Circulating transforming growth factor-β and aortic dilation in repaired hearts: abridged secondary publication

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KEY MESSAGES

1. Compared with healthy controls, patients with repaired tetralogy of Fallot had significantly higher circulating levels of transforming growth factor-β1, metalloproteinase-2, and metalloproteinase-9, whereas patients with atrial switch operation or Fontan procedure had significantly higher circulating levels of metalloproteinase-2.
2. The circulating transforming growth factor-β1 level correlated significantly with metalloproteinase-9 and aortic sinus dimension.
3. The ascending aortic dimensions were significantly greater, and elastic properties were significantly worse in all patient groups, compared with healthy controls.
4. Aortic stiffness correlated positively with sinus dimension and negatively with indices of systemic ventricular systolic and diastolic deformation.

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Introduction

Progressive dilation of the aortic root is a concern in patients with congenital heart disease. Histological abnormalities of the aortic root as characterised by medionecrosis, fibrosis, and fragmentation of elastic fibres have been documented in different congenital heart lesions including tetralogy of Fallot (TOF) with or without pulmonary atresia, complete transposition of the great arteries (TGA), and functionally univentricular hearts.[1] Understanding of vascular remodelling in such patients may shed light on therapies for progressive aortic root dilation and the risk of its associated complications.

The transforming growth factor-beta (TGF-β) superfamily of cytokine is important in vascular remodelling.[2] Increased circulating level of TGF-β1 in patients with Marfan syndrome is associated with aortic root dilation and is predictive of cardiovascular events including aortic dissection, and the need for aortic root replacement.[3] Overexpression of TGF-β1 has been found in the ascending aorta of those with congenital heart disease.[4] We hypothesised that the circulating TGF-β1 level is increased and associated with aortic root dilation in patients with repaired hearts at risk of ascending aortopathy. We aimed to determine the circulating levels of TGF-β1 and their associations with aortic dilation and elastic properties in patients who underwent surgery for congenital heart disease.

Methods

This study was approved by the Institutional Review Board, and all patients (or parents of minors) gave written informed consent. Adolescent and adult patients with congenital heart disease who underwent repair for TOF, arterial or atrial switch operation for TGA, or Fontan procedure for functionally univentricular heart were recruited. Staff members of the hospital and their relatives and friends were recruited as healthy controls.

Vascular and echocardiographic assessments were performed using the Vivid 7 ultrasound machine after resting for >15 minutes. The size of the aortic root was measured at diastole from the parasternal long-axis view at four levels: annulus, sinus of Valsalva, sinotubular junction, and proximal ascending aorta. Severity of aortic regurgitation was assessed using Doppler colour flow mapping. Elastic properties of the ascending aorta were determined using M-mode echocardiography based on the parasternal long-axis view. Ascending aortic systolic and diastolic dimensions and systolic and diastolic blood pressures were measured to assess aortic elastic properties: strain, distensibility, and stiffness. The brachial-ankle pulse wave velocity was determined using an automated device.

Systemic ventricular function was assessed using tissue-Doppler and two-dimensional speckle tracking echocardiography. The peak systolic myocardial tissue velocity, peak early and late diastolic myocardial tissue velocities, early/late ratio, and myocardial isovolumic acceleration were calculated. For patients with Fontan procedure, the mean right and left-sided annular tissue velocities was used for analysis. Global longitudinal and

circumferential strain and strain rate of the systemic ventricle were assessed using two-dimensional speckle tracking echocardiography.

Total TGF-β1 level was measured using commercially available assay. Serum levels of metalloproteinase-2 (MMP-2) and metalloproteinase-9 (MMP-9) were also determined using enzyme-linked immunosorbent assay.

The aortic dimensions were measured by a single investigator to avoid inter-observer bias. Cardiovascular indices and circulating levels of TGF-β1, MMP-2, MMP-9 among different groups were compared using analysis of variance. Cardiovascular and blood indices between the control group and each of the patient cohorts were compared using unpaired Student's *t* test. Associations between TGF-β1 and MMP levels and those between TGF-β1 and aortic dimensions and elastic properties were assessed using Pearson correlation analysis. Multivariate analysis was performed to identify significant correlates of circulating levels of TGF-β1. A P value of <0.05 was considered statistically significant. Statistical analyses were performed using SPSS (Windows version 19; IBM Corp, Armonk [NY], US).

Results

Of 209 patients invited, 109 (66 males and 43 females) agreed to participate, whereas 36 (16 males and 20 females) healthy controls were recruited. Of the 109 patients, 46 underwent surgical repair for TOF (43 had TOF with pulmonary stenosis and 3 had TOF with pulmonary atresia), 21 underwent arterial switch operation for TGA, 15 underwent atrial switch operation for TGA, and 27 underwent Fontan procedure. The mean follow-up duration was 24.0±5.7 years.

Compared with controls, patients had significantly larger aortic annulus, sinus of Valsalva, sino-tubular junction, and proximal ascending aorta (all P<0.05, Table 1).

Compared with controls, all four patient groups had significantly lower aortic strain (all P<0.001) and aortic distensibility (all P<0.001), and greater aortic stiffness index (all P<0.001) [Fig.]. For all patient groups and controls, aortic sinus dimension correlated with aortic strain (r= -0.50, P<0.001), distensibility (r= -0.51, P<0.001), and stiffness index (r=0.48, P<0.001). However, the mean right and left brachial-ankle pulse wave velocities, which reflect the composite effects of central and peripheral arterial stiffness, were similar among different patient groups and controls.

Doppler assessment of systemic ventricular inflow revealed significantly lower peak systemic atrioventricular inflow velocity at early diastole, and lower peak systemic atrioventricular inflow velocity

TABLE 1. Demographic and clinical data and aortic root dimensions

Clinical variable	Patients with congenital heart disease who underwent				Healthy controls (n=36)*
	Repair for tetralogy of Fallot (n=46)*	Arterial switch operation for complete transposition of the great arteries (n=21)*	Atrial switch operation for complete transposition of the great arteries (n=15)*	Fontan procedure (n=27)*	
No. of males: females	23:23	12:9	13:2	18:9	16:20
Age at surgery, y	4.22±2.65	0.04±0.02	1.32±1.17	4.79±3.28	
Duration after repair, y	25.1±6.6	21.5±2.3	29.1±3.9	20.9±3.7	
Age at study, y	29.6 ±7.2	21.9±2.2	30.6±4.5†	26.1±4.6	26.9±7.4
Weight, kg	59.5±13.2	59.9±11.0	61.4±8.7	55.1±13.2	59.9±14.5
Height, m	1.6±0.1	1.7±0.1	1.7±0.0	1.6±0.1	1.6±0.1
Body mass index, kg/m^2	22.2±4.0	22.1±4.3	21.8±2.9	20.4±3.8	21.9±3.6
Body surface area, m^2	1.6±0.2	1.7±0.1	1.7±0.1	1.6±0.2	1.6±0.2
Systolic blood pressure, mmHg	113±10	119±12†	120±9†	110±13	107±10
Diastolic blood pressure, mmHg	65±5	68±8†	69±5	66±5†	62±6
Annulus, cm	2.21±0.41†	2.45±0.36†	2.40±0.34†	2.33±0.52†	1.90±0.23
Sinus of Valsalva, cm	3.36±0.37†	3.38±0.38†	3.32±0.33†	3.32±0.52†	2.63±0.35
Sino-tubular junction, cm	2.83±0.43†	2.96±0.45†	2.66±0.35†	2.66±0.50†	2.05±0.36
Ascending aorta, cm	2.80±0.44†	3.04±0.48†	2.76±0.41†	2.68±0.52†	2.11±0.39

* Data are presented as mean ± standard deviation

† P<0.05 compared with controls

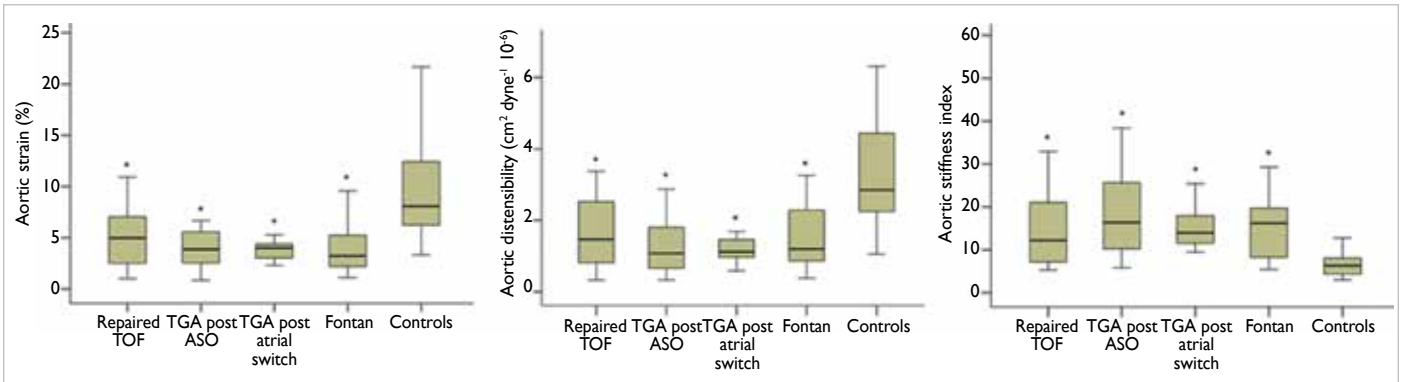

FIG. Aortic elastic properties in patients and controls.

at early/late diastole ratio in patients with Fontan procedure, compared with controls (both P<0.001, Table 2). Tissue Doppler echocardiography revealed evidence of worse systolic and diastolic function of the systemic ventricle in all patient groups, compared with controls, as demonstrated by the significantly lower systolic annular myocardial velocity, early diastolic annular myocardial velocity, and systemic ventricular myocardial isovolumic acceleration (all P<0.01). Strain imaging revealed primarily impairment of systolic and diastolic deformation in the longitudinal dimension of the systemic ventricle in all patient groups who had significantly reduced global systolic longitudinal strain and strain rate, and early and late diastolic strain rates (all P<0.001).

For all patient groups, aortic stiffness was negatively associated with systemic ventricular global longitudinal systolic strain (r= -0.37, P<0.001), systolic strain rate (r= -0.29, P<0.001), and early diastolic strain rate (r= -0.32, P<0.001) [Table 2]. Furthermore, aortic stiffness correlated negatively with systemic atrioventricular valvar tissue velocities including the systolic annular myocardial velocity (r= -0.34, P<0.001) and early diastolic annular myocardial velocity (r= -0.30, P=0.001).

The circulating levels of TGF-β1, MMP-2, and MMP-9 differed significantly among patient groups (P<0.001). Patients with repaired TOF had significantly higher circulating levels of TGF-β1 (P=0.005), MMP-2 (P=0.001), and MMP-9 (P<0.001), compared with controls (Table 2). Additionally, patients with atrial switch operation for TGA (p=0.034) or Fontan procedure (p<0.001) had significantly higher MMP-2 levels, compared with controls.

In patients, the circulating TGF-β1 level correlated significantly with MMP-9 (r=0.44, P<0.001) [but not MMP-2 level] and the size of the aortic sinus (r=0.22, P=0.035). There were no significant associations between circulating levels of TGF-β1, MMP-2, MMP-9, and indices of aortic elasticity.

Multivariate analysis revealed that the diagnosis of TOF was an independent correlate (β=6.82, P<0.001) of higher circulating TGF-β1 levels, after adjusting for other congenital heart disease categories, age, sex, aortic sinus dimension, and aortic stiffness index.

Discussion

Compared with healthy controls, patients with repaired TOF had significant higher circulating levels of TGF-β1, MMP-2, and MMP-9. Among patients with congenital heart disease, circulating TGF-β1 was associated with magnitude of aortic sinus dilation and positively correlated with MMP-9. Furthermore, increased aortic stiffness was associated with greater aortic root dilation and worse systemic ventricular systolic and diastolic function. Subgroup analysis showed increased TGF-β1, MMP-2, and MMP-9 levels in patients with repaired TOF, whereas MMP-2 levels also increased in patients with arterial switch operation or Fontan procedure. Circulating TGF-β1 level was associated with the size of aortic sinus. Thus, there is possible perturbation of the TGF-β1 signalling pathway with consequential increased MMP activities in the pathogenesis of aortopathy in patients with repaired TOF, and possibly in patients with TGA after atrial switch operation or Fontan procedure.

Our findings suggest that modulation of the TGF-β1 signalling pathway by angiotensin-converting enzyme inhibitor and angiotensin-receptor blocker[1,5] and MMP inhibition by doxycycline[1] may be useful in the management of congenital heart disease–related aortopathy, in particular in patients with repaired TOF.

Limitations to this study include the lack of a longitudinal trajectory of circulating TGF-β1 and

TABLE 2. Echocardiographic indices of systemic ventricular function

Systemic ventricular function variable	Patients with congenital heart disease who underwent				Healthy controls (n=36)*
	Repair for tetralogy of Fallot (n=46)*	Arterial switch operation for complete transposition of the great arteries (n=21)*	Atrial switch operation for complete transposition of the great arteries (n=15)*	Fontan procedure (n=27)*	
Systemic atrioventricular inflow Doppler indices					
Peak systemic atrioventricular inflow velocity at early diastole (E), cm/s	93.3±22.0	98.6±21.8	91.8±21.6	64.1±13.1†	97.3±20.2
Peak systemic atrioventricular inflow velocity at late diastole (A), cm/s	47.5±14.1	44.8±14.2	49.7±18.4	46.3±9.3	46.4±9.9
E/A ratio	2.1±0.7	2.5±1.2	2.0±0.6	1.5±0.4†	2.2±0.7
E deceleration time, ms	171.6±44.7	163.1±32.9	140.5±34.3†	174.8±51.8	162.8±31.3
Systemic atrioventricular annular tissue velocities					
Systolic annular myocardial velocity, cm/s	5.6±1.7†	5.9±2.2†	5.0±0.9†	3.7±0.9†	7.9±1.5
Early diastolic annular myocardial velocity (e), cm/s	10.6±3.1†	11.5±2.6	5.4±1.9†	5.9±1.4†	12.1±2.3
Late diastolic annular myocardial velocity (a), cm/s	4.5±1.9†	4.5±1.5†	4.7±1.8	4.2±1.3†	5.7±1.2
e/a ratio	2.8±1.2†	2.9±1.2†	1.4±0.5†	1.6±0.6†	2.2±0.6
E/e ratio	9.3±2.9	8.8±3.2	18.5±8.2†	10.4±2.7†	8.3±2.1
Myocardial isovolumic acceleration, m/s^2	0.6±0.3†	0.9±0.6†	0.7±0.4†	0.9±0.4†	1.2±0.4
Subpulmonary atrioventricular annular tissue velocities					
Systolic annular myocardial velocity, cm/s	6.0±1.5†	4.7±1.3†	4.9±1.3†	-	10.0±1.6
Early diastolic annular myocardial velocity (e), cm/s	7.8±3.2†	8.9±2.0†	7.2±2.8†	-	11.9±2.1
Late diastolic annular myocardial velocity (a), cm/s	4.1±1.9†	5.2±1.1†	3.7±0.9†	-	7.1±1.7
e/a ratio	2.5±1.9†	1.8±0.5	2.1±1.0	-	1.8±0.5
Myocardial isovolumic acceleration, m/s^2	0.4±0.4†	1.0±0.4†	0.9±0.8†	-	1.8±0.6
Systemic ventricular longitudinal deformation					
Global longitudinal strain, %	13.5±3.1†	13.0 ±2.4†	12.1±1.9†	12.1 ±2.6†	17.4±2.3
Systolic strain rate, /s	0.72±0.17†	0.69±0.14†	0.62±0.12†	0.69±0.11†	0.92±0.15
Early diastolic strain rate, /s	1.00±0.31†	1.16±0.22†	0.80±0.20†	0.81±0.28†	1.53±0.33
Late diastolic strain rate, /s	0.51±0.17†	0.48±0.12†	0.42±0.14†	0.51±0.21†	0.63±0.15
Systemic ventricular circumferential deformation					
Global longitudinal strain, %	16.7±3.57	14.5±4.2	13.5±5.4	15.1±3.0	16.3±2.4
Systolic strain rate, /s	0.94±0.20	0.92±0.22	0.81±0.23†	0.75±0.22†	1.01±0.21
Early diastolic strain rate, /s	1.65±0.43†	1.32±0.31	1.30±0.71	1.20±0.44	1.35±0.31
Late diastolic strain rate, /s	0.33±0.17†	0.37±0.15	0.38±0.23	0.41±0.18	0.43±0.17
Transforming growth factor-β1, ng/mL	38.7±9.1†	30.5±10.1	34.4±6.1	33.8±10.9	32.6±9.9
Matrix metalloproteinase-2, ng/mL	234.0±41.5†	219.2±37.8	227.1±36.0†	261.7±39.1†	204.3±33.2
Matrix metalloproteinase-9, ng/mL	325.0±151.0†	163.1±97.3	197.5±71.0	226.9±193.4	196.3±113.6

* Data are presented as mean ± standard deviation

† P<0.05 compared with controls

MMP levels, the small sample size, the modest correlation between aortic stiffness and indices of ventricular deformation and between circulating TGF-β1 level and aortic sinus dimension, the undetermined origin of the circulating TGF-β1 and MMP levels, and the difficulty in assessing anteriorly located aorta in a few patients with atrial switch operation for TGA. Nonetheless, our findings provide basis for medical therapies that target at the TGF-β1 signalling pathway and MMP activation in the management of aortopathy.

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Disclosure

The results of this research have been previously published in:

1. Cheung YF, Chow PC, So EK, Chan KW. Circulating transforming growth factor-β and aortic dilation in patients with repaired congenital heart disease. Sci Rep 2019;9:162.

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