Ultra-structural changes of coronary arterial endothelium with myocardial bridge in human

Sann Lin Ko, Thin Thin Win, Khine Zaw Oo, Tun Tun Win, Aye Moe Moe Kyaw

ABSTRACT

Aims: The study aimed to compare the scanning electron microscopic gradings of the endothelium and calcium depositions between proximal, under-bridging, distal, and control segments of the coronary artery with myocardial bridge (MB).

Methods: The autopsy of 45 adult hearts with MB among the 243 cases was collected from North Okkalapa General and Teaching Hospital, Yangon. Then, the length and thickness of the MB were measured. The endothelial gradings and elements composition of each specimen was examined by a scanning electron microscope (Phenom Pro X) with energy dispersion X-ray spectrometry (EDS) operated at 15 kV.

Results: In the present study, 45 bridges (18.5%) were observed among the 243 hearts. They were located on the left anterior descending artery (LAD) in all cases (100%). The mean length of the MB was 18.3 ± 11.7 mm (range 1.5–59 mm). The mean thickness of the MB was 2.8 ± 1.5 mm (range 1–6.3 mm). In comparing endothelial cell gradings and calcium deposition of four segments, the proximal segment was significantly different from the under-bridging segment, distal segment, and control segment. The under-bridging segment was significantly different gradings from the distal segment (p < 0.05).

Conclusion: The proximal segment was the most endothelial damage site due to hemodynamic effect of the MB. The under-bridging segment was the least endothelial damage site which might be due to the atheroprotective effect of the MB in under segment.

Keywords: Calcium, Coronary artery, Endothelium, Myocardial bridge, Ultra-structural

INTRODUCTION

The myocardial muscle fiber over the segment of the coronary artery was known as a “myocardial bridge” (MB). The common affected location of MB was the middle parts of the left anterior descending artery (LAD) [1–4]. In autopsy studies, the frequency of MB was 4.7–86% [5, 6].

The proximal segment of MB was more prone to arteriosclerosis [7]. Angiographic studies assumed that...
MB was not associated with atherosclerosis changes of the coronary artery. There was a complex relationship between MB and major cardiovascular events that depended on the anatomical properties of MBs [6]. Meta-analysis review suggested that more studies need to find out the association between myocardial bridging and myocardial infarct, and sudden death [8].

Many researchers concluded that hemodynamic changes occurred in the different segments of the coronary artery with MB. Endothelial dysfunctions more occurred in the proximal LAD but less in the middle or under-bridging segment of LAD due to different effect of wall shear stress (WSS) and local tensile stress (TS) [9–13]. Jain et al. [14] described that the different blood flow velocities of the LAD were both systole and diastole. In recent years, there had been an increasing amount of literature with regard to the histopathology, pathophysiology, electron microscopic study, and element analysis of MB. Tohno et al. [15] described that the accumulation of the elements was different in the proximal segment of MBs and under-bridging segments of the LAD. In the experimental study, abnormal hemodynamic effect appeared in the proximal segment of the myocardial segment during high-pressure compression [16].

Therefore, the findings of the present study provided information for incidence, location, length, and thickness of the bridging segments as well as the ultra-structural findings and intimal calcification of the endothelium of the coronary artery with or without MB.

**MATERIALS AND METHODS**

In this cross-sectional analytic study, 45 hearts with myocardial bridging, males 30 (67%) and females 15 (33%), the age ranged from 24 to 85 years (mean 52 years), were collected from North Okkalapa General and Teaching Hospital during December 2017 to December 2019. Ethical approval for this study was obtained from the Institutional Review Board of Defence Services Medical Academy and Defence Services Medical Research Center (FWA-00023030, IORG-0009413, IRB-00011205). The length and thickness of MB were measured with the aid of Vernier Callipers, with 0.01 mm precision. Then, the four pieces of the coronary artery were taken in proximal (segment 1), under (segment 2) and distal segments (segment 3) of the coronary artery with MB, and segment from a coronary artery without MB in the same autopsy hearts to be used as control (segment 4).

**Scanning electron microscopy (SEM) and energy dispersion X-ray spectrometry (EDS) analysis**

The sample coronary arterial tissues were fixed in a 2.5% glutaraldehyde solution with Na-phosphate at pH 7.2. Dehydration was done with a graded ethanol series and a critical-point dryer (Quorum K850). The sample was coated with 5 nm of gold by using a sputter coater (Quorum Q150R ES). The entire endothelial surface of four segments of each specimen was examined with a scanning electron microscope (Phenom Pro X) operated at 15 kV. Energy dispersion X-ray spectrometry (EDS) examined the element deposition of each specimen. The specimens were observed by researchers and blinded the data of manipulation group.

**Statistical analysis**

Data were composed in Microsoft Excel. Data analysis was carried out by Wilcoxon signed-rank test. P value of less than 0.05 was considered to be significant.

**Scanning electron microscopic endothelium gradings (SEM grading)**

Grading “0”—This grading included normal endothelial cells and there was no defect in endothelial cell junction.

Grading “1”—In this grading, no defect was found in endothelial cell junction but the cells were unusually raised above the normal endothelium surface. Leucocytes more frequently adhered to the surface membrane. Leucocytes transition may present or not.

Grading “2”—The two endothelial cells that had wider cell junction and everted cell margin.

Grading “3”—An endothelial lesion that had isolated detachment of endothelial cells. Most of the area had a normal endothelial cell but some focal surfaces exposed subendothelial connective tissue.

Grading “4”—Most of the area (two or more focal area) was covered by subendothelial tissue.

**RESULTS**

**The location, length, and thickness of MBs in human adult autopsied hearts**

In the present study, 45 bridges (18.5%) were observed among the 243 autopsy hearts. There were located on the LAD in all cases (100%). Myocardial bridge was observed in males 30 (67%) and females 15 (33%), the age ranged from 24 to 85 years (mean 52 years). There were no double MBs in LAD. All MBs were located in the middle part of the LAD (Figure 1). The presence of a collateral branch was found in the LAD of three autopsied hearts. The collateral branch arose before the LAD entered to the myocardial bridging segment. The mean length of the MB was 18.3 ± 11.7 mm (range 1.5–59 mm). The mean thickness of the MB was 2.8 ±1.5 mm (range 1–6.3 mm). The length of the MB is described in detail in Table 1.
Scanning electron microscopic findings of endothelium at proximal, under, distal, and control segments

The scanning electron microscopic features of endothelial cells were varied in proximal, under, distal, and control segments. The mean and sum of endothelial grading scores of proximal segments were 2.02 and 91, respectively. In the case of advanced endothelial grading (endothelial gradings “3” and “4”), the subendothelial connective tissue was exposed at the detached area of the coronary arterial endothelium. The subendothelial connective tissue with the huge calcified plaque was covered at the proximal segment of the MB (Figure 2). These types of severe endothelial damage (endothelial gradings “3” and “4”) were found in the proximal segment (38%), distal segment (11%), and control segment (7%). There was no endothelial grading 3 and grading 4 in under bridging segment (segment 2).

In the endothelium of under bridging segment (segment 2), the range of endothelial grading score was “0–2.” The mean and sum of endothelial grading score was 0.67 and 30, respectively.

In the case of lower endothelial grading (endothelial gradings 1 and 2), the shape of endothelial cells was dehiscent endothelial junction or everted cell margin. There were leucocytes, probably monocytic, adherent to an intact endothelial surface. There was increased cellularity such as platelets. Most of the cells aligned along the main axis of blood flow (Figure 2).

The energy dispersion X-ray spectrometry analysis of proximal, under, distal, and control segments

In the energy dispersion X-ray spectrometry analysis, the Calcium element was found six cases in the proximal segment (segment 1), one case in segments 2 and 3, and three cases in segment 4, respectively. Iron (Fe) was detected in four cases in segment 1 and one in segment 2. The other elements such as sodium (Na), phosphorus (P), copper (Cu), and sulfur (S) were detected. In energy dispersion X-ray spectrometry analysis of case number 37, energy dispersion X-ray spectrometry (EDS) spikes showed carbon (C), calcium (Ca), oxygen (O), nitrogen

Table 1: Length of myocardial bridge among the collected human adult autopsy hearts (mm)

<table>
<thead>
<tr>
<th>Length (mm)</th>
<th>Middle part of LAD</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10 mm</td>
<td>7</td>
<td>15.6</td>
</tr>
<tr>
<td>10–19</td>
<td>19</td>
<td>42.2</td>
</tr>
<tr>
<td>20–29</td>
<td>12</td>
<td>26.6</td>
</tr>
<tr>
<td>30–39</td>
<td>4</td>
<td>8.9</td>
</tr>
<tr>
<td>&gt;40</td>
<td>3</td>
<td>6.7</td>
</tr>
</tbody>
</table>

Abbreviation: LAD: left anterior descending artery.

Figure 1: Long myocardial bridging on the left anterior descending artery. MB length (10 mm) and thickness (2 mm).

Figure 2: Scanning electron micrograph of proximal, under-bridging, distal, and control segments of case number 37. (A) All surface area covered by subendothelial connective tissue, no endothelial cell, calcified plaque (black arrow head) found at proximal segment of myocardial bridge (grade 4). (B) Normal endothelial integrity but the cells were strikingly raised above the surface (grade 1). (C) Cells detachment cannot be seen but rouge endothelial surface at distal segment of myocardial bridge (grade 1). (D) Noted rough endothelial surface (grade 1).
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(N), and phosphorus (P). The energy dispersion X-ray spectrometry for calcium (Ca) was 1.27% atomic concentration (3.66% weight concentration) and phosphorus (P) was 0.84% atomic concentration (1.88 % weight concentration) (Figure 3).

Comparing the scanning electron microscopic gradings and the energy dispersion X-ray spectrometry analysis of endothelium between four segments

Comparing to scanning electron micrographic endothelial gradings of four segments, segment 1 was significantly different from segment 2, segment 3, and segment 4. Segment 2 was significantly different from segment 3. Segment 4 was not significantly different from both segment 2 and segment 3 (p 0.05) (Figure 4).

Comparing to the energy dispersion X-ray spectrometry findings of four segments, calcium deposition in segment 1 was significantly different from segment 2, segment 3, and segment 4. Segment 2 was a similar deposition of calcium in segments 3 and 4. Segment 3 was not significantly different from segment 4 (p 0.05).

DISCUSSION

The results of the present study were consistent with the reviews of Möhlenkamp et al. [5] and Ishikawa et al. [6] demonstrated that the frequency of MB was 4.7–86%. In the present study, MBs were found over the left anterior descending branch of the left coronary artery (100%) and no MB on other coronaries arteries. These results were in keeping with previous studies [1–4]. In this study, the collateral branch arose before the LAD entered to the myocardial bridging segment. There might be collateral circulation for the MB.

The present study compared the data from the meta-analysis review on MB of Hostiuc [17] who reviewed the thickness of the MB of 39 studies (11 autopsy and 28 computed tomography (CT) studies). The overall mean MB thickness of the meta-analysis review was 2.63 mm (2.48–2.85). The present study was consistent with meta-analysis review on autopsy studies which was 2.80 mm (2.39–3.22). The present study was thicker MB than 2.57 mm (2.31–2.89) in CT studies. The range of MB thickness in the present study was from 1 to 6.3 mm which was wider than the previous researches which were the range of MB, from 1 to 4 mm [18–22]. This study had a narrower range of MB thickness than 0.5 to 9.1 mm [4] and ranged from 5 to 27 mm observed by Loukas et al. [23]. In the previous autopsy and angiography studies reported the length of the MB were varied from 0.5 to 69 mm [1, 18, 21, 22, 24–26]. The range of length of the present study was 1.5–59 mm. The findings of the present study supported the results of previous studies.

The severe endothelial damage (SEM grades 3 and 4) of the coronary artery with MB in the present study reflected SEM grading of Davies et al. [27] and Hangler et al. [28] who described as subendothelial connective tissue covered all luminal surface of the coronary artery. Most cases of the proximal segment (38%) of the present study reflected! "{\textdegree} Ding et al. [16] who verified the effect of the MB led to irregular hemodynamics at the proximal end of the under bridging segment of the coronary artery. The present study confirmed the severity or higher grading of endothelial injury which reflected the degree of compression of MB over the coronary artery.

The results of the present study also supported the findings of many previous works. The previous researches described that the hemodynamic changes simulated WSS alteration in the different segments of the vessel including proximal, middle, and distal segments in patients with MB. Endothelial dysfunctions of the proximal LAD were associated with the site of lower WSS. There was non-endothelial dysfunction in the middle segment of LAD at greater WSS [9–12]. The present study confirmed the report of Jain et al. [14] in which the blood flow velocity could be increased under bridging segment of LAD at both systole and diastole. According to the pathophysiology review, it might be due to structural and functional changes of endothelial cells and smooth muscle cells induced by higher local TS described by Haga et al. [13].
The mean and sum grading scores of segment 1 were higher than segments 2–4. The endothelial grading scores of segment 2 were “0–2.” There was no advanced endothelial grading (grades 3 and 4) in under segment. Most of the distal and control segments of MB cases were grade 2. Some of the distal and control segments had a major defect in endothelial integrity. The SEM findings of the present study reflected the work of Ishikawa et al. [29], who stated that the quantity of intimal thickening of the coronary artery segments was depending on the thickness and length of MB. The SEM findings of the present study matched the findings of Davies et al. [27] and Hangler et al. [28] who described the SEM features of endothelial injury.

This study supported the work of Tohno et al. [15] who described that the calcium (Ca), phosphorus (P), zinc (Zn), and sodium (Na) were not uniform coronary artery with myocardial bridging cases. The elements accumulation was higher in the proximal part of MB than in the distal part of MB in comparison with under bridging segment of the LAD artery but there was no detailed description of element deposition of under segment. The present study was also agreed with the findings of Ge et al. [30] who described that calcium deposit with an eccentric plaque located at the proximal segment to the bridge especially in the systolic phase. In the animal study, calcium elements were not deposited in an early stage of lesions but they were found in the advance stage of the lesion as hydroxyapatite [31]. Therefore, the calcium elements were not found in all advance endothelial grading cases of the present study. They would depend on the type of calcification and method of analysis. In the present study, all iron (Fe) detected cases were SEM grade 4. This study is consistent with that of Salonen et al. [32] who reported iron might be involved in the atherogenesis process and a possible risk factor in coronary heart disease.

The present study confirmed the experiment of Ding et al. [16] who revealed that oscillatory shear stress which was significantly greater in the proximal segment of MB than the distal segment of MB under the 50% compression on the coronary artery. There were higher WSS at the distal segment of MB than the proximal segment of MB. The proximal end of MB was higher the oscillatory stress than other segments, at experimental 80% compression pressure [33]. The solid mechanical forces induced stresses which caused plaque growth and fissure at the proximal segments of MB [30]. These hemodynamic mechanisms played an important role in endothelium damage of the MB.

CONCLUSION

The under-bridging segment was the least endothelial damage site which might be due to the atheroprotective effect of the MB in under segment. The proximal segment was the most endothelial damage site. The distal segment had lower endothelial grading than the proximal segment and higher endothelial grading than both of the under bridging segment and control segment. It might be the different blood flow and shear stress in these segments.

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Thin Thin Win – Conception of the work, Drafting the work, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

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Authors declare no conflict of interest.

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All relevant data are within the paper and its Supporting Information files.

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