Comparison of Heart Valve Circumference Examined Before and After 10% Formalin Fixation

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ABSTRACT

Objective: To compare the heart valve circumference before and after 10% formalin fixation.

Materials and Methods: The study analyzed 63 Thai human cadaveric hearts. Each heart valve circumference was separately measured in the fresh state by specifically designed equipment. After that, the hearts were fixed in 10% formalin for 3 days. Then each heart valve circumference was measured by the same equipment and by the thread and ruler technique. The results were analyzed using SPSS package to find the association between the heart valve circumference before and after formalin fixation.

Results: This study showed that the average circumferences of the heart valve measured in the fresh state were 13.329 cm in the tricuspid valve, 10.617 cm in the mitral valve, 8.416 cm in the pulmonic valve, and 7.122 cm in the aortic valve. The average circumferences of the heart valve measured after 10% formalin fixation were 11.019 cm in the tricuspid valve, 8.714 cm in the mitral valve, 6.751 cm in the pulmonic valve, and 6.089 cm in the aortic valve. The average ratios of the heart valve circumference measured fresh and after 10% formalin fixation were 0.8267 in the tricuspid valve, 0.8235 in the mitral valve, 0.8050 in the pulmonic valve, and 0.8573 in the aortic valve. There were significant differences in the heart valve circumference between the fresh state and after formalin fixation ($p < 0.001$).

Conclusion: This study revealed important information on the dimensional changes of all the formalin-fixed heart valves. We found that the heart valve shrank after formalin fixation, with the formalin-fixed hearts an estimated 0.8 times smaller than the fresh cadaveric hearts.

Keywords: Heart valve circumference; formalin fixation (Siriraj Med J 2021; 73: 478-484)

INTRODUCTION

The number of people with cardiovascular disease is increasing globally and it is now one of the leading causes of death worldwide, not only in industrial countries but also in developing countries, such as Thailand.[1,2] Valvular heart disease represents an important public health problem and its rising incidence is leading to an increased mortality rate among the general population.[3,4] Acute rheumatic fever and chronic rheumatic heart diseases, for example, were responsible for 115 deaths in 2013, leading to a death rate of 0.2 per 100,000 persons per year in Thailand; while the number of deaths had increased to as many as 226 persons in 2017, leading to a death rate of 0.3 per 100,000 persons per year.[1]

The heart valves play a significant role in controlling blood circulation between the heart chambers and systemic circulation. The four heart valves are the tricuspid valve (TV), pulmonic valve (PV), mitral valve (MV), and aortic valve (AV). The cardiac valves are affected by various factors, including genetics, aging, sex, and lifestyle, as well as by infection. Pathological changes of the heart valves can affect the circulation, such as regurgitation

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and stenosis. People with severe heart valve disease may need to undergo a surgical procedure to replace the damaged valve with an artificial heart valve.

Knowledge of the cardiac anatomy plays an important role in cardiothoracic surgery as well as in understanding the pathophysiology of various cardiac diseases. Several studies from a variety of countries and based on different ethnicities have been performed to find the average heart valve circumference to aid more accurately estimating the circumference needed for an artificial heart valve.[5-15] In general, previous studies took measurements on formalin-fixed hearts. However, it is well known that formalin fixation can shrink fresh tissues in an unpredictable manner. Consequently, in this study we thought it would be interesting to study the comparison between the heart valve circumference before and after 10% formalin fixation. In addition, this study aimed to compare different methods of measuring the heart valve circumference.

MATERIALS AND METHODS

This study was approved by the Human Research Ethics Committee of the Faculty of Medicine Siriraj Hospital, Mahidol University (Si 483/2020(IRB2) on July 20, 2020). This descriptive study included a total of 63 hearts, which were collected from Thai adult male and female cadavers at the Department of Forensic Medicine, Faculty of Medicine Siriraj Hospital from 1 August 2020 to 31 December 2020. Hearts with disease, trauma, or postmortem changes were excluded from this study.

The heart samples were collected from dead bodies with known age, sex, weight, and height. The heart was removed from the pericardial cavity. The great vessels connecting the heart with the other organs were separated and cut without damage to the valvular structure. Then, the heart chamber was cut in a horizontal line through the left and right ventricle, and blood clots were removed to show all the heart valves. Measurement of each valve circumference was performed using specially designed equipment (Method 1). This cone-shaped equipment (Fig 1) was fully accredited and designed to directly measure the exact size of a heart valve circumference.

Next, each heart was fixed in 10% formalin solution for three days. A thread was used to hold the heart within the formalin to maintain the normal shape of the heart. The circumference of all the formalin-fixed heart valves was observed again and measured with the same equipment (Method 2). Subsequently, the heart chambers were routinely opened to expose the opened valve annulus, then the thread was placed along the boundary of each valvular annulus and measured with a ruler (Fig 2) (Method 3). All the data were recorded in millimeters to 2 decimal points.

All the data were collected in Excel 2016 and analyzed using the SPSS package (PASW 18.0 for Windows). Quantitative data according to the average and the standard deviation of the data were calculated. To determine whether the fresh and formalin-fixed heart samples, as well as the measurements obtained by the direct and thread and ruler techniques, were statistically different ($p < 0.05$), and ANOVA or non-parametric Freidman tests were performed on the data. Lastly, assessment of the intra-observer and inter-observer variability in the measurements was also conducted with intra-class correlation coefficients.

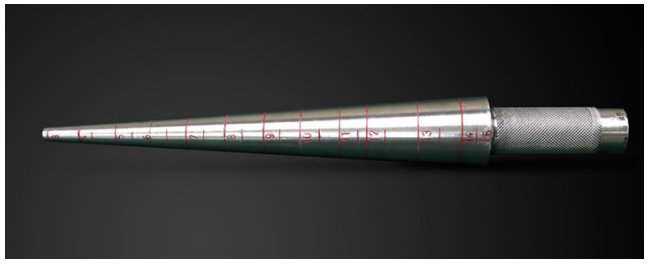

Fig 1. Specially designed cone-shaped equipment.

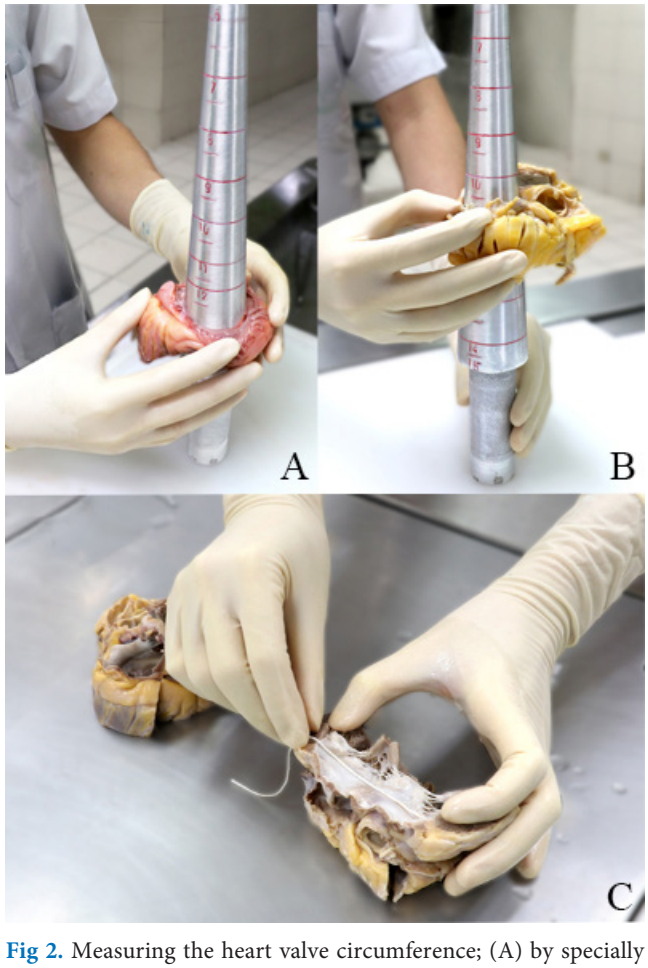

Fig 2. Measuring the heart valve circumference; (A) by specially designed equipment in the fresh state; (B) by specially designed equipment after formalin fixation; (C) by the thread and ruler method after formalin fixation.

RESULTS

In this study, 63 hearts were examined to observe the normal values regarding the heart valve circumferences. The males included in this study were aged between 14 and 64 years old, and the females between 16 and 68 years old. In the study sample, the mean age of the male and female cadavers was 34.31 ± 14.2 years old and 41.58 ± 15.85 years old, respectively. A summary of the descriptive data from the heart valve circumference of the mixed group (both male and female) is provided in Table 1. All the measurements were found to be normally distributed. This study showed that the average circumference of the tricuspid valve was the highest, while the average circumference of the aortic valve was the lowest. Among the measurements of the heart valve circumference, this study indicated that the heart valves after 10% formalin fixation were smaller than those before fixation.

Differences between the measurements were also explored. Comparisons of the measurements taken before and after 10% formalin fixation with the different methods are shown in Table 2. The circumference ratios of all the valves after 10% formalin fixation compared with those before 10% formalin fixation were less than 1 throughout all the valves ($p < 0.001$ by t-test). The average ratios of heart valve circumference measured by the specially designed equipment before and after 10% formalin fixation were 0.8267 in the tricuspid valve, 0.8235 in the mitral valve, 0.8050 in the pulmonic valve, and 0.8573 in the aortic valve (Table 2).

Different plots were conducted to explore the agreement between Method 2 and Method 3 (Fig 3). A Bland-Altman plot was used in this study since neither of the two methods are reference techniques. Almost all the measurement values were within a 95% confidence interval of the mean difference, implying that the variability of the measurements was low. In addition, intra-class correlation coefficients (ICCs) were determined to identify the relationship between the direct measurement and the measurement by the thread and ruler method. This study showed excellent reliability between the two types of measurement. The ICC of the tricuspid valve was 0.952, the mitral valve was 0.958, the pulmonic valve was 0.947, and the aortic valve was 0.956 (Fig 3).

The reliability of this study, particularly the intra- and inter-observer variations, was also evaluated to exclude the bias of heart valve circumference measurement by human error. Measurements were performed on 15 samples using all the measurement methods and the results compared with previous results. Based on Kappa statistics, the average values of ICC were more than 0.9 for both the intra- and inter-observer reliabilities. Therefore, this study showed excellent reliability of all the measurements.

TABLE 1. Descriptive statistics for all the heart valve circumferences; Method 1 = Measurement with specially designed equipment before 10% formalin fixation; Method 2 = Measurement with specially designed equipment after 10% formalin fixation; Method 3 = Measurement with the thread and ruler technique after 10% formalin fixation.

Heart valve (n = 63) Type	Method	Mean (cm)	Standard deviation (cm)	Minimum (cm)	Maximum (cm)
Tricuspid Valve (TV)	1	13.33	0.92	11.00	15.00
	2	11.02	1.10	8.60	13.70
	3	10.81	1.02	8.50	13.50
Mitral valve (MV)	1	10.62	1.13	8.20	13.50
	2	8.71	0.99	7.00	12.00
	3	8.65	0.91	7.20	12.20
Pulmonic Valve (PV)	1	8.42	1.01	6.80	10.70
	2	6.75	0.80	5.00	8.70
	3	6.66	0.70	5.30	8.10
Aortic Valve (AV)	1	7.12	0.79	5.50	9.00
	2	6.09	0.70	4.90	8.30
	3	6.00	0.69	5.00	7.80

TABLE 2. Descriptive data of the proportions among the different measurement methods.

Ratio of Different Methods	Mean	Standard Deviation	Mean of Standard Error	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
						Lower	Upper
TV_2 / TV_1	0.83	0.06	0.01	0.00	-0.17	-0.19	-0.16
TV_3 / TV_1	0.81	0.06	0.01	0.00	-0.19	-0.20	-0.17
MV_2 / MV_1	0.82	0.07	0.01	0.00	-0.18	-0.20	-0.16
MV_3 / MV_1	0.82	0.06	0.01	0.00	-0.18	-0.20	-0.17
PV_2 / PV_1	0.81	0.07	0.01	0.00	-0.20	-0.21	-0.18
PV_3 / PV_1	0.80	0.07	0.01	0.00	-0.20	-0.22	-0.19
AV_2 / AV_1	0.86	0.07	0.01	0.00	-0.14	-0.16	-0.13
AV_3 / AV_1	0.85	0.07	0.01	0.00	-0.15	-0.17	-0.14

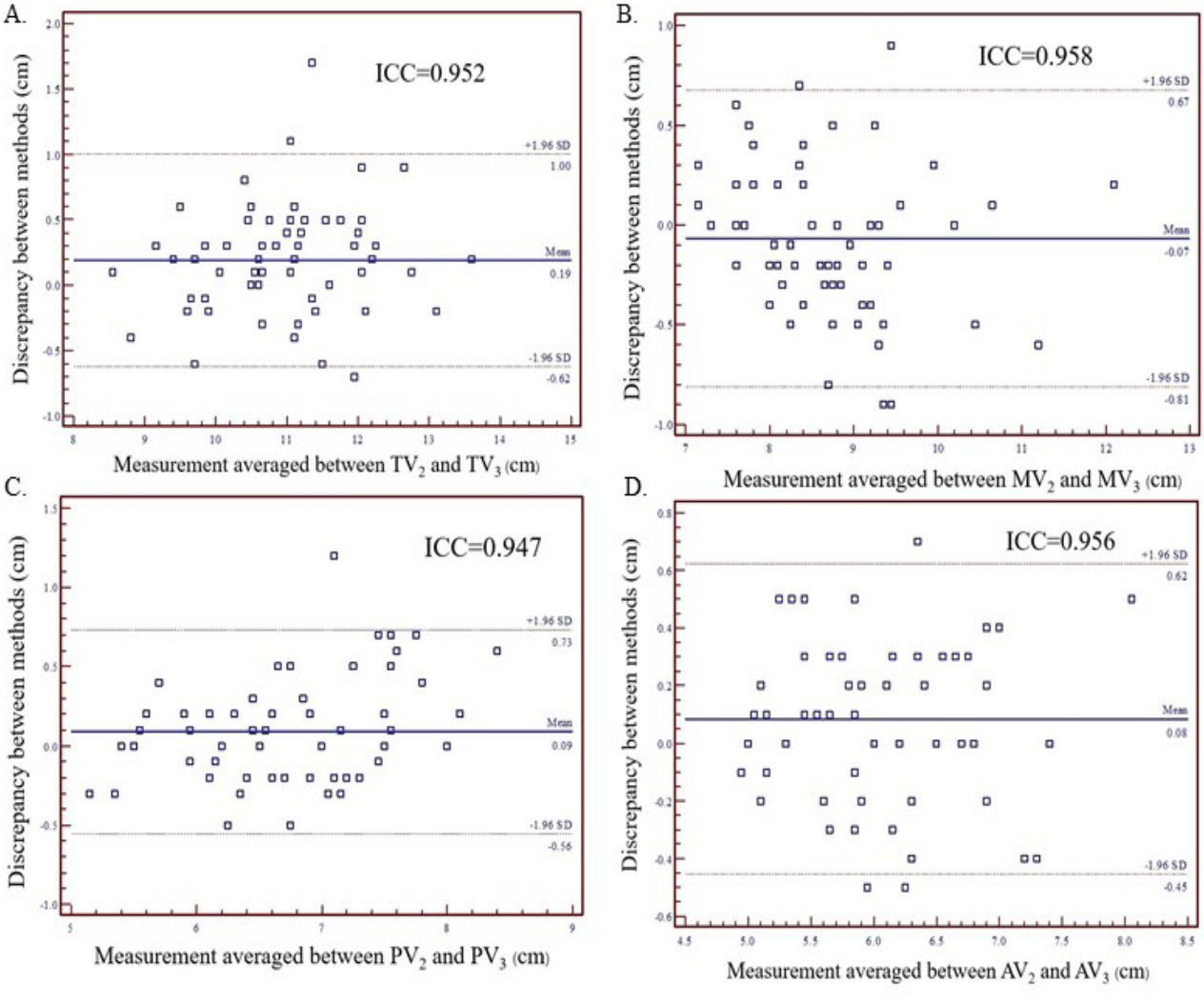

Fig 3. Differences between Methods 2 and 3: (A) Tricuspid valve; (B) Mitral valve; (C) Pulmonic valve; (D) Aortic valve; ICC: Intra-class correlation coefficient.

DISCUSSION

It is well known that tissues shrink after formalin fixation. With reference to previous studies, however, a variable reduction in the percentage of tissue shrinkage was observed.[16-20] Most of these works used different soft tissues as their samples and they reported different results. Pritt *et al*. reported that there was no significant change in 96% of the breast tumor size between the fresh and fixed states.[17] Also, Jonmarker *et al*. found no significant decrease in prostate tissue diameter after formalin fixation, but observed a significant weight loss.[18] In contrast, a different result was found by Hsu *et al.* in their study, whereby they found that the measured dimensions of the oral cavity mucosa and tongue muscle shrank by 30.7% after formalin fixation.[19] It was also reported that the mean diameter of breast cancer tissues decreased by 4.5% after formalin fixation.[20] Lastly, the average shrinkage of the maximum diameter of head and neck cancer samples was 4.4% in the study by Chen *et al.*[16] In our study, the average heart valve circumference after formalin fixation was 15-20% smaller than that before formalin fixation. Regarding the variable shrinkage reported in the aforementioned works, it is worth mentioning that possibly the constitution and type of tissue (e.g., amount of elastic tissue or fat) may have had an effect on the overall degree of shrinkage.[16]

Many studies have been carried out in formalin-fixed hearts rather than fresh human hearts.[6,9-11,13,15] Formalin is widely used to preserve tissues for routine histological examination. The most common formula is 10% formalin, consisting of 3.7% formaldehyde in water with 1% methanol.[16] When using to preserve tissues, the process includes a two-phase fixation. The first phase is the alcohol fixation phase, described as alcohol-induced dehydration and hardening of the tissue. Shrinkage of the tissue occurs during this phase. Subsequently, this is followed by the cross-linking phase formed by a cross-linking of formaldehyde and peptide. There are two factors that influence formaldehyde fixation: penetration and fixation.[21] The former is the potential of the solution to penetrate the tissue, while the latter is the ability of the formaldehyde to form cross-linking. The completion of formalin fixation is dependent on many factors, such as the temperature, pH, time, type of tissue, and the concentration of formaldehyde.[21-22] Therefore, it can be assumed that the average heart valve circumference varies among different studies using different fixative methods, as we found in this study.

Various techniques have been conducted using a variety of methods, such as the thread and ruler method, the direct measurement, echocardiography, and computer software. Currently, there is no standard method to measure heart valve circumference. With reference to previous studies[5-8,10,12,13], the thread and ruler technique was the most popular practice to evaluate the heart valve circumference. Because none of the above-mentioned studies provided data on direct measurement, no comparisons can be made. In this study, we measured the heart valve circumferences by the direct measurement using a specially designed instrument and by the thread and ruler technique. The results showed that there was excellent reliability between the two types of measurement. Thus, we recommend the direct measurement method for assessing the heart valve circumference because of its easy manipulation.

The next issue we considered was that of the variation in heart valve circumference. Westaby *et al.*[11] reported a wide individual variation in heart valve circumference and identified that the size of the heart valve circumference was unrelated to the body habitus. Many studies have performed detailed analyses of the heart valve circumferences in various populations (Table 3). However, the data in the previous studies showed some different results compared with this study. Before formalin fixation, the average heart valve circumference in this study was slightly higher than in almost all the previous studies[5-14], but lower than that reported by Tei *et al.*[15] and coincided with those reported by Jatene *et al.*[8] and Alison *et al.*[23] Nevertheless, the average heart valve circumference after formalin fixation was slightly lower or similar to the above-mentioned studies. Why there was such a difference between our study and previous studies might be explained by the different socio-economic status and population groups from which the hearts were obtained as well as by the factors affecting formalin fixation, as mentioned above.

To the best of our knowledge, this study is the first study to investigate the effects of formalin fixation on heart valve samples. Previous studies were conducted using formalin-fixed hearts and these may be different from the values from fresh hearts. In this study, an effort was made to describe this finding, which might be used to compare the valve orifice size before and after formalin fixation. Thus the data from this study can be taken as a useful guide in medical practice, especially being a reference in the diagnosis and treatment of valvular heart disease. This study should help in the choice of prosthetic replacement as well. It is realistic to use a mean circumference of each heart valve as a guide for valve surgery. The measurements that we have taken will help a surgeon to estimate the correct size of prosthesis to fit accurately in the valve orifice in a person. Further

TABLE 3. Comparison with the previous studies' heart valve measurements.

Study	Nationality	Heart status	Measurement Method	Heart valve	Circumference (cm)	
					Mean	S.D.
Ilankathir[4]	India	Fresh	Thread	TV	10.37	-
				PV	6.82	-
				MV	8.29	-
				AV	7.54	-
Deopujari *et al*.[6]	India	Fresh	Thread	MV	8.27	1.25
Jatene *et al*.[7]	Brazil	Fresh	Thread	AV	7.38	1.01
Udhayakumar and Yasawardene[11]	Sri Lanka	Fresh	Thread	AV	6.47	0.70
Alison *et al*.[22]	U.S.	Living	Echocardiograph	MV	10.70	1.46
Tei *et al*.[14]	U.S.	Fresh	Ruler	TV	13.50	0.80
				MV	11.40	0.70
		Formalin-fixed	Ruler	TV	12.20	0.80
				MV	10.70	0.50
Gupta *et al*.[8]	India	Formalin-fixed	Image analysis	MV	9.11	0.44
Nayak *et al*.[9]	India	Formalin-fixed	Thread	MV	7.92	0.50
Westaby *et al*.[10]	U.S.	Formalin-fixed	Ruler	TV	11.63	1.39
				PV	7.63	0.93
				MV	9.79	1.23
				AV	7.28	0.92
Garg *et al*.[5]	India	Formalin-fixed	Thread	PV	6.50	0.59
Lama *et al*.[12]	Nepal	Formalin-fixed	Thread	TV	11.22	0.20
				MV	9.22	1.49
The present study	Thailand	Fresh	Equipment	TV	13.33	0.92
				PV	8.42	1.01
				MV	10.62	1.13
				AV	7.12	0.79
		Formalin-fixed	Equipment	TV	11.02	1.10
				PV	6.75	0.80
				MV	8.71	0.99
				AV	6.09	0.70
			Thread	TV	10.81	1.02
				PV	6.66	0.70
				MV	8.65	0.91
				AV	6.01	0.69

studies concerning a survey of a larger sample size and comparisons with radiological and echocardiographic examinations should be conducted.

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