Transforming growth factor-ß/Smad signaling function in the aortopathies

Fator transformador de crescimento-ß/Smad como via de sinalização em aortopatias

Shi-Min Yuan¹, Jun Wang², Xiao-Nan Hu³, De-Min Li⁴, Hua Jing⁵

Abstract

Objective: Transforming growth factor (TGF-ß)/Smad signaling pathway in aortic dissection patients and normal subjects has not been previously described. The present study was designed to evaluate the TGF-ß/Smad signaling expressions in the patients with acute type A aortic dissection in comparison with those in the patients with thoracic aortic aneurysm and with coronary artery disease, and (or) the healthy subjects.

Methods: Consecutive surgical patients for acute type A aortic dissection (20 patients), aortic aneurysm (nine patients) or coronary artery disease (20 patients) were selected into this study. Blood samples (4 ml) were obtained from the right radial arterial indwelling catheter after systemic heparinization prior to the start of cardiopulmonary bypass in the operating room. Twenty-one young healthy volunteers without underlying health issues who donated forearm venous blood samples (4 ml) were taken as control. The surgical specimens of the aortic tissues were obtained immediately after they were severed during the operations of the replacement of the aorta in the patients with aortic dissection or aortic aneurysm. In patients receiving coronary artery bypass grafting, the tiny aortic tissues were taken when the punch holes of the proximal receiving coronary artery bypass grafting, the tiny aortic tissue was lower in the aortic dissection than in the aortic tissue of the aortic dissection patients and normal subjects has not been previously described. The present study was designed to evaluate the TGF-ß/Smad signaling expressions in the patients with acute type A aortic dissection in comparison with those in the patients with thoracic aortic aneurysm and with coronary artery disease, and (or) the healthy subjects.

Results: Quantitative real-time reverse transcription polymerase chain reaction showed that TGF-ß, mRNA were upregulated in all surgical groups (1.59 ± 0.33 vs. 1.45 ± 0.34 vs. 1.48 ± 0.48, P < 0.05). Western blot revealed that the expressions of TGF-ß, TGF-ß receptor I, Smad2/3, Smad4 and Smad7 were positive in the aortic tissues of all three investigated groups. Of the quantitative relative gray scales, a significant reverse correlation was noted between TGF-ß, and Smad2/3 (Y = -0.8552X + 1.6417, r = -0.759, P < 0.0001), and a close direct correlation between Smad4 and Smad7 (Y = 0.5905X + 0.2805, r = 0.781, P < 0.0001) in the Aortic Dissection Group. In the Aortic Aneurysm Group, Smad4 and Smad7 were also closely correlated (Y = 0.5228X + 0.1642, r = 0.727, P = 0.026), and in the Coronary Artery Disease Group, TGF-ß, and Smad7 were much significantly correlated (Y = 0.5301X + 0.5758, r = 0.917, P < 0.004). By enzyme-linked immunosorbent assay, TGF-ß, level of the aortic tissue was lower in the aortic dissection than in the aortic aneurysm and coronary artery disease groups with no statistical significance (319.52 ± 129.21 pg/mg protein vs. 324.09 ± 49.70 pg/mg protein vs. 304.15 ± 29.39 pg/mg protein, P > 0.05). The plasma TGF-ß, levels were 1158.30 ± 11.54 pg/ml, 1170.27 ± 8.26 pg/ml, 1225.00 ± 174.42 pg/mL and 1160.25 1170.27 ± 8.26 pg/ml. In particular, the linear correlations of the relative grayscales between different proteins of each group, and those correlations between the quantitative TGF-ß, by enzyme-linked immunosorbent assay and the time interval from the onset to surgery or the maximal dimensions of the aorta of the aortic dissection group were assessed.

Conclusions: The TGF-ß/Smad signaling pathway is significantly upregulated in acute type A aortic dissection patients compared with the coronary artery disease patients, and the aortic tissue of the aortic dissection patients and normal subjects has not been previously described. The present study was designed to evaluate the TGF-ß/Smad signaling expressions in the patients with acute type A aortic dissection in comparison with those in the patients with thoracic aortic aneurysm and with coronary artery disease, and (or) the healthy subjects.
INTRODUCTION

The transforming growth factor (TGF)-β family, including TGF-β1, TGF-β2, and TGF-β3, is a group of pleiotropic secreted cytokines with a broad spectrum of biologic functions. Of them, TGF-β1 is a secreted protein with many cellular functions, including cell growth, cell proliferation, cell differentiation and apoptosis. In humans, TGF-β1 is encoded by the TGF-β1 gene, either stimulating or inhibiting cell growth depending upon the cellular context [1]. TGF-β1 can modulate cell differentiation and proliferation in an auto- or paracrine manner [2]. In vascular smooth
muscle cells, TGF-β may upregulate fibronectin and connective tissue growth factor expressions via activation of Smads, and thus promote the deposit of extracellular matrix [3]. The receptors including TGF-β receptor (TßR) I and TßRII are glycoproteins of 55 kDa and 70 kDa, respectively, with core polypeptides of 500-570 amino acids [4]. Smads are molecules of 42-60 kDa, with two homology domains at the amino and carboxy terminals termed as terminal Mad-homology domains MH1 and MH2 [5]. Smads can be divided into three classes, receptor-regulated Smads (R-Smads), co-mediator Smads (Co-Smads) and inhibitory Smads (I-Smads). R-Smads are directly phosphorylated and activated by TßRI kinases. Smad2 and Smad3 are involved in TGF-β signaling transduction and Smad1, Smad5 and Smad8 in bone morphogenenic protein signaling transduction [6]. Smad4 was termed as DPC4 (deleted in pancreatic carcinoma locus 4), which was a candidate tumor suppressor gene in chromosome 18q21 frequently subjected to mutation or deletion in pancreatic cancer [7]. Smad2/3 and Smad4 are just the factors of the signaling pathway favoring the deposit of extracellular matrix mediated by TGF-β [3]. Smad6 and Smad7 inhibit TGF-β signaling as negative regulators [6].

Elevated TGF-β1 mRNA was noted in alveolar macrophages of lung tissue from patients with idiopathic pulmonary fibrosis [8], in the hepatic tissue of experimental alcoholic hepatic disease [9], and in the kidney of chronic allograft nephropathy characterized by fibrosis [10]. Many human malignancies including ovarian cancer [11], hepatocellular carcinoma and prostate cancer [12], were associated with overexpressions of TGF-β1 mRNA and protein, showing close relations to the progress of the disease [11]. Experiments on mammary cancer demonstrated absence of TGF-β1 reactivities resulted from TßR II or Smad4 generic products [13]. Studies have suggested that colon cancer might be associated with mutations of TßRII, Smad2 or Smad4 resulting in a poor response to TGF-β stimulus [5].

Aortopathies including aneurysm, dissection, and rupture of the aorta, is a pathological process incorporating vascular damage, repair and remodeling [14,15]. This complex process may incorporate enhanced TGF-β signaling function and damaged TGF-β receptors [4]. In either nontransmural infarct rat model [16] or myocardial infarct patients [17], TGF-β1 mRNA expressions were increased by 2-4 folds 2-10 days after infarction. In the atherosclerotic lesions, TGF-β was taken as a vascular protecting agent, while TßRs might be adverse factors in angioplasty as it has been observed that TGF-β increased 10 folds and TßRII increased 3 folds within 24 hours following vascular damage, and activin receptor-like kinase 5 increased twice 8 hours after arterial damage [18]. Even though TGF-β signaling in thoracic aortic aneurysm of different etiologies (Marfan’s syndrome, bicuspid aortic valve, or degenerative) has been sufficiently investigated [14,19,20], however, the TGF-β/Smad signaling pathway in aortic dissection has not been previously described, and moreover the exact mechanisms of TGF-β/Smad signaling responsible for the development of these aortic disorders still remain uncertain [5]. The present study was designed to evaluate the TGF-β1 signaling function of aortic dissection in comparison to aortic aneurysm, coronary artery disease, and healthy individuals by way of biomolecular studies.

METHODS

Patients and sampling
From October 2008 to March 2010, consecutive surgical patients for acute type A aortic dissection (20 patients), aortic aneurysm (nine patients) or coronary artery disease (20 patients) who had blood samples and/or surgical specimens of the aortic tissues available were selected randomly into this study, while the Marfan patients were excluded. The surgical patients were comparable in terms of their age and gender. Blood samples (4 ml) were obtained from the right radial arterial indwelling catheter after systemic heparinization prior to the start of cardiopulmonary bypass in the operating room. Twenty-one young healthy volunteers without underlying health issues donated forearm venous blood (4 ml) as control samples. Blood samples were centrifugated at 3000 × g for 5 min, and plasma was collected and stored at -80°C until detection. The surgical specimens of the aortic tissues were obtained immediately after they were severed during the operations of the replacement of the aorta in the patients with aortic dissection or aortic aneurysm. In patients receiving coronary artery bypass grafting, the tiny aortic tissues 0.2–0.4 cm in size were taken when the punch holes of the proximal anastomosis on the anterior wall of the ascending aorta were made. The aortic tissues were stored at -80°C, and were thawed for RNA, protein, or supernatant preparations until detection of TGF-β1 mRNA by quantitative real-time reverse transcription polymerase chain reaction (RT-PCR), of TGF-β1, TßRI, Smad2/3, Smad4 and Smad7 by Western blot, and of TGF-β1 by enzyme-linked immunosorbert assay (ELISA), respectively. The patients’ demographics were listed in Table 1.

RT-PCR
RNA samples were treated with DNase I to remove genomic DNA contamination before reverse transcription processing. A total of 2-5 µg of RNA from each sample was reverse transcribed into cDNA using the SuperScript™ III first-strand synthesis system (Invitrogen) according to the manufacturer’s suggested protocol. Quantitative RT-PCR
reactions were designed and prepared with a KeyGen reaction kit in a final volume of 20 µl containing 1 µl of reverse-transcribed total RNA, 2 µl of primers, and 10 µl of KeyGen Real-time PCR Master Mix (SYBR Green) (KeyGEN Bio, Nanjing, China). PCR reactions were carried out in capillaries in a DA7600 LightCycler instrument (Da An Gene Co., Ltd. of Sun Yat-sen University, Guangzhou, Guangdong, China) and were cycled 40 times. The primers of TGF-ß1 were designed and synthesized by KeyGEN Bio, Nanjing, China as sense 5’-CAAGCAGAGTAGTACACACAGCAT-3’ and antisense 5’-TGCTCCACTTTAACTTGAGCC-3’, along with those of the internal control GAPDH as sense 5’-GGAAAGTTGAGGTGGCGAGTCA-3’; and antisense 5’-GTCATTGATGCAACAATATCCACT-3’. The thermal cycling conditions consisted of a pre-incubation for 5 min at 95°C, followed by 40 cycles of denaturation for 15 s at 95°C, annealing for 30 s at 60°C and extension for 30 s at 72°C, and a final extension for 10 min at 72°C. All experiments were done in triplicate to verify the results. The relative expression of TGF-ß1 mRNA to GAPDH mRNA was calculated.

Western blot

Protein extracts (10 mg) of the aortic tissue were denatured in sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) loading buffer and separated by 12% SDS-PAGE. Proteins were transferred to a microporous polyvinylidene difluoride membrane (PVDF) membrane using an electroblotting apparatus and incubated for 1 h at room temperature with 0.5% bovine serum albumin. Membranes were stained with Ponceau S dye, to check for equal loading and homogeneous transfer. The following primary antibodies were utilized: TGF-ß1(Y369) (Bioworld Technology, Inc., Louis Park, MN, USA), TßRI (E161) (Bioworld Technology, Inc., Louis Park, MN, USA), Smad2/3 (S2) (Bioworld Technology, Inc., Louis Park, MN, USA), Smad4 (L43) (Bioworld Technology, Inc., Louis Park, MN, USA), Smad7 (M09) (Abgent Primary Antibody Company, 10239 Flanders Court, San Diego, CA 92121, USA). Filters were washed and developed using an enhanced chemiluminescence (ECL) system (Amersham Life Science). The optical densities were obtained by scanning densitometry, after normalization for nuclear or

<table>
<thead>
<tr>
<th>Variables</th>
<th>Aortic Dissection</th>
<th>Aortic Aneurysm</th>
<th>Coronary Artery Disease</th>
<th>Healthy Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case, n</td>
<td>20</td>
<td>9</td>
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<td>Female gender, n</td>
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<td>3</td>
<td>2</td>
<td>2 (plasma)</td>
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<td>Age, year</td>
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<td>46.20 ± 11.16</td>
<td>60.33 ± 4.87</td>
<td>28.17 ± 2.61</td>
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<td>Symptom, n</td>
<td>Chest pain (18), chest distress (2)</td>
<td>Chest pain (3), chest distress (2), palpitation (1), laryngeal discomfort (1), abdominal pain (1), asymptomatic (1)</td>
<td>Chest pain (21)</td>
<td></td>
</tr>
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<td>Hypertension, n</td>
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<td>6</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus, n</td>
<td>2</td>
<td>0</td>
<td>9</td>
<td></td>
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<tr>
<td>Renal failure</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
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<tr>
<td>Cardiovascular medication, n</td>
<td>12</td>
<td>6</td>
<td>18</td>
<td></td>
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<tr>
<td>Smoker, n</td>
<td>5</td>
<td>2</td>
<td>12</td>
<td></td>
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<tr>
<td>Operation, n</td>
<td>Replacement of the aorta (ascending/arch/descending) with/without aortic valve replacement/stent graft deployment (20)</td>
<td>Replacement of the ascending aorta (3), Replacement of the ascending aorta and aortic valve (2), aortic arch replacement (1), thoracic and abdominal aorta replacement (1), descending aorta replacement (1), Bentall procedure (1)</td>
<td>Off-pump coronary artery bypass (15), coronary artery bypass grafting (5), beating heart coronary revascularization (1)</td>
<td></td>
</tr>
<tr>
<td>Disease course, month</td>
<td>0.13 ± 1.66</td>
<td>62.82 ± 168.61</td>
<td>41.88 ± 49.74</td>
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<tr>
<td>Survival, %</td>
<td>100</td>
<td>85</td>
<td>100</td>
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cytoplasmatic housekeeping gene product (β-actin). The grayscales of the graphs were analyzed using Quantity One software (BIO-RAD Laboratories). Relative grayscales in contrast to those of β-actin were calculated and analyzed.

ELISA

The expression of TGF-β₁ was determined with commercially available ELISA kit (Human TGF-β₁ ELISA Kit, Cat number: KGEHC107b, KeyGen Biotech Co. Ltd., Nanjing, China) for the detection of the plasma and aortic tissue supernatant by sandwich ELISA according to specialized procedures described in the instructions for users of the product.

Statistics

Data were expressed as mean ± standard deviation. Intergroup comparisons of quantitative variables were made by using one-way ANOVA model, and meanwhile by rank sum test as well. A two-tailed P value less than 0.05 was considered significant. The linear correlations of the relative grayscales between different proteins of each group, and those correlations between the quantitative TGF-β₁ by ELISA and the time interval from the onset to surgery or the maximal dimensions of the aorta of the Aortic Dissection Group were assessed. |r| < 0.3 was taken as a non-significant correlation, while 0.3≤|r|<0.5, 0.5≤|r|<0.8, and |r|≥0.8 were taken as a slight, middle, and striking correlation, respectively.

Ethics

This study was approved by the institutional ethical committee, and was conducted following the guidelines of the Declaration of Helsinki. Informed consent was obtained from each patient before commencing treatment.

RESULTS

Quantitative RT-PCR

The melting curves showed the changing rate of the relative fluorescence units (RFU) with time (T) (-d(RFU)/dT) on the Y-axis versus the temperature on the X-axis displayed a single peak at the melting temperature (Tm) of 87°C for the samples, and of 84°C for the control, respectively (Fig. 1). The expressions of TGF-β₁ mRNA were positive in all three groups. The results of TGF-β₁ mRNA were calculated quantitatively by 2-ΔΔCT method, however, they did not show any intergroup differences (1.59 ± 0.33 vs. 1.45 ± 0.34 vs. 1.48 ± 0.48, P > 0.05 by rank sum test).

Western blot

Western blot assay revealed TGF-β₁, TβRI, Smad2/3, Smad4 and Smad7 were positive in all three groups (Fig. 2). Smad4 was weakly present in the aortic tissues of the coronary patients. In spite of scanty of significant intergroup differences, quantitative results of relative grayscales of the five investigated proteins showed TGF-β₁ was less pronounced in the aortic dissection than in the aortic aneurysm or coronary artery disease group, and a more pronounced TGF-β₁ was present in the latter group than others. The expressions of Smad2/3 was somehow higher in the aortic dissection than in the aortic aneurysm and coronary patients, and Smad4 were the highest in the Aortic Dissection Group. TβRI and Smad7 expressions were similar in all three groups (Fig. 3, Table 2).

Fig. 1. The melting curves of (A) the samples, and (B) the control. Note the melting temperatures were 87°C and 84°C, respectively.
Of the quantitative relative grayscales, a significant reverse correlation was noted between TGF-β, and Smad2/3 ($Y = -0.8552X + 1.6417, r = -0.759, P < 0.0001$), and a close direct correlation between Smad4 and Smad7 ($Y = 0.5905X + 0.2805, r = 0.781, P < 0.0001$) in the aortic dissection group. In the aortic aneurysm group, Smad4 and Smad7 were also closely correlated ($Y = 0.5228X + 0.1642, r = 0.727, P = 0.026$), and in the coronary artery disease group, TGF-β, and Smad7 were much significantly correlated ($Y = 0.5301X + 0.5758, r = 0.917, P = 0.004$) (Fig. 4).

**ELISA**

The expressions of TGF-β, in the aortic tissue were $319.52 \pm 129.21 \text{ pg/mg protein}$, $324.09 \pm 49.70 \text{ pg/mg protein}$, and $304.15 \pm 29.39 \text{ pg/mg protein}$ in the three groups, respectively. Despite no significant differences, a less pronounced elevation could be seen in the aortic dissection in comparison to either aortic aneurysm or coronary artery disease group (Fig. 5).
Table 2. Non-significance of relative net grayscales of Western blot assay by ANOVA

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± standard deviation</th>
<th>P value</th>
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<tbody>
<tr>
<td></td>
<td>AD</td>
<td>AA</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>0.95 ± 0.64</td>
<td>1.10 ± 0.55</td>
</tr>
<tr>
<td>TßRI</td>
<td>0.75 ± 0.51</td>
<td>1.02 ± 0.54</td>
</tr>
<tr>
<td>Smad2/3</td>
<td>0.81 ± 0.46</td>
<td>0.57 ± 0.37</td>
</tr>
<tr>
<td>Smad4</td>
<td>0.82 ± 0.68</td>
<td>0.59 ± 0.27</td>
</tr>
<tr>
<td>Smad7</td>
<td>0.99 ± 0.65</td>
<td>1.01 ± 0.57</td>
</tr>
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</table>


Plasma TGF-β1 values were 1158.30 ± 11.54 pg/ml, 1170.27 ± 8.26 pg/ml, 1225.00 ± 174.42 pg/mL and 1160.25 ± 13.01 pg/mL in the four groups, respectively. A similar but less pronounced increasing trend was found to that in the supernatant of the aortic tissues in the aortic dissection and the aortic aneurysm groups (Fig. 6). However, the plasma TGF-β1 level was remarkably enhanced in the coronary patients, and significant intergroup differences were present by rank sum test (P < 0.025).

The time interval from the onset to surgery was 4.76 ± 7.85 days (range: 8 hours to 1 month) in patients with aortic dissection. This time interval did not correlate with aortic or plasma TGF-β1 values (aorta: Y=23.757X + 827.68, r² = 0.0411, r = -0.203, P = 0.420; plasma: Y=0.3148X + 1156.70, r² = 0.0324, r = 0.180, P = 0.670), neither did the maximal dimension of the thoracic aorta with aortic or plasma TGF-β1 (aorta: Y=145.52X + 1807.67, r² = 0.0400, r = -0.200, P = 0.493; plasma: Y=1.9537X + 1145.03, r² = 0.0649, r = 0.255, P = 0.626) (Fig. 7).
DISCUSSION

Studies on TGF-β signaling revealed that Smad4 was unlikely to be involved in matrix contraction induced by TGF-β, whereas Smad2/3 was distributed in the cytoplasm but relatively lower in the nucleus [21]. On the contrary, Smad7 overexpression may inhibit the TGF-β-induced fibronectin and connective tissue growth factor expressions [3]. Nevertheless, the intensity and duration of TGF-β signals and Smad2/3 nuclear translocation may largely depend on the regulation by Smad7 on the one hand [21], and Smad7 overexpression may prevent injury-induced α-smooth muscle actin expression as well [22]. Besides, Smad7 overexpression may remarkably reduce the β-galactose-labelled cells in the neointima, decrease the loss of the lumen, reduce the collagen content of the vascular adventitia, and delay the process of vascular fibrosis following balloon angioplasty [23].

In aortic dissection, Smad4 may promote, while Smad7 may abolish, this signaling pathway, leading to matrix degradation by attenuating laminin expression and increasing expression of matrix metalloproteinases, making the balance between deposition and degradation a shift to the latter. Similar to what has been described previously, upregulations of TGF-β, and Smad2, Smad3 and Smad7 may be responsible for cardiac hypertrophy induced by abdominal aortic constriction in the rat models [24]. In addition, Smad4 was upregulated as well, despite few other studies have directly investigated this issue, but an attenuated expression of Smad4 in a murine model of thoracic aortic aneurysm with enhanced other ligands of the signaling pathway has been reported [25]. In the vascular smooth muscle cells, in the condition of angiotensin II stimulation, a rapid Smad2 phosphorylation, nuclear translocation of phosphorylated-Smad2 and Smad4 might occur [26]. In contrast, Smad4 functional loss may result in increased laminin expression and decreased expression of matrix metalloproteinases, which, with increased levels of laminin α1, cause excessive basement membrane deposition [27].

Madri et al. [28] found in the balloon-injured rat carotid artery model the neointima of the arteries showed intense staining of TGF-β, at 10 weeks after vascular injury. Majesky et al. [29] also observed an increased TGF-β1 in neointimal
smooth muscle cells with antecedent transcripts for TGF-β, 6 hours after balloon injury, serum TGF-β1 between the patients with abdominal aortic aneurysm and the subjects without an aneurysm did not display any significant difference (32.6 ± 9.9 ng/mL vs. 33.2 ± 8.3 ng/mL, P = 0.098) [30]. However, TGF-β1 might be released from the platelets into the serum when blood coagulates, and this would largely influence the serum detection [31]. Therefore, one should always bear in mind such influence factors when confronting TGF-β1 results detected by ELISA especially when the patients are at risks of coagulopathies.

TGF-β1 mRNA can be upregulated in cancer and disorders involving fibrotic process, and it is especially more expressed in malignant than in benign lesions. In comparison with non-atherosclerotic disease, atherosclerotic aortic smooth muscle cells showed much more TGF-β1 mRNA expressions. In this study, TGF-β1 mRNA was expressed in all the aortic tissues of the patients of each group, with a slight higher level in the aortic dissection than in the aortic aneurysm and coronary artery disease group, but lack of significant differences. The results indicated that TGF-β1 may participate the development of the aortopathies, with no difference in the extent at the genetic level while displaying its major biological function. But the potential disparities of the functioning ways in various aortopathies could not be excluded. Anyway, interruption of TGF-β1/Smad signaling pathway at the genetic level might represent an alternative of reversing the pathological process of these lesions [32].

Subtraction of the background gray levels may facilitate correct measurement of the grayscale at each pixel across the image in immunostaining [33] and Western blot analyses [34], and maximize the signal strength and minimize the non-specific bands [35]. We therefore adopted positive net grayscale in evaluating the positiveness of quantitative Western blot results, from which we noted the close correlations between Smad4 and Smad7 of the aortic dissection and aortic aneurysm patients, which may indicate an intense abolishing effect of Smad7 in the signaling transduction. However, such a relation was scanty in coronary artery disease patients, indicating a less inhibitory effect of Smad7 associated with atherosclerotic changes. The negative regressions between TGF-β1 and Smad2/3 in aortic dissection highlighted a probable impetus of matrix degradation. Background noise is often associated with the problematic samples such as plasma, serum or cell culture. It may influence on all values, but influence more on the lower and non-expressed genes at a large extent [36]. Our Western blot disclosed an enhanced TGF-β1/Smad transduction in the aortopathies, including aortic dissection, aortic aneurysm and atherosclerosis. Furthermore, TGF-β1 was less pronounced in the aortic dissection than in the aortic aneurysm or coronary artery disease group, and a more pronounced TGF-β1 was present in the latter group than others. The expressions of Smad2/3 was somehow higher in the aortic dissection than in the aortic aneurysm and coronary patients, and Smad4 were the highest in the Aortic Dissection Group, but was weakly present in the aortic tissues of the coronary patients. TßRI and Smad7 expressions were similar in all three groups. Linear correlations revealed a somehow damaged TGF-β1 in the aortic dissection. We postulated that TGF-β1/Smad signaling transduction varied in various aortopathies: R-Smad was slightly upregulated, Co-Smad was remarkably upregulated and I-Smad was moderately upregulated in the aortic dissection; and R-Smad and Co-Smad moderately attenuated and I-Smad enhanced in the aortic aneurysm, while Co-Smad was remarkably attenuated in the coronary patients.

In this study, the ELISA showed a distinguished increase of TGF-β1 in the aortic tissue in the Aortic Aneurysm Group, and a distinguished increase of TGF-β1 in the plasma in the coronary artery disease group, indicating TGF-β1 might be expressed in the aortic tissues prior to its release into the circulation. As such, TGF-β1 upregulation may play a role in inhibiting the progression of aortic dilation as described in the literature [37].

There were four limitations confronted in this study that should be mentioned: small sample, small aortic tissues from the coronary patients, the lack of normal aortic tissues from heart transplant donors, and the different sources of healthy controls for blood and aorta sampling. Further studies on larger patient population and sufficient sampling sources can be helpful for obtaining more precise information.

**CONCLUSION**

In conclusion, TGF-β1/Smad signaling transduction varied in the functioning way in different aortopathies. In patients with aortic dissection, the signaling was enhanced, in comparison to aortic aneurysm and coronary artery disease, characterized by a less pronounced TGF-β1 expression, but a somehow pronounced I-Smad and Co-Smad upregulation, suggesting a prominent matrix degradation in aortic dissection, but a prominent matrix deposition in the aortic aneurysm and coronary artery disease.

**REFERENCES**


