL cholesterol level was calculated using Friedwald's formula. Estimation of TGF-β was done with DRG TGF-β1 ELISA kit on the Triturus automated ELISA machine using Enzyme Immunoassay methodology.

STATISTICAL ANALYSIS

The statistical software SPSS pc+ (Statistical Package for Social Science) was used for statistical analysis. Mean and Standard deviation were estimated from the sample each study group. The mean values were compared by student's t-test to calculate the p value. P value < 0.01 was considered significant. Thus increased BMI, total Cholesterol, Triglyceride, LDL Cholesterol, decreased HDL Cholesterol and decreased TGF- β were found to be significant contributing factors for the occurrence of Atherosclerotic Peripheral Vascular Disease. Using Pearson's Correlation analysis we observed a strong positive correlation between the levels of TGF-β and HDL-C, and a negative correlation between TGF-β and Triglyceride. Patients with multi – vessel disease (i.e) bilateral PVD, associated CAD/ CVD had significantly lower level of TGF-β than those with unilateral PVD.

Table−1: Comparison of parameters of Controls and of Patients with Atherosclerotic PVD

S.No	Particulars	Controls (n=50)	Cases (n=50)
1	Age in years	51.96	51.98#
2	Body Mass Index	23.37	25.69**
3	Total Cholesterol (mg/dL)	157.52	195.14**
4	Triglyceride (mg/dL)	113.20	190.06**
5	LDL (mg/dL)	90.10	119.68**
6	HDL (mg/dL)	44.59	37.47**
7	TGF-β (pg/mL)	467.78	182.82**

p value <0.0001 for TGF-β.

Table−2: Comparison of Levels of TGF-β between bilateral PVD & unilateral PVD patients

		Mean	Std. Deviation	p value
TGF-β	Bilateral PVD	166.9	50.3	0.001**
	Unilateral PVD	204.9	81.7	

Table−3: Comparison of Levels of TGF-β between CAD & Non CAD patients

	CAD	Mean	Std. Deviation	p value
TGF-β	Yes	137.4	24.28	0.0001**
	No	182.82	67.30	

Table−4: Comparison of Levels of TGF-β between CVA & Non CVA patients

	CVA	Mean	Std. Deviation	p value
TGF-β	Yes	140.8	34.39	0.0001**
	No	182.82	67.30	

Table−5: Correlation between other lipid parameters with TGF-β in controls and cases (Pearson Correlation)

S.No	Parameter	Controls	Cases
1	Total Cholesterol	0.089(0.536)	-0.141(0.327)
2	Triglyceride	0.031(0.827)	-0.477(0.0004) **
3	LDL	0.045(0.758)	-0.084 (0.563)
4	HDL	0.261 (0.139)	0.362(0.009)**

R value (p value)# Not significant; * significant (p<0.05); ** highly significant (p<0.01)

DISCUSSION

While the role of TGF- β in atherosclerosis has been studied extensively, there have been few studies relating it to atherosclerotic PVD. Since TGF-β has shown to be associated independently with hypertension and the pathogenesis of hypertension is both interlinked and many a times confounding with atherosclerosis, it was decided to exclude systemic hypertension from this study. While this study has proven beyond doubt that TGF-β is an anti atherogenic cytokine, it doesn't shed much light on the actual pathogenic mechanisms leading to its decrease of blood levels in atherosclerotic PVD patients due to the complexity of the web of interacting pathways controlling blood vessel wall structure. However, it has suggested that reduced TGF-β activity is a consequence of a range of environmental and genetic factors associated with the development of atherosclerosis. Is there a "safe" value or "cut-off" value for TGF- β below which a person is prone to develop atherosclerotic plaques? Does ethnicity have an effect on TGF- β levels? Research shows that activation of TGF-β and hence its blood levels can be decreased markedly by lipoprotein (a), a genetically determined cholesterol rich lipoprotein, high concentration of which has emerged as a prominent and independent risk factor for the development and progression of atherosclerosis [20]. Studies aimed at exploring the pathogenic relationship between lipoprotein (a) and TGF- β have to be conducted in different populations to identify novel pathways that may be manipulated for treating atherosclerotic patients. Further research works and clinical trials focused on identifying strategies to improve the TGF-β activity and develop TGF- β stimulating drugs can be performed. These are the future scopes for this study.

CONCLUSION

Transforming Growth Factor beta (TGF-β) plays a vital role in many of the normal physiologic and also pathologic events of the system. It is a potent anti-inflammatory, immunosuppressive and pro-fibrotic cytokine, with major effects on the biology of vascular smooth muscle cells. These properties of TGF-β are highly suggestive of a potential antiatherogenic role for this cytokine. Indeed, serum TGF-β is markedly depressed in patients with advanced and severe atherosclerosis. Its level in the blood is influenced by a wide variety of conditions and factors.

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