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Original Article

Aortic mineralisation in children with congenital cardiac disease

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Abstract *Background:* Congenital cardiac diseases are the most frequent congenital malformations. In adult patients, the mineralisation of the aorta due to cardiovascular disease is very common, but vascular mineralisation in paediatric cardiopathies is a topic less studied. This study shows that children with a complex congenital cardiopathy show a high degree of vascular mineralisation in the ascending aorta. This can be part of the cardiac failure pathophysiology due to congenital cardiopathies. *Objective:* The aim of this study was to determine the presence and degree of vascular mineralisation in samples of the ascending and descending aorta of children with complex congenital cardiopathies. *Design:* We conducted a cross-sectional study. *Subjects:* We obtained 34 vascular tissues from the autopsies of 17 children with congenital cardiac disease. *Methods:* We used a scanning electron microscope with an energy-dispersive X-ray spectroscopy in order to analyse the vascular tissues. *Results:* The amount of minerals was two times higher in the ascending aorta than in the descending aorta of children with congenital cardiac disease. *Conclusions:* The study provides evidence that vascular mineralisation can start at an early age, and that it is higher in the ascending aorta than in the descending aorta.

Keywords: Paediatric cardiology; aortic mineralisation; congenital cardiac disease

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CONGENITAL CARDIAC DISEASES ARE THE MOST frequent congenital malformations.[1,2] The definition most commonly used to describe them, proposed by Mitchell et al,[3] states that it refers to an evident structural anomaly of the heart or of the large intrathoracic vessels with a real or potential repercussion. The reported prevalence of congenital cardiac disease for 1000 live births goes from 2.1 in New England in the United States of America, 2.17 in Toronto in Canada to 10.6 in Japan, and 12.3 in Florence in Italy.[4,5] The prevalence of congenital cardiac disease in Mexico is unknown, but it can be said that it is the second cause of mortality in infants under 1 year of age.[6,7]

In Mexico, an analysis of 2257 patients with congenital cardiopathy performed at the Cardiology Hospital of the National Medical Center Siglo XXI revealed that patent ductus arteriosus represented 20% of all cases, followed by interatrial communication

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Table 1. Age and type of complex congenital cardiopathy of the patients included in the study.

Number	Age	Type of congenital cardiopathy
1	1 month	Dextroisomerism
2	1 month	Dextroisomerism
3	4 months	Double-inlet left ventricle
4	1 month	Pulmonary atresia and intact ventricular septum
5	15 months	Pulmonary atresia and intact ventricular septum
6	2 days	Hypoplastic left heart
7	3 days	Hypoplastic left heart
8	5 months	Pulmonary atresia and ventricular septal defect
9	10 months	Pulmonary sling
10	10 months	Double-outlet right ventricle
11	3 months	Double-outlet right ventricle
12	1 year	Transposition of the great arteries
13	3 years	Double-outlet right ventricle
14	3 months	Interrupted aortic arch type A – ventricular septal defect
15	2 months	Total anomalous pulmonary veins connection – pulmonary veins stenosis
16	4 months	Atrioventricular septal defect
17	6 months	Atrioventricular septal defect

with 16.8%, interventricular communication with 11%, tetralogy of Fallot and pulmonary atresia with ventricular septal defect with 9.3%, aortic coarctation and pulmonary stenosis with 3.6% each, and total anomalous connection of pulmonary veins with 3%.[8,9] The classical observed pathophysiological patterns in the paediatric population are the volume and pressure overload, as congenital cardiac disease depicts a high degree of variability, not only in cardiac malformations, but also in clinical manifestations.

Elasticity and distensibility are altered in the presence of aortic vascular calcification, which increases the workload of the heart and triggers cardiac failure.[10]

Mineralisation is a biological process in which many of the minerals found in the vascular wall correspond to calcium and phosphates (hydroxyapatite); however, many other minerals such as silicium and iron can also be found.[11]

Vascular and bone mineralisation share physiological mechanisms that reflect the similarity of the paracrine signals coming from the osteoblasts, condrocytes, and osteoclasts. Inflammation, turbulent blood flow, hyperphosphataemia, lysis of elastin, and oxidative stress all favour remodellation of the vascular matrix through an increment in the synthesis of bone morphogenetic protein-2, -4, and Wnt signalling cascade. In addition, these pathological processes also deteriorate the mechanisms that limit mineral deposits, such as inhibiting the osteochondrogenetic differentiation and depuration of the vesicular matrix.[12]

Even if mineralisation of tissues is recognised as a consequence of the inflammation generated by metabolic, mechanical, haemodynamic, and/or infectious disorders, and is generally associated with advanced age,[13] children with different congenital cardiac disease could present diverse degrees of vascular mineralisation influencing cardiovascular function. On the other hand, volume and pressure overloads, characteristic of the different cardiopathies, could favour the development of vascular mineralisation. Therefore, our objective was to determine the presence and degree of vascular mineralisation in samples of the ascending and descending aorta in children with diverse congenital cardiac disease.

Materials and methods

We conducted a cross-sectional study, analysing vascular tissue from 17 children with complex congenital cardiopathies. Table 1 shows the age and type of complex congenital cardiopathies included in the analysis.

Vascular tissues were obtained from autopsies at the Instituto Nacional de Cardiología "Ignacio Chávez". We dissected sections of 0.5 centimetres of the ascending and descending aorta.

The aorta sections, preserved in formaldehyde, were dried at room temperature for 24–48 hours and placed on glass coverslips, covered with graphite, and studied under a scanning electron microscope, Japanese Electronic and Optical Laboratory, Model JEOL JXA8900-R, with an energy-dispersive X-ray spectrometer, at an acceleration voltage of 20 kilo electro volt, an acquisition time of 30–60 seconds, and a 20 nanoampere current. Digital images are obtained with a resolution of 1024×1024 pixels. The images of this mapping were processed with

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the IMAGE-PRO PLUS 4.1 software; the mineral deposits contrast with the image background. To avoid bias, the same dimension of a 100 square micrometre area was always analysed.

Statistical analysis

Data are expressed as mean, that is, standard error. Groups were compared with the *t*-test for paired samples and differences were considered significant if p value was less than or equal to 0.5.

The study was conducted with the approval of the Review Board of the National Institute of Cardiology, México.

Results

Our comparison of the presence of minerals in the ascending aorta with a mean of 23.07, Figure 1, with that of the descending aorta with a mean of 11.38, Figure 2, of patients with congenital cardiac disease also revealed statistically significant differences in p value equal to 0.036, with a greater amount of minerals in the ascending aorta.

Table 2 shows different types of minerals found in the ascending and the descending aortas. Results are in percentage of every element per studied field. Results are expressed in percentage of weight ratios of each mineral.

Table 3 provides the energy-dispersive X-ray spectrometer of the corresponding Figures 1 and 2. Results are expressed in percentage of weight ratios of each mineral.

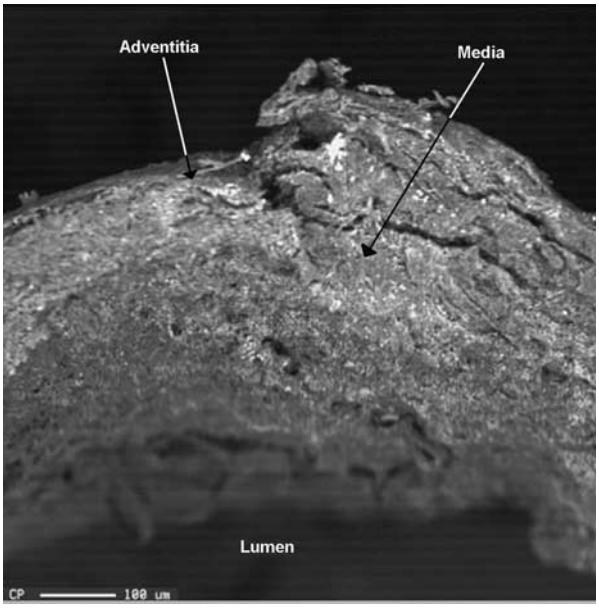

Figure 1.
Minerals in the ascending aorta.

Discussion

The use of the electron probe microanalysis[14] allowed us to recognise the presence of different minerals in the studied aortic tissues. The technique consists of bombarding the sample with an electron beam in a high-vacuum electronic column; the signals emitted by the sample are semi-quantitatively analysed through energy-dispersive X-ray spectroscopy, which detects the dispersion of the characteristic X-rays of the different elements constituting the sample. It enables the identification of the

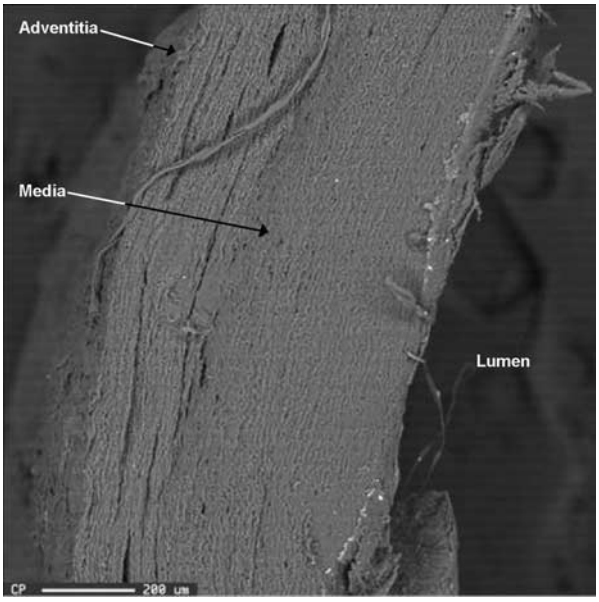

Figure 2.
Minerals in the descending aorta.

Table 2. Mineralisation according to chemical element in the descending and ascending aorta of the patients with congenital cardiopathy.

	Descending aorta	Ascending aorta	
Element	**Mean (SE)**	**Mean (SE)**	**p**
Silicon	4.88 (0.63)	5.15 (0.53)	0.689
Sulphur	20.42 (2.95)	24.61 (3.01)	0.374
Chlorine	8.60 (3.53)	5.68 (2.29)	0.504
Calcium	36.21 (5.15)	27.80 (3.47)	0.196
Zinc	2.01 (0.92)	6.24 (2.64)	0.160
Aluminium	3.15 (0.45)	2.70 (0.52)	0.333
Phosphorus	9.64 (1.27)	11.41 (1.52)	0.345
Iron	4.48 (1.16)	4.84 (1.51)	0.848
Potassium	1.09 (0.33)	1.37 (0.33)	0.572
Magnesium	2.22 (0.45)	2.26 (0.49)	0.959
Manganese	1.39 (0.41)	1.92 (0.38)	0.227
Sodium	1.15 (0.29)	0.50 (0.33)	0.059
Nickel	3.31 (0.96)	4.32 (1.53)	0.561
Chromium	1.46 (0.57)	1.18 (0.60)	0.777

Results are expressed in percentage of weight ratios of each mineral

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Table 3. Energy-dispersive X-ray spectometer of Figures 1 and 2.

	Figure 1	Figure 2
Element	Ascending aorta*	Descending aorta*
Silicon	3.37	6.81
Sulphur	38.67	24.27
Chlorine	0.00	3.50
Calcium	18.03	20.03
Zinc	0.00	0.00
Aluminium	3.88	4.50
Phosphorus	12.53	10.69
Iron	0.00	9.68
Potassium	3.71	1.17
Magnesium	0.00	6.55
Manganese	3.49	2.34
Sodium	0.00	0.00
Nickel	9.59	10.48
Chromium	6.74	0.00

*Results are expressed in percentage of weight ratios of each mineral

chemical composition of solid materials without destroying them at very low concentrations, in the order of 0.2% for the analysis to be reliable, by comparing the characteristic energy of the X-rays of the sample with that of reference standards or internal patterns of the instrument.

This technological resource is issued mainly in geophysics and palaeontology, but as far as we know it is the most sensitive method to quantify minerals in a tissue. With just one determination, the content of the different minerals can be obtained, as it has a high sensitivity and spatial resolution and can be applicable in the realm of vascular mineralisation studies in the future.

Results show a higher degree of vascular mineralisation in the ascending aorta than in descending aorta of the children with complex congenital cardiopathy.

Congenital cardiac diseases, from a haemodynamic point of view, are characterised by a volume overload in the ascending aorta. The haemodynamic demand of the cardiovascular system requires the aorta to store energy during systole and to release it during diastole, while minimising cardiac work. The loss of elasticity and aortic performance fostered by the aortic vascular mineralisation increases the speed of the arterial pulse wave, resulting in increased systolic pressure. This also increases cardiac work, promoting left ventricular hypertrophy, cardiac failure, and diastolic dysfunction.[10]

It has been described that mineralisation, predominantly by the presence of calcium in the ascending aorta, originates ejection resistance by the left ventricle, mainly due to the loss of elasticity at the root of the aorta, thus promoting ventricular hypertrophy, diastolic dysfunction, and cardiac failure, independently from the presence of other factors.[15,16] In contrast to the children with congenital cardiac disease, vessel calcification in adults with atherosclerosis is found mainly in the root and in the aortic arch, and less frequently in the descending aorta. It is considered that the most influential factors are variations in the ejection pressure of the left ventricle, alterations in systemic pressure, and the mechanical disorders of the aortic valve.

Our results are in accordance with those of Krefting et al,[17] who studied the presence of minerals in patients with coarctation of the aorta and found different elements: Na, Mg, P, S, Cl, K, and Ca. These authors described that Ca and P were higher in the ascending aorta, which, anatomically, is subjected to a greater pressure.

On the other hand, the studied patients with complex congenital malformations present tissular hypoxia, which could contribute to the vascular mineralisation mechanism. It has been demonstrated in pre-osteocyte cultures that bone mineralisation decreases in a hypoxic environment[18] and favours a similar mechanism described in osteoporosis, in which the deposit of minerals decreases in the bone tissue and the vascular deposit increases.[19] It is necessary to develop more studies to elucidate this hypothesis.

Conclusion

The ascending aorta of children with complex congenital cardiopathy shows a high degree of vascular mineralisation as compared with the descending aorta without differing in the type of mineral elements.

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