Histopathological Changes in Aorta and Pulmonary Artery in Patients with Dextro-Transposition of Great Arteries undergoing Arterial Switch Operation: A Prospective Study

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Abstract

Background: Following the arterial switch operation (ASO), there is a risk of neo-aortic root enlargement, and aortic regurgitation in follow-up. This study is intended to study whether abnormalities in the histopathological findings of the neo-aorta at the time of the arterial switch operation could lead to these complications.

Patients and methods: Between 1st April 2015 to 31st October 2016, 83 consecutive patients undergoing the arterial switch operation were included in this prospective cohort study. A sufficient representative sample of tissue obtained from the native pulmonary artery and the native aortic root, was fixed in 10% neutral buffer formalin and subjected to histopathological examination. These tissues were conservatively processed and paraffin blocks were made, sections were cut and stained by Hematoxylin & Eosin, Vanhoeff elastic, Van Giessen, Masson's trichrome stains. Elastic tissue and smooth muscle was systematically accessed, in the native aorta and native pulmonary artery. Simultaneously a note was made of all the patients pre-operative characteristics, etiology, associated cardiac anomalies, echocardiogram findings, cardiac catheterization data (if performed), intra operative details. Comparisons were made between histo-pathological findings of native aorta and native pulmonary artery, comparison with controls available in the literature, post-operative course, follow up, and follow-up echocardiographic data.

Results: Eighty-three patients had an ASO for TGA between 1st April 2015- 31st October, 2016. We examined elastic lamellar count of native aorta and native pulmonary artery and compared it with the findings described in the literature. It was found that elastic lamellar counts were similar in both the neo-aorta and the neo-pulmonary artery. Early in age, they were similar histopathologically. With increasing age, it was observed that lamellar count decreases in the neo-pulmonary artery.

Conclusions: There were no gross differences between native pulmonary artery and native aorta histopathology but an inverse relation was found between advancing age and elastic lamellar counts in both structures signifying the need for an early arterial switch to prevent long-term complications.

Introduction

The neonatal arterial switch procedure (ASO) introduced by Norwood and Castaneda demonstrated that corrective neonatal surgery could be performed with low mortality and it is now the preferred surgical option for transposition of great arteries with or without an intact ventricular septum ventricular septal defect [1-2]. ASO, unlike the Mustard and Senning operation provides a more anatomical & physiological correction. The surgical approach is a matter of debate in children, who present after first few weeks of age, particularly with an intact ventricular septum (IVS) because of concerns over the ability of the left ventricle (LV) to handle the systemic circulation. For patients, who present after first few weeks of life with a regressed LV, primary ASO in such subsets has found limited application [3]. In such settings, the atrial switch operation (SSO) or LV preparation followed by ASO has been recommended [4,5]. On comparing the of primary ASO with the two-stage approach, the results of late follow-up have shown increased incidence of neo-aortic regurgitation, reduced left ventricular systolic performance and right ventricular outflow tract obstruction. Dilatation of the neo-aortic root has been reported in more than two-third of patients following ASO [6,7]. The natural history of neo-aortic root dilatation in this setting is unclear; previous studies have documented indexed neo-aortic root dimensions to progressively increase over time [8]. Neo-aortic valve regurgitation may also be an important late complication, and although uncommon, the need for aortic valve repair or replacement has been reported in several series late after ASO [9].

Larger vessels especially aorta and pulmonary arteries have a common structural plan in that they are composed of three concentric coats: (a) Tunica intima: consists of the endothelial lining and its basement membrane and a delicate layer of loose sub-endothelial connective tissue (b)Tunica media: composed predominantly of elastic lamella with intervening smooth

muscle fibers along with a variable amount of reticular and elastic fibers (c) Tunica adventitia: consists predominantly of fibrous connective tissue. The internal elastic lamella is a layer of elastic tissue that forms the outermost part of tunica intima. It separates tunica intima from tunica media. Each lamella and the adjacent zone containing smooth muscle cells, which synthesize the connective tissue matrix, is a lamella unit [10]. Aorta and pulmonary artery being elastic arteries have a prominent component of elastic tissue, which can be objectively assessed by counting the number of elastic lamella. A review of literature shows that at birth there are about 35 elastic lamina arranged in the aortic media, which initially increase in number after birth and then becomes steady [10]. We therefore hypothesized that alterations in some of the histological components could be responsible for neo-aortic root dilatation and aortic regurgitation after the ASO in older patients. Therefore, the present study was undertaken to determine the risk factors associated with neo-aortic root dilatation and neo-aortic valve regurgitation with special reference to histopathological changes in the native pulmonary artery and native aorta while performing ASO. We compared these findings with historical controls in the literature that had a normal aorta and the pulmonary artery. The ultimate aim was to identify risk factors for neo-aortic root dilatation, namely histo-pathological abnormality, surgical technique, post-operative management and follow up of the conditions associated with the procedure.

Patients and Methods

83 consecutive patients undergoing ASO between 1st April 2015 & 31st October 2016 at All India Institute of Medical Sciences New Delhi, India, was included in this cohort. The study protocol was duly approved by the ethics committee of the Institute. (Ref. no: IESC/T-67;21.01.2015). Informed consent was obtained from the parents of all the patients included in the study. A note was made of all the patients' pre-operative characteristics, echocardiography findings, cardiac catheterization data (if performed), and intra operative details.

Surgery and Anesthesia

Routine anesthesia and surgical techniques with standard cardiopulmonary bypass and myocardial protection were used in all 83 patients. Intra-operative transesophageal echocardiogram (TEE) was performed, wherever possible to confirm the diagnosis and to assess the LV function and shape and to evaluate associated anomalies. Surgery was performed in the standard manner through a midline sternotomy. All the patients underwent primary ASO under moderately hypothermic (28° C) cardiopulmonary bypass.

Histopathological Analysis

At ASO, biopsy samples were obtained from the native proximal aorta (future neo RVOT) and native proximal pulmonary artery (future neo aorta). Three to four mm repetitive samples of tissue were obtained from the native pulmonary artery and the native aortic root. These were fixed in 10% neutral buffer formalin and were sent to the Department of Pathology.

Subsequently these tissues were routinely processed and paraffin blocks were prepared. Four to five micron thick sections were cut and were stained by Hematoxylin & Eosin, Verhoeff's van Gieson (VVG) and Masson trichrome (MT) stains. Elastic tissue and smooth muscle components were systematically analyzed, in the native aorta and the native pulmonary artery by VVG and MT stains respectively. Histopathological examination of native aorta and native pulmonary artery was performed and compared. Each lamella count was performed by two observers simultaneously using VVG stain under light microscope. The tissue was scored by counting the number of elastic lamina count under light microscope in the native aortic media as well as native pulmonary artery. Ultra structural analysis of one sample was performed by using electron microscope to highlight the structural components of native aorta and native pulmonary artery for collagen and elastic tissue.

After operation, subsequently, post-operative course, follow-up and follow-up echocardiographic data was noted in detail. The observed details included: aortic annulus size, aortic valve, pulmonary annulus size, pulmonary valve, dimensions of ascending aorta and aortic arch dimensions

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 23 (IBM Corp., Armonk, N.Y. USA. As is known, the sample size of any study determines the amount of sampling error inherent in a test result. Other things being equal, effects are harder to detect in smaller samples. Increasing sample size is often the easiest way to boost the statistical power of a test. To achieve a minimum statistical power of 0.9, the sample size for this study was calculated by using the formula:

$$SampleSize = \frac{Z^2 * (P) * (1 - P)}{c^2}$$

Where:

Z = Z value (e.g. 1.96 for 95% confidence level)

p = percentage picking a choice, expressed as decimal

(.5 used for sample size needed)

c = confidence interval, expressed as decimal

(e.g., .04 = ±4)

Values for normally distributed continuous variables are expressed as mean± standard deviation (SD). Nonparametric variables are expressed as median values and range. Discrete variables are expressed as percentages. Outcomes between patients and controls were compared using a paired and non-paired t test. P < 0.05 was considered statistically significant.

Results

A total of 83 consecutive patients undergoing ASO were studied. Patients were divided in 2 groups, Group A (TGA with IVS) and Group B (TGA with VSD).

A total of 52 (62.6%) had associated VSD and 31 (37.3%)

had intact ventricular septum. Other associated conditions found were Tausig-Bing anomaly (03/83, 3.6%), double outlet right ventricle (DORV, n=04/83, 4.8%), additional ventricular defect (VSD, n=4/83, 4.8%), left ventricular outflow tract obstruction (LVOTO, n= 2/83, 2.4%), pulmonary stenosis (PS, n= 2/83, 2.4%), anomalous coronary artery (n=4/83, 4.8%) and regressed LV (n=5/83, 6 %).

Out of the 83 patients, 68 (82%) were male. Mean age was 55±8 days (range 10days—5years). Preoperative baseline and clinical characteristics, intra-operative data and postoperative variables have been summarised in Tables 1 and 2. Fourteen patients presented with documented preoperative Lower Respiratory Tract Infection (LRTI). There was no increased predilection of infection in any of the 2 groups. Balloon atrial septostomy was performed in 19 (61.3%) of 31 children with IVS. These patients were operated within 2 weeks of the Balloon atrial septostomy.

Table 1: Pre-operative Baseline characteristics of the patients.

Characteristics	All Patients n=83	TGA-Intact Ventricular Septum(IVS) n=31	TGA-Ventricular Septal Defect(VSD) n=52
Age (months), median (range)	10days- 5 years	10days- 9months	27days- 5years
Gender			
Male	68	27	41
Female	15	4	11
Associated malformations			
Tausig Bing anomaly	3	0	3
Double Outlet Right Ventricle	4	0	4
Additional Ventricular septal defect	4	0	4
Left Ventricular Outflow Tract Obstruction	2	0	2
Pulmonary Stenosis	2	0	2
Anomalous Coronary Artery	4	1	3
Regressed Left Ventricle	5	4	1
Lower Respiratory Tract Infection	14	8	6

Table 2: Intra-operative and Post-operative data

	TGA-Intact Ventricular Septum(IVS)	TGA-Ventricular Septal Defect(VSD)
Intra-operative Data		
Aortic Cross Clamp time (min) (mean±SD)	70.2±8.8	76.5±8.5
Cardio Pulmonary Bypass time (min) (mean±SD)	98.7±13.8	106±18.7
ICU Stay		
Mechanical Ventilation (hours) (mean±SD)	63.8±6.5	62.5±7.9
Inotropic support (hours) (mean±SD)	82±29.1	84±25.1
ICU Stay (days) (mean±SD)	4.3±1.7	4.7±3.3
Hospital Stay (days) (mean±SD)	12.5±3.8	12.8±3.6
Early deaths		
Left Ventricle Failure	1	3
Sepsis	2	3

Histopathological Evaluation

Histopathological examination of native aorta and native pulmonary artery was performed and compared. Elastic tissue and smooth muscle components were systematically analyzed in the native aorta and the native pulmonary artery by VVG and MT stains respectively. Lamella count was evaluated in each case (Native Aorta (NA) and Native Pulmonary Artery (NPA), simultaneously by two individuals.

We grouped the patients according to their age in various age groups (Table 3).

Twenty-eight patients who were below the age of one month, thirty-two patients were between >1-3 months, ten patients were between > 3-6 months of age, five patients were > 6-9 months, four patients were > 9-12 months, four patients were above 12 months of age.

We counted the number of elastic lamina in aortic media under light microscope and plotted a graph of the numbers according to the advancing age.

Table 3 shows the lamella counts in native aorta and native pulmonary artery according to age and groups. Because there was minimal variation of lamella count in aortic media in specific age group we have presented the mean values.

Figure 1 depicts the graphical distribution of patients in six age groups (X-axis) and their corresponding lamella counts (Y-axis) in native aorta and native pulmonary artery respectively. Light microscopic evaluation did not reveal any evidence of cystic medial necrosis (mucoid extracellular matrix accumulation), smooth muscle disarray or loss of smooth muscle nuclei (H & E: Figures 2a and 2b; VVG: Figure 3).

Table 3: Showing elastic lamella count in Native Aorta & Native Pulmonary Artery in various age groups

Age	No. of patients	Elastic Lamella Count in Native Aorta	Elastic Lamella Count in Native pulmonary Artery
Up to 1 month	28	57 ± 3	56 ± 3
> 1-3 months	32	53 ± 3	52 ± 4
> 3-6 months	10	48 ± 3	47 ± 4
> 6-9 months	5	45 ± 2	43 ± 2
> 9-12 months	4	44 ± 3	42 ± 3
> 12 months	4	43 ± 2	41 ± 2

Figure 1: Graphical representation of elastic lamella count in native aorta and in native pulmonary artery

Ultra structural examination of native aorta and native pulmonary artery was done in a one patient using electron microscope (Figure 4). There was no significant ultra-structural alteration. Elastic tissue component in a collagenous background was seen (Figures 5 and 6).

There was minimal difference between the Group A and Group B in terms of aortic cross clamp and cardiopulmonary bypass times but there was no difference in duration of mechanical ventilation, inotropic support, ICU stay or hospital stay. Extracorporeal membrane oxygenation support (ECMO) was instituted in 8.4% (7/83) patients. Special precaution was needed while weaning the children with regressed LV from mechanical ventilation. In five children, the LV was unable to maintain an adequate cardiac output that was needed to support the work of breathing while weaning. These children required mechanical ventilation for a prolonged period. All of these children recovered over a period of 1 week and were slowly weaned off from mechanical ventilation. Gradual weaning from the ventilator and inotropic support depending on the hemodynamic response was the cornerstone in the management of these patients.

Figure 2a: Photo micrograph showing light microscopic features of native pulmonary artery, H&E (10X)

Figure 2b: Photo micrograph showing light microscopic features of native aorta, H&E (10X)

Figure 3: Light microscopic depiction of VVG stain (10X) high lighting elastic lamella of native pulmonary artery

Figure 4: Light microscopic depiction of VVG stain (10X) high lighting elastic lamella of native aorta.

Figure 5: Electron microscopic view of native aorta showing collagen.

Figure 6: Electron microscopic view of native pulmonary artery showing elastic tissue in the vessel wall.

The improvement in the surgical outcome as seen has been due to precise expertise in intra-operative technique and postoperative intensive care management of such critically ill infants, including the use of ECMO.

Discussion

Since, the arterial switch operation (ASO) was first described by Jatene and his colleagues in 1975. It has become the surgical procedure of choice for repair of transposition of the great arteries (TGA) [11]. Peri-operative mortality has significantly reduced in the more recent era, and several case series have described with good long-term survival in patients up to 30 years after the ASO [12]. However, these long-term studies have also revealed important late complications that contribute to late morbidity and the need for reoperation. These late complications include coronary artery insufficiency, right ventricular outflow tract obstruction, and problems with the native pulmonary root and pulmonary valve in the systemic position functioning as the neo-aortic root and the neo-aortic valve, respectively [13-15]. Dilation of the neo-aortic root has been reported in more than two-thirds of patients after ASO [16]. The natural history of neo-aortic root dilation in this setting is unclear. Previous studies have demonstrated data, with reports of the indexed neo-aortic root dimensions progressively increasing over time [17]. Neo-aortic valve regurgitation may also be an important late complication, and although uncommon, the need for aortic valve repair or replacement has now been reported in several series late after

ASO [18]. The function of the aortic root, including the pulmonary valve, that has become the new aortic valve, plays a crucial role in the long-term follow-up after the ASO. Disproportionate dilatation of the neo-aortic root and insufficiency has been reported [19]. The technique of the ASO however introduces possible growth interference at different levels, such as the pulmonary-aortic anastomosis and the introduction of aortic tissue due to coronary implantation in neo-aorta. This interference may have detrimental effect on the aortic valve, the neo-aortic (pulmonary) sinuses and the aortic root [20]. In the present study, we sought to identify the possible aetiology of neo-aortic root dilation and neo-aortic valve regurgitation in patients with TGA repaired with ASO at our institution. We also intended to determine the risk factors for the development of the late complications. The reason for analysing the tissue of native pulmonary artery and native aorta was to observe for any histopathological changes in them. We observed the changes in each tissue and compared the neo-aortic and neo-pulmonary specimens and compared them with varying ages at ASO. There is paucity of data on the histopathological changes of NA and NPA in the available literature. To the best of our knowledge, there is no report on such changes published in the English literature therefore we were unable to compare these results with any other case series. Our study did not show any significant histological changes like cystic medial necrosis (MEMA), smooth muscle disarray, loss of smooth muscle cell nuclei, in any of the specimen of NA and NPA. The elastic lamella count in NA and NPA did not show any significant difference; however, there was a trend of decrease in elastic lamella count, as compared to advancing age of the patient, during primary surgical intervention. The normal elastic lamella count of NA is around 35 at birth [21-22]; contrary to these observations, we found an increase in elastic lamella count of NA and NPA in patients of d TGA. This has therapeutic implications because if the ASO is performed in older patients, there is increased predisposition to dilatation of the aortic root because of a gradual diminution of the elastic lamella in these patients.

Study Limitations

This was not a case controlled study and the patients undergoing the ASO were not matched with age and gender matched healthy controls. However, this study is unique because it provides a baseline data on histopathological findings in d-TGA and future studies can use these comparisons which we made to address the issue of clinical relevance of our findings.

Conclusion

We did not observe any differences in the elastic lamella counts in both the neo-aorta and the neo-pulmonary artery in patients undergoing the ASO in patients in their neonatal period. However with increasing age, the lamella counts were found to show a progressive decrease in the neo aorta and neo-pulmonary artery. Whether the increment in elastic lamella count in d TGA in neonates is a remodeling process due to underlying congenital disorder or not, remains unanswered. A timely ASO can therefore be advocated to reduce the incidence of neo-aortic dilatation and neo-aortic insufficiency.

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