

Morphometry of the coronary artery and heart microcirculation in infants

A. Avirmed, A. Auyrzana, D. Nyamsurendejid, E. Tumenjin, S. Enebish, D. Amgalanbaatar

Department of Anatomy, Health Sciences, University of Mongolia, Ulaanbaatar, Mongolia

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Knowledge of morphometric quantities of coronary arteries in infants is an increasingly vital component in managing congenital and acquired heart disease. Because of considerable heterogeneity of coronary vasculature, what is considered atypical and aberrant or insignificant anatomy is often unclear. The purpose of our present study is to define normal infant anatomy. This was done by focusing on the segment analysis of coronary arteries in infants. Segment analysis was used to define an accurate definition of the length and diameter of the coronary network. The lengths, widths, and numbers of collateral branches of the coronary arteries were measured. The coronary vessels of 40 infant hearts were visualised postmortem by injection of the coronary arteries with X-ray opaque dye for the imaging study. Also, black ink cast and silver impregnation specimens were studied. The longest segment of the circumflex branches of left coronary arteries was the second; the lengths were $9066.6 \pm 1828 \mu\text{m}$. The length of I, III, and IV were $7366 \pm 378.7 \mu\text{m}$, $7536.6 \pm 1533.8 \mu\text{m}$, $4476.6 \pm 690.9 \mu\text{m}$, respectively. The lengths of the circumflex branch of the coronary artery were longer than that of the others; it is joined with the anterior interventricular branch of the coronary artery in the dorsal wall of the left ventricle. Rates of branching and ramification were low, and the number of lateral branches was low. (Folia Morphol 2012; 71, 2: 93–99)

Key words: heart, coronary arteries, black ink, X-ray examination, morphometric study

INTRODUCTION

The human coronary arteries and their branching characteristics have been the subject of particular attention among many researchers [13, 15, 17–22, 25, 30, 31, 33–37]. The finer details may be appropriate for the interest of anatomists and radiologists. In clinical practice, however, surgeons need only to be concerned with some of the more constant and important features. The branching characteristics of the coronary arterial tree play an important role in cardiac surgeries such as a heart pacemaker implantation, angioplasty, and even in stent placement [16].

The main branch of the right coronary artery (RCA) was short at the base of the heart. In newborns, lateral branches of the RCA were short, scattered, and curved. Analysis of the data suggests a new anatomical system for classifying the vasculature of the coronary arteries in newborns [8].

It is difficult to evaluate the microcirculation of the heart in infants. The available knowledge base is inadequate at present. The black ink cast technique has been applied most frequently to the studies of microcirculation. In the present study we focused on learning about the architecture of small coronary arteries in infants. The eventual focus will

Address for correspondence: A. Avirmed, Department of Anatomy, Health Sciences, University of Mongolia, Street of Choidog, 976 Ulaanbaatar, Mongolia, e-mail: avirmed_baba@yahoo.com

be on the modification of the silver impregnation method proposed by Kuprianov, in which the anatomical sections are pre-treated with silver nitrate. This procedure enhances the penetration and improves the detection of the structures of the walls of blood micro vessels [1–6].

In accordance with guidelines for research involving human subjects or human biological materials, the aims and procedures of the study were approved by the ethics committee of the Health Sciences University of Mongolia.

MATERIAL AND METHODS

The research study was implemented at the Department of Morphology, Health Sciences University of Mongolia, Maternal and Child Health Research Centre, Mongolia and the National Forensic Medicine Bureau of Mongolia. The study obtained 40 human hearts (19 male and 21 female infants) from cadavers of infants who died with non-cardiovascular disease. Thanks to the cooperation of organisations mentioned above we fulfilled the following criteria for obtaining hearts: (1) We obtained organs within 4 hours after death, and after the above-mentioned ethical criteria were fulfilled, the hearts were examined; and (2) If coronary arteries had any macroscopic signs of congenital anomaly, the organ was excluded from the study. The morphometric study was done by the method recommended by B.B. Bunnak (1941), A.I. Abrikosov (1936), and by G.G. Avtandilov (1936).

To reach the goal of defining the microcirculation, we used the black ink cast method in some hearts to assess the microcirculation, the modified silver impregnation method in some of the hearts to define the wall structure of the micro blood vessels, and the coronary angiography method in the rest of the hearts to determine the anatomy of the coronary arteries and their main branches.

Although the black ink cast method was used to define microcirculation, it did not reveal information about the wall structure of the heart micro blood vessels. The silver impregnation technique is most frequently applied for this purpose. It more effectively evaluates wall structure of the blood micro vessels.

We used the coronary angiography method in some hearts to determine the great arteries such as RCA and left coronary artery (LCA) and its derived branches.

Coronary angiography method

The heart was isolated and immediately processed. The ascending aorta was opened and 2–3 glass

cannulas were introduced into the RCA and LCA. The cannulas were ligated tightly in the bulbous aorta where the coronary artery originated and connected to a plastic connector. One end of each tube was connected to a 10-mL syringe. We perfused the cardiac vascular system with 10 mL of 5% contrast media of (10.0 g glycerin, 1.0–2.0 g PbO_2) at 80–100 mm Hg pressure (B. Dagdanbazar, B. Purevsuren). In some selected cases barium sulphate was used as the opaque media. Then X-ray films were taken using an URD-110 X-ray unit. The methods of opening and taking X-ray films were first described by Reiner and Schlesinger. All the procedures were performed under the control of an X-ray technician.

Black ink cast

By the method of Ogner, the first cut was performed at the level of the pericardial sac. Then the pulmonary artery was removed and the ascending aorta was cut through the anterior and posterior walls. Then cannulas were introduced into the RCA and LCA. The great vessels were tied to prevent black ink from flowing out of the vessels. Also, we put cannulas into the RCA and LCA, and the cannulas were ligated tightly. Injection with a water suspension of black ink (1:3) was performed under manometric control. To improve the results, the ink was filtered three times prior to the injection. After the infusion procedure with black ink, the cannulas were removed and the vessels were ligated.

After fixation in 10% formaldehyde for 14 days, the heart tissue was cut with a specially adapted circular saw into 1 cm³ blocks and then into 30–60 μm thin blocks. In this way we studied the microcirculation of the heart.

Silver impregnation method by Kuprianov

The heart tissue was cut with a specially adapted circular saw into 1 cm³ blocks and then into 30–60 μm thin blocks. The following steps were used for the silver impregnation method of Kuprianov.

1. Fix the heart tissue blocks in 2% formaldehyde (PH = 7.2) for one day.
2. Rinse with tap water 2–3 times.
3. Gently submerge in distilled water 2–3 times.
4. Then the specimens were stained in a 10–15% silver nitrate solution and transferred into a container at 37°C.
5. The specimens were rinsed with distilled water for 3 minutes.

6. Specimens were kept in 1% formic acid (PH = 7.2) until the remnants of soft tissue could not be seen.
7. The specimens were kept in silver nitrate for 2 minutes.
8. After that the specimens were pre-treated in 0.5% formic acid
9. The specimens were then placed in 0.5% formic acid until the colour turned yellow. If the specimens were thin, they were kept until they turned a brown colour.

Data management

Data analysis was done with a computer program, SPSS version 11. A p value was used to indicate statistical significance for all the estimated parameters.

RESULTS

We assessed the diameters of arteria coronaria dextra (RCA), ramus circumflexus (circumflex branches of LCA), and ramus interventricularis anterior (anterior interventricular branches of LCA) using segment analysis, according to WHO recommendations. We divided the whole vessel into 4 segments and measured the diameter, length, and number of collateral branches of each segment. The lengths and widths of each segment were calculated using the following formula:

$$t = \frac{M_1 - M_2}{m_1^2 - m_2^2}$$

where: t — student t test; M_1 — arithmetic mean at the beginning of the vessel; M_2 — arithmetic mean at the end of the vessel; m_1 — error of the arithmetic mean at the beginning of the vessel; m_2 — error of the arithmetic mean at the end of the vessel.

Arteria coronaria dextra (RCA)

The RCA originates from the bulbous of the aorta and runs in the sulcus coronarius cordis to reach the crux (junction of the atrioventricular groove and the posterior interventricular sulcus) of the heart. It supplies blood to the inferior (diaphragmatic) right ventricular wall and often the posterior one third of the interventricular septum as well as the free wall of the right ventricular through its right ventricular (acute marginal) branches.

The main branch of the RCA was short at the base of the heart. In infants, the lateral branches of the RCA were short, scattered, and curved. This produced poor blood supply to the apex of the heart.

The number of lateral branches of the RCA was increased. The longest segment was segment I; its length was $12013.3 \pm 601.4 \mu\text{m}$ and its diameters were $963.3 \pm 92.5 \mu\text{m}$; the lengths of II, III, and IV were $8953.3 \pm 949.3 \mu\text{m}$, $10143.3 \pm 3530 \mu\text{m}$, and $7196.6 \pm 2542.2 \mu\text{m}$, respectively; the diameters were $793.3 \pm 92.5 \mu\text{m}$, $623.3 \pm 92.5 \mu\text{m}$, and $453.3 \pm 92.5 \mu\text{m}$, respectively. The diameter of the collateral branches of the RCA ranged from $113.3 \pm 23.1 \mu\text{m}$ to $736.6 \pm 92.5 \mu\text{m}$.

We identified the correlation between each of the lengths of the I, II, III, and IV segments and the diameters of the I, II, III, and IV segments of the RCA. The first and second segments showed a negative correlation between length and diameter ($r = 0.42, 0.97$), but the III and IV segments showed a positive correlation between the length and diameter ($r = 0.3, 0.93$) of the RCA.

The left main coronary artery

The left main coronary artery branches off the bulbous aorta, then it further divides into two major branches: anterior inter-ventricular branch and a circumflex branch. The left main coronary artery was not clearly distinguished with the LCA in adults. For this reason we measured the LCA via those two arteries.

Ramus circumflexus (circumflex branch of LCA)

The circumflex branch of the LCA courses along the left sulcus coronarius cordis, around the obtuse margin, and continues posteriorly toward the crux of the heart. The circumflex branch of the LCA reaches the crux of the heart and supplies the posterior wall of the left ventricle (Fig. 1).

Sometimes it joins with the posterior interventricular branch of the coronary artery in the dorsal wall of the left ventricle. The rates of branching and ramification were low and the number of lateral branches was low. The longest segment of the circumflex branch of the LCA was the second; the length was $9066.6 \pm 1828 \mu\text{m}$ and the diameter was $736.6 \pm 46.2 \mu\text{m}$. The lengths of I, III, and IV were $7366 \pm 378.7 \mu\text{m}$, $7536.6 \pm 1533.8 \mu\text{m}$, and $4476.6 \pm 690.9 \mu\text{m}$, respectively, and the diameters were $906.6 \pm 46.2 \mu\text{m}$, $566.6 \pm 46.2 \mu\text{m}$, and $396.6 \pm 46.2 \mu\text{m}$, respectively. The lateral branches increased in number in the fourth segments. The diameters were ranged from $141.6 \pm 23.1 \mu\text{m}$ to $566.6 \pm 46.2 \mu\text{m}$. We identified the correlation between each of the lengths of the I, II, III, and IV segments and the diameters of the I, II, III, and IV segments of

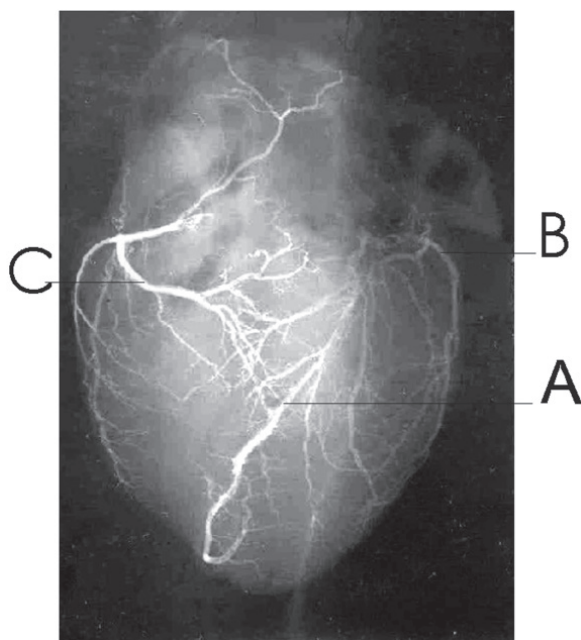


Figure 1. The X-ray film of coronary artery in infants; anterior position; A — anterior interventricular branch of the left coronary artery; B — circumflex branch of the left coronary artery; C — posterior interventricular branch of the right coronary artery.

the circumflex branch. The first segment showed a negative correlation between length and diameter ($r = 0.52$), but the II, III, and IV segments showed a positive correlation between the length and diameter ($r = 0.3, 0.66, 0.93$) of the circumflex branch.

Ramus interventricularis anterior (anterior interventricular branch of LCA)

The anterior interventricular branch is longer than other branches and supplies blood to the 2/3 part of the septum interventricularis. It gets to the apex of the heart then supplies the posterior wall of the left ventricle. The longest segment was the second segment; the length was $24820 \pm 6921.2 \mu\text{m}$ and the diameter was $623.3 \pm 46.2 \mu\text{m}$. The lengths of I, III, and IV were $20513.3 \pm 5972.5 \mu\text{m}$, $5043.3 \pm 1139.9 \mu\text{m}$, and $3570 \pm 764.4 \mu\text{m}$, respectively; the diameters were $793.3 \pm 46.2 \mu\text{m}$, $453.3 \pm 46.2 \mu\text{m}$, and $283.3 \pm 46.2 \mu\text{m}$, respectively. The lateral branches increased in number in the first segments. The diameters ranged from $113.3 \pm 23.1 \mu\text{m}$ to $736.6 \pm 46.2 \mu\text{m}$.

We identified the correlation between each length of the I, II, III, and IV segments and the diameter of the I, II, III, and IV segments of the anterior interventricular branch. The first segment showed a negative correlation between length and diameter ($r = 0.41$), but the II, III, and IV segments showed

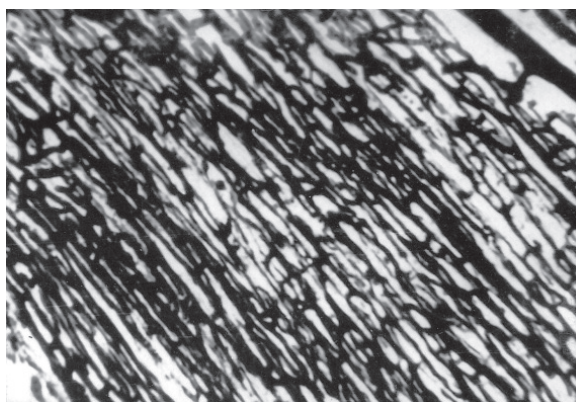


Figure 2. Dichotomous deletion of arteries in external longitudinal layer of the right ventricle in infants. Black-ink cast (1:3), magnification $\times 40$.

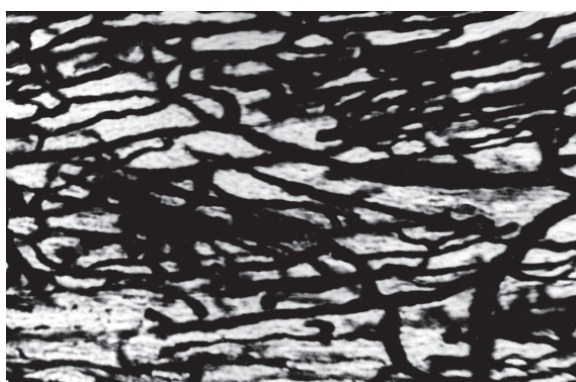


Figure 3. Artery network in circular layer of the left heart ventricle in infants. Black-ink cast (1:3), magnification $\times 30$.

a positive correlation between the length and diameter ($r = 0.41, 0.3, 0.54, 0.99$) of the anterior interventricular branch.

Arteriole and meta-arteriole

The arterioles were distributed at an acute angle to the muscle fibres. Therefore, the arterioles were not distinguished from each other in their diameters. Those arterioles with tiny diameters gave off a dichotomous branch before ramification into meta-arterioles (Fig. 2). This branching pattern was seen in the circular and internal longitudinal layer of the left myocardium (Fig. 3). In silver impregnated specimens, a layer of smooth muscle cells with their nuclei was seen in the walls of the arterioles. This was dependent on the diameter of the arterioles. As it narrowed, the distribution of the nuclei changed from an irregular shape into a spiral shape (Fig. 4). The diameters of the arteriole and meta-arterioles in the layers of the heart myocardium in infants are shown in Table 1.

Table 1. The lengths and diameters of blood micro vessels of the cardiac muscle in infants

Values	Myocardium of left ventricle			Myocardium of right ventricle			Inter ventricular septum	
	External	Circular	Internal	External	Circular	Internal	Right part of heart	Left part of heart
The diameters of arterioles [μm]	23 \pm 0.3	23 \pm 0.2	23 \pm 0.4	24 \pm 0.3	23 \pm 0.3	23 \pm 0.2	22 \pm 0.2	24 \pm 0.4
The diameters of meta-arterioles [μm]	12.7 \pm 0.2	12.2 \pm 0.3	12.7 \pm 0.2	12.9 \pm 0.2	14 \pm 0.2	11.6 \pm 0.2	13 \pm 0.3	13 \pm 0.2
The diameters of capillaries [μm]	4.6 \pm 0.2	4.5 \pm 0.14	4.7 \pm 0.2	4.7 \pm 0.2	4.8 \pm 0.2	4.6 \pm 0.2	4.4 \pm 0.3	5.0 \pm 0.2
The lengths and widths of capillary loop [μm]	18 \pm 0.5	15.7 \pm 0.5	17 \pm 0.8	16 \pm 0.6	16 \pm 0.4	17 \pm 0.5	14 \pm 0.5	17 \pm 0.6
	82 \pm 0.7	82 \pm 1.3	83 \pm 1.8	74 \pm 1.8	79 \pm 1.8	81 \pm 1.3	73 \pm 3.6	79 \pm 3.1



Figure 4. Bifurcation of artery in external longitudinal layer of the left heart ventricle in infants. Black-ink cast (1:3), magnification $\times 30$.

Capillaries

The capillaries of the right ventricle were similar to those in the left ventricle. As reported below, they formed a loop-like network. This network of capillaries is frequently oriented parallel to the muscle fibres. In the myocardium of the right ventricle, the capillaries were of diameter approximately $4.6 \pm 0.2 \mu\text{m}$ in the internal longitudinal layer of the myocardium. In the external oblique layer of the right myocardium the capillaries were $4.7 \pm 0.2 \mu\text{m}$, and in circular layer the diameter was $4.75 \pm 0.2 \mu\text{m}$. The capillaries emitted a series of tree-like dichotomous branches; its diameters are described in detail in Figure 2 and Table 1.

Capillaries emerge from arterioles or meta-arterioles and are not on the direct flow route from arteriole to venula. At their sites of origin, there is a ring of smooth muscle fibres called sphincter arteriola precapillares that controls the flow of blood entering a capillary.

DISCUSSION

The key observation of this study is the segment analysis of RCA and LCA and their derived branches, such as the circumflex branch and anterior interventricular branch of the LCA in infants. On other hand, the study facilitated the classic description of coronary arteries in infants in terms of diameters, lengths, number, and their derived quantities. The main feature of our segment analysis is that it provides a means of using the average morphometric parameters, those available to the management of the cardiac surgery and radiology, also to make cardiac simulations [26–29, 32]. The segments of vessels help to provide morphometric measurements with statistical significance. There are many excellent studies

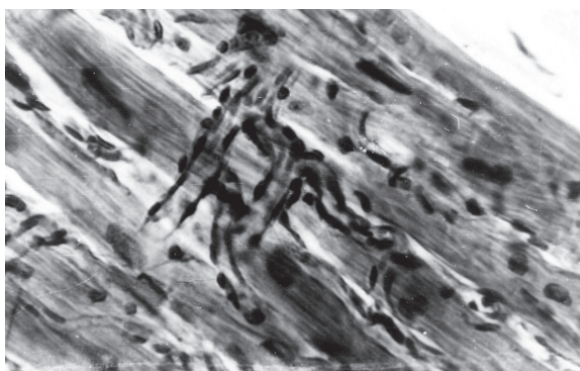


Figure 5. Meta-arterioles begin from the arterioles in longitudinal layer of left ventricle in infants. Silver impregnation of the vessels was performed using one of the recommended methods by Kuprianov, magnification $\times 150$.

done in different species, which have dealt with segment analysis of the myocardium [1–6, 10, 12, 14–23, 25]. In particular the segment analysis of the coronary artery has been evaluated in several studies [33–37], but those studies do not provide the necessary information for appropriate systematisation of coronary artery measurements.

Morphometric quantities of microcirculation in infants are studied by modified silver impregnation method. Understanding the heart microcirculation is important in guiding management of sudden death of infants; also, it enhanced understanding to improve operative outcomes [7, 24]. We determined the anatomical data of microcirculations in infants, studied by silver impregnation (Fig. 5). The arterioles gave off a dichotomous branching pattern in the internal longitudinal layer of the left myocardium. This arrangement was also reported by Phipps.

In the myocardium of the right ventricle, the capillaries were of diameter approximately $4.6 \pm 0.2 \mu\text{m}$ in the internal longitudinal layer of the myocardium. In the external oblique layer of the right myocardium the capillaries were $4.7 \pm 0.2 \mu\text{m}$ and in circular layer the diameter was $4.75 \pm 0.2 \mu\text{m}$. This is similar to the data of Zamir and Chee.

In contrast, the present results show that at least for the small arterioles of the terminal coronary bed, which supply the heart muscle, they were not identical to each other, ramified two or three times, and gave a dichotomous branch. The capillaries of the right ventricle were similar to those in the left ventricle; they formed a loop-like network. This network of capillaries is frequently oriented parallel

to the muscle fibres. This finding is a new aspect of the coronary artery distribution and is in accordance with findings reported in a recent study by Zamir (1996) although his findings refer to the RCA whereas our study was focused on the RCA and LCA. Hence, the parameters evaluated in this study will serve as a new basis for cardiovascular modelling [9, 11]. We suggest that the circumflex branch of LCA reaches the crux of the heart and supplies the posterior wall of the left ventricle. Sometimes it joins with the posterior interventricular branch of the coronary artery in the dorsal wall of the left ventricle. This is an unusual course of the circumflex branch of LCA and it does not occur in adults. This further study is planned and will be performed in the near future.

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