Histopathological Characteristics of Distal Middle Cerebral Artery in Adult and Pediatric Patients with Moyamoya Disease

Yasushi TAKAGI,[1] Yulius HERMANTO,[1] Jun C TAKAHASHI,[2] Takeshi FUNAKI,[1]
Takayuki KIKUCHI,[1] Yohei MINEHARU,[1] Kazumichi YOSHIDA,[1]
and Susumu MIYAMOTO[1]

[1]Department of Neurosurgery, Kyoto University Graduate School of Medicine,
Kyoto, Kyoto;
[2]Department of Neurosurgery, National Cerebral and Cardiovascular Center,
Suita, Osaka

Abstract

Moyamoya disease (MMD) is a unique progressive steno-occlusive disease of the distal ends of bilateral internal arteries and their proximal branches. The difference in clinical symptoms between adult and children MMD patients has been well recognized. In this study, we sought to investigate the phenomenon through histopathological study. Fifty-one patients underwent surgical procedures for treatment of standard indications of MMD at Kyoto University Hospital. Fifty-nine specimens of MCA were obtained from MMD patients during the surgical procedures. Five MCA samples were also obtained in the same way from control patients. The samples were analyzed by histopathological methods. In this study, MCA specimens from MMD patients had significantly thinner media and thicker intima than control specimens. In subsequent analysis, adult ($\geq$ 20 years) patients had thicker intima of MCA compared to pediatric (< 20 years) patients. There is no difference in internal elastic lamina pathology between adult and pediatric patients. Our results indicated that the pathological feature of MMD in tunica media occurs in both adult and pediatric patients. However, the MMD feature in tunica intima of MCA is more prominent in adult patients. Further analysis from MCA specimens and other researches are necessary to elucidate the pathophysiology of MMD.

Key words: moyamoya disease, histopathological study, adult, pediatric, middle cerebral artery

Introduction

Moyamoya disease (MMD) is an idiopathic progressive steno-occlusive of bilateral intracranial internal carotid arteries and their proximal branches, resulting in secondary formed of an unusual vascular network at the base of brain (moyamoya vessels).[1] MMD is most prevalent in Japan and East Asian countries, however its presence has been observed in various population around the world.[2] Together with the finding of familial cases, it potentially indicates a genetic factor in its pathogenesis.[3,4]

Histopathological findings of carotid termination in MMD patients revealed fibrocellular intimal thickening and tortuousness of the internal elastic lamina.[5–7] In addition, the decrease in the media thickness was reported.[8,9] Moyamoya vessels commonly have

histopathological changes, including fibrin deposits, fragmented elastic lamina, attenuated media, thrombosis, and the formation of microaneurysms.[5,10] Therefore, these histopathological changes might be closely associated with the onset of ischemic and hemorrhagic stroke.[10]

In contrary to pediatric patients, who commonly present with ischemic symptoms, about one half of adult patients have intracranial hemorrhage that seriously affects their prognosis.[1,2,11] In addition, due to the rarity of pathological examination of MMD in children and the difficulty to harvest the involved arteries during surgery, many MMD examinations usually relied on adult autopsy and extracranial specimens.[5–7,10,12] To this end, the pathological difference between adult and pediatric patients remains undefined. In this study, we collected tiny tips of MCA walls from MMD patients, during superficial temporal artery-middle cerebral artery (STA-MCA) bypass and

analyzed histologically to explore the difference of vascular pathology between adults and children.

Methods

I. Sample preparation

During STA-MCA bypass surgery, an 11-0 nylon monofilament was passed around the MCA wall of the recipient MCA (M4 portion, 0.5 mm–1.0 mm in diameter). Then the vessel was pulled up by lifting the monofilament with forceps (Fig. 1e), and the operator performed arteriotomy with microscissors. Collected tissues were fixed and used for further experiments.

II. Patients

Fifty-one patients underwent surgical procedures for treatment of standard indications of MMD at Kyoto University Hospital (Kyoto, Japan). All patients were diagnosed as definite MMD based on the diagnostic criteria of Japanese research committee on MMD. Fifty-nine specimens were obtained from the patients during the surgical procedures. Control MCA samples were also obtained in a similar way from the control subjects. In this study, patients ≥ 20 years were classified as adult, while < 20 years as pediatric. Clinical data of five control patients were as follows: age 67.4 ± 4.3 [mean ± standard deviation (SD)], four males and one female, internal carotid artery occlusion four, and glioblastoma one. As for MMD patients, the clinical data were summarized as shown in Table 1. Adult patients from 21 years to 67 years old and pediatric patients from 3 years to 17 years old were included in this study.

III. Histological analysis

All specimens were fixed in 10% formalin overnight and then embedded in paraffin the next day. The specimens were stored at room temperature. In each case, multiple, sequential, 6-μm-thick tissue sections cut from paraffin blocks were deparaffinized in xylene, rehydrated, and stained with hematoxylin and eosin. The sections were analyzed under a BX51
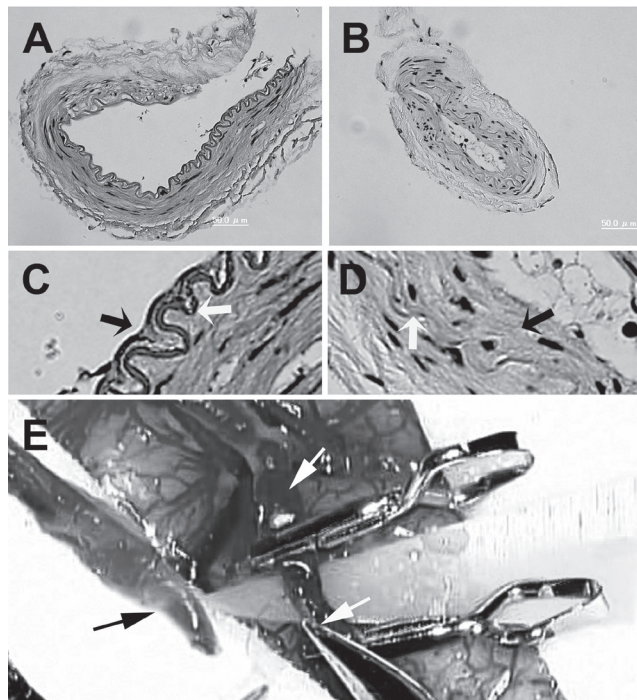

Fig. 1 **A: Low power microscopic view of middle cerebral artery from control patient. B: Low microscopic view of middle cerebral artery from the patient with moyamoya disease. C: High power microscopic view of middle cerebral artery from control patient.** *Black arrow* **indicates the intima and** *white arrow* **indicates the internal elastic lamina. D: High power microscopic view of middle cerebral artery from moyamoya patient.** *Black arrow* **indicates the intima and** *white arrow* **indicates the internal elastic lamina. E: Intraoperative view.** *Black arrow* **indicats superficial temporal artery. Upper** *white arrow* **indicates middle cerebral artery and lower** *white arrow* **indicates tiny piece of middle cerebral artery which is collected as sample for further histopathological study.**

Table 1 Summary of cases

	Adult (≥ 20 years)	Pediatric (< 20 years)
No. of patients	35	16
No. of cases	42	17
Age	42.95 ± 12.64	7.82 ± 5.03
Sex		
Male	10 (23.8%)	7 (41.2%)
Female	32 (76.2%)	10 (58.8%)
Onset		
Present	24 (57.1%)	14 (82.4%)
Without	18 (42.9%)	3 (17.6%)
Infarct	11 (26.2%)	4 (23.5%)
Hemorrhage	3 (7.1%)	0 (0.0%)
Suzuki stages		
6	3 (7.1%)	
5	1 (2.4%)	
4	6 (14.3%)	3 (17.6%)
3	32 (76.2%)	14 (82.4%)
CBF (Power's classification)		
0	2 (5.1%)	
1	19 (48.7%)	5 (31.3%)
2	18 (46.2%)	11 (68.8%)

CBF: cerebral blood flow.

fluorescent microscope (Olympus Optical Co., Tokyo) for hematoxylin-eosin stained samples. Under a BX51 fluorescence microscope, the histological images were captured with a computer. Then intimal and medial thickness were analyzed by using an NIH Image-ImageJ analyzing system.

IV. Statistical analysis

Data were presented as mean ± SD. Mann-Whitney and Chi-square tests were used for statistical analysis (Sofastats 1.4.3, Paton-Simpson, Auckland, New Zealand). $P < 0.05$ was considered statistically significant.

Results

I. Thickness of intimal and media

In comparison with control MCA, the vascular walls of MCA from MMD had thicker intimal. On the contrary, media became thinner. The mean thickness of the MMD intimal was significantly higher than the control (Fig. 1a, b and Table 2; MMD, 17.48 ± 11.20 µm; and control, 7.90 ± 1.53 µm; $p = 0.029$), whereas the mean thickness of the MMD media was significantly less (Fig. 1c, d and Table 2; MMD, 26.74 ± 10.27 µm; and control, 56.70 ± 10.43 µm; $p < 0.001$).

Then, we analyzed the thickness of MCA intimal and media between adult and pediatric patients of MMD. In this study, there was a significantly higher intimal thickening in adult compared to pediatric patients (Table 3: adult, 20.09 ± 11.78 µm; pediatric, 11.66 ± 7.07 µm, $p = 0.006$). The finding was not obtained in media thickness, in which adult had no significantly thicker media compared to pediatric (Table 4: adult, 28.04 ± 10.60 µm; pediatric, 23.53 ± 8.88 µm, $p = 0.110$). In our subsequent analysis about the intimal thickness between adult and pediatric patients compared to control, we found that only intimal thickness in adult was thicker (Table 5: adult, 20.09 ± 11.78 µm; control 7.90 ± 1.53 µm, $p = 0.006$).

II. The internal elastic lamina pathology

In MMD specimens, the internal elastic lamina of MCA was commonly found abnormal. The common

Table 2 Intimal and media thickness of middle cerebral arteries of patients with moyamoya disease

	MMD	Control	p value
Intimal thickness (µm)	17.48 ± 11.20*	7.90 ± 1.53	0.029
Media thickness (µm)	26.74 ± 10.27*	56.70 ± 10.43	< 0.001

*: $p < 0.05$, MMD: moyamoya disease, statistic test: Mann-Whitney U test.

Table 3 Intimal thickness in relation with clinical characteristics

Clinical characteristics	Intimal thickness (µm)	
	Adult (≥ 20 years)	Pediatric (< 20 years)
Average	20.09 ± 11.78*	11.66 ± 7.07
Onset		
Symptomatic	21.56 ± 10.29	12.61 ± 7.48
Asymptomatic	18.26 ± 13.50	7.25 ± 0.90
CBF		
0	18	
1	16.33 ± 10.71	7.80 ± 4.52
2	23.20 ± 11.34	12.86 ± 7.69
Suzuki		
6	18.13 ± 4.42	
5		
4	11.00 ± 4.88	11.63 ± 7.58
3	22.03 ± 12.29	11.83 ± 5.13

CBF: cerebral blood flow, *$p = 0.006$, statistic was tested with Mann-Whitney U test.

Table 4. Media thickness in relation with clinical characteristics

Clinical characteristics	Media thickness (µm)	
	Adult (≥ 20 years)	Pediatric (< 20 years)
Average	28.04 ± 10.60	23.53 ± 8.88
Onset		
Symptomatic	28.95 ± 8.66	23.38 ± 9.14
Asymptomatic	26.83 ± 12.92	24.25 ± 9.33
CBF		
0	23.63 ± 10.78	
1	25.87 ± 7.77	20.10 ± 4.94
2	30.86 ± 13.11	25.14 ± 10.38
Suzuki		
6	24.25 ± 7.00	
5	18.00	
4	21.71 ± 7.17	23.93 ± 9.75
3	29.90 ± 11.01	21.67 ± 2.67

CBF: cerebral blood flow.

irregularities of internal elastic lamina were elongated, fragmented, and disappearance (Fig. 1c, d). In this study, we did not observe any significant difference in internal elastic lamina pathologies of MCA between adult and pediatric patients. The frequency

Table 5 Intimal and media thickness of middle cerebral arteries of adult and pediatric moyamoya disease patients in comparison with control

	Adult (≥ 20 years)	Control	p value
Intimal thickness (μm)	20.09 ± 11.78*	7.90 ± 1.53	0.006
Media thickness (μm)	28.04 ± 10.60*	56.70 ± 10.43	< 0.001

	Pediatric (< 20 years)	Control	p value
Intimal thickness (μm)	11.66 ± 7.07	7.90 ± 1.53	0.480
Media thickness (μm)	23.53 ± 8.88*	56.70 ± 10.43	< 0.001

*: $p < 0.05$, Statistic test: Mann-Whitney U test.

of abnormal internal elastic lamina is summarized in Table 6.

Discussion

In this study, we obtained a large number of surgically sampled MCA specimens from patients with MMD. The MCA of MMD patients had a thinner media and thicker intimal than the control ones. In addition, surgeons who operate on MMD patients are well aware of the thinner wall and smaller diameter of MCA that brings technical difficulty while performing direct bypass.[9,13] Together, the findings in line with previous reports using autopsy samples indicates that the pathology of MMD extends beyond the carotid termination and its proximal branches. Therefore, MCA of MMD patients are regarded as a valuable resource for MMD research.

We observed that MCA from adult patients had higher intimal thickness compared to pediatric patients. However, the phenomenon was not obtained in media thickness of MCA, in which adult and pediatric had no difference. In addition, we also did not obtain difference in intima and media thickness according to other clinical variables. Thus we emphasized age categorizing as an important factor. To this end, the Japan nationwide epidemiological survey of MMD reported that the distribution of age at onset had two peaks: children at 10 years of age and adults around 40 years of age.[2,14,15] Those reports suggest that there is a different onset MMD between adult and pediatric patients, thus adult MMD does not necessarily evolve from childhood lesion.

In addition, we conducted further analysis to observe whether MCA of adult or pediatric was different from the controls. We found that both adult

Table 6 The characteristics of internal elastic lamina

	Adult (≥ 20 years)	Pediatric (< 20 years)	p value
Elongated	12 (29.3%)	7 (41.2%)	0.379
Fragmented	17 (41.5%)	7 (41.2%)	0.984
Disappearance	13 (31.7%)	7 (41.2%)	0.490

Statistic test: Chi-square.

and pediatric patients had a thinner media, but only adult patients had a thicker intima compared to control. This finding suggests that the tunica media abnormality occurred both in adult and pediatric patients of MMD. Otherwise, the tunica intima thickening of MCA is more profound in the adults.

Adult and pediatric MMD not only differ in the age of onset, but also in the clinical symptoms. Hemorrhage manifestation in MMD is commonly found in adult, while in pediatric it is very rare, pediatric often manifests with ischemic symptoms.[2,10,11] In addition, sequential progression of MMD is detectable in pediatric but rarely in adult.[16,17] Younger patients and underlying disease have been reported predisposition factors of rapid progression of MMD.[18]

As limitation, our control MCAs were obtained from adult patients, thus we could not exclude the potential role of growth to MCA pathology. Since we could not compare the MCA thickness of pediatric MMD with normal pediatric. However, intimal thickening is often found in association with atherosclerosis,[19] vasculitis,[20] aging,[21] and other vascular disorders.[22] Our control samples may have atherosclerosis and are from aged patients. In this way, our control samples are not true control not only for pediatric but also for adult patients. This is also a limitation of this study.

In this study, the internal elastic lamina of both adult and pediatric patients exhibit irregularities such as elongated, fragmented, and disappearance. We might speculate that MMD pathology extends to distal MCA of both adult and pediatric, but the distinct feature of cerebrovascular physiology contribute to specific remodeling in different age groups. Further researches are also needed to investigate the precise molecular action that perhaps being regulated differently in adult and children.

In summary, MCA intima thickness of adult MMD was thicker compared to pediatric patients. While, there is no difference in tunica media pathology between adult and pediatric patients. Therefore, further analysis from MCA specimens and other researches are necessary to elucidate the pathophysiology of MMD.

Conflicts of Interest Disclosure

All authors have no conflicts of interest in this manuscript.

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Address reprint requests to: Yasushi Takagi, MD, PhD, Department of Neurosurgery, Kyoto University Graduate School of Medicine, 54 Kawahara-cho, Sakyo, Kyoto, Kyoto 606-8507, Japan.
e-mail: ytakagi@kuhp.kyoto-u.ac.jp