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The Role of miR-26a, miR-29a and miR-448-3p in the Development of Cerebral Aneurysm

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ABSTRACT

AIM: To elucidate the role of microRNAs (miRNAs) in the development of cerebral aneurysms.

MATERIAL and METHODS: This study compared the expression levels of miR-26a, miR-29a and miR-448-3p in 50 samples each of cerebral aneurysm tissues and normal superficial temporal artery tissues. The miRNA expression levels were also compared in terms of aneurysm location and rupture status, i.e., presence or absence of rupture.

RESULTS: Expression levels of miR-26a, miR-29a and miR-448-3p were increased in aneurysm tissues compared with normal vascular tissues. No significant difference was found in the miRNA expression levels with respect to aneurysm location or rupture status.

CONCLUSION: This study showed that miR-26a, miR-29a and miR-448-3p overexpression could play an important role in intracranial aneurysm development independent of aneurysm location and rupture status. miR-26a, miR-29a and miR-448-3p could act as potential therapeutic targets in patients with intracranial aneurysms; however, further studies are needed on this issue.

KEYWORDS: MicroRNAs, miR-26a, miR-29a, miR-448-3p, Cerebral aneursyms

INTRODUCTION

Cerebral aneurysms result in the abnormal expansion of arterial structures and weakening of arterial walls. The most common pathological manifestation is the loss of the tunica media of the artery. Its worldwide incidence rate is within 2%–3%, but in adult autopsy series, it is 1%–5% (8,19). Although several theories have been suggested for its pathophysiology, it is not yet fully understood. Smoking, hypertension and stress are factors involved in the development of aneurysms (2,14). Its concomitance with polycystic kidney disease, Marfan syndrome, Ehlers–Danlos syndrome and fibromuscular dysplasia suggests the involvement of genetic factors. Moreover, recent studies have shown the aetiological role of gene polymorphism (1). The rupture of cerebral aneurysms leads to high rates of mortality and morbidity. Thus, complete elucidation of the aetiopathogenesis is necessary to improve treatment models.

MicroRNAs (miRNA) are small noncoding RNAs of approximately 22 nucleotides in length. They regulate eukaryotic gene expression through posttranscriptional mechanisms such as translation suppression or target mRNA degradation. The role of miRNAs in gene expression regulation has been understood in the recent past, and research on this issue became popular today. New miRNAs are being identified every day, and the effects of miRNAs on gene expression levels are gaining popularity in several research fields, especially in cancer research (4). Recent studies have revealed that miRNAs are important posttranscriptional regulators that ensure the maintenance of cardiovascular homeostasis and participate in the mechanism underlying intracranial aneurysm (IA) development (17). Therefore, this study was conducted to elucidate the role of miRNA in IAs.

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miR-29a

miRNAs are known to play a regulatory role in vascular disease pathology and are considered novel biomarkers (5). miR-29a is one of the three members of the miR-29 family that is involved in the fibrotic response induced by collagen I, collagen III, fibrin-1 and elastin-1 and the regulation of gene transcripts that encode extracellular matrix proteins (20). It promotes colorectal cancer metastasis by regulating matrix metalloproteinase-2 and E-cadherin (21). Recent studies have shown that miRNAs is important in the development of IA (6). miR-29a acts as a gene modulator in the development and ageing of the aorta and plays a role in IA progression. miR-29a overexpression is believed to have a potential role in IA development through the regulation of the extracellular matrix, which subsequently leads to vascular wall rupture (18). A study involving mouse models of IA revealed that miR-29a expressed 200 times more in mutant mice than in normal mice (9). In vitro experiments have shown that the downregulation of miR-29a decreases the apoptosis of smooth muscle cells in the human brain, whereas miR-29a overexpression increases the apoptosis of these smooth muscle cells. In vivo studies on IA have also suggested that miR-29a overexpression could regulate apoptosis through mitochondrial pathways (24).

miR-26a

miR-26a, a highly conserved miRNA, is involved in modulating the functions of vascular smooth muscle cells and their proliferation and apoptosis. miR-26a dysregulation is believed to be associated with various vascular pathologies (12). A study also reported that mirR-26a may help regulate cellular oxidative stress (16). Vascular smooth muscle cell apoptosis is an important cellular mechanism underlying the onset of various vascular diseases (10).

miR-448-3p

miR-448 is an important miRNA. Its expression decreases in cancers such as hepatocellular carcinoma, Hodgkin's lymphoma and breast, stomach, ovarian and colorectal cancers. It is also involved in tumour suppression (15). miR-448 is overexpressed in the smooth muscle cells of coronary arteries with atherosclerotic plaques compared with the smooth muscle cells of normal coronary arteries. The ectopic expression of miR-448 has been shown to induce the proliferation and migration of vascular smooth muscle cells in atherosclerotic plaques by targeting *MEF2C* mRNA and suppressing *MEF2C* expression (23). miR-448-3p is an inhibitory miRNA with low expression in tumour cell arrays. A study showed that miR-448-3p plays an inhibitory role in IA development, and its overexpression prevents IA development through the downregulation of macrophage-mediated inflammation (22).

MATERIAL and METHODS

Tissue Samples

The study was approved by the ethics committee of the Adana City Training and Research Hospital. Informed consent was obtained from all patients included in the study.

The study included a total of 100 patients, of which 50 were diagnosed with aneurysms and presented to the brain and nerve surgery clinic between January 2019 and August 2020 and 50 underwent cranial surgery for reasons other than vascular pathologies.

During surgery, the aneurysm was clipped and cut for sample collection. Superficial temporal artery (STA) samples were also obtained from patients who underwent surgery in our clinic during the same period and had pathologies such as tumours and haemorrhages that required STA dissection. No further incision was performed for STA sampling in any patient. Patients aged < 18 years and those with genetic diseases were excluded from the study. All the collected samples were immediately placed in 1.5-mL Eppendorf tubes containing 0.5 mL of RNA preservative solution and stored in a refrigerator at –20°C.

miRNA Isolation

To isolate miRNA from brain tissues, GeneMATRIX Universal RNA/miRNA Purification Kit (EURX Cat No. E3599) was used. Isolation was performed following the manufacturer's protocol. To homogenise the samples, 30 µL of buffer A solution that came with the kit was added to the homogenisation spin column and kept for 10 min at room temperature. The tissue was homogenised using liquid nitrogen and transferred to a 2-mL Eppendorf tube. Then, 200 µL of Lyse ALL and 300 µL of RL buffer were added to it and mixed using a vortexer. It was centrifuged at maximum speed for 3 min. The supernatant obtained after homogenisation was transferred to the spin column and centrifuged at 12,000 × g for 2 min. Then, 1.2 mL of 96% ethanol was added to the filtrate using a pipette, and 600 µL of the resultant mixture was added to the RNA-binding spin column. It was centrifuged at 11,000 × g for 1 min, and the supernatant was removed. It was centrifuged once more for 1 min at 11,000 × g. Subsequently, wash miRNA buffer was added, and it was centrifuged at 11,000 × g for 1 min. The supernatant was removed, and wash miRNA buffer was added again. This was centrifuged at 11,000 × g for 1 min, and the supernatant was removed and centrifuged again at 11,000 × g for 1 min. The content of the spin column was transferred to a new Eppendorf tube. Then, 50 µL of RNase-free water was added, and it was centrifuged for 2 min at 11,000 × g.

cDNA Synthesis, Real-Time Polymerase Chain Reaction (PRC) Steps and Analysis

To prepare poly(A) reaction mixture, 0.5 µL of 10× poly(A) buffer, 0.5 µL of ATP, 0.3 µL of poly(A) enzyme and 1.7 µL of RNase-free water were added to a 1.5-mL centrifuge tube for each sample and mixed using a vortexer. Then, 2 µL of each sample was added to a reaction plate, and 3 µL of the poly(A) reaction mixture was added to each sample. The reaction plate was covered with an optical film and then vortexed. The plate was placed in a thermal cycler at 37°C for 45 min and at 65°C for 10 min. To prepare the ligation reaction mixture, 3 µL of 5× DNA ligase buffer, 4.5 µL of 50% PEG 8000, 0.6 µL of 25× ligation adaptor, 1.5 µL of RNA ligase and 0.4 µL of RNase-free water were added to a 1.5-mL Eppendorf tube for each sample and vortexed. Thereafter, 10 µL of

the ligation reaction mixture and 5 µL of the poly(A)-tailing reaction product were added to each plate. The reaction plate was covered with an optical film and then vortexed. The plate was placed in a thermal cycler at 16°C for 60 min. To prepare the reverse-transcription (RT) reaction mixture, 6 µL of 5× RT buffer, 1.2 µL of dNTP mixture (25 mM of each dNTP), 1.5 µL of 20× universal RT primer, 3 µL of 10× RT enzyme mixture and 3.3 µL of RNase-free water were added into a 1.5-mL centrifuge tube for each sample and then vortexed. Then, 15 µL of the RT reaction mixture and 15 µL of the adaptor ligation reaction product were added into each plate. The plate was placed in a thermal cycler at 42°C for 15 min and 85°C for 5 min. To prepare the miR-Amp reaction mixture, 25 µL of 2× miR-Amp Master Mix, 2.5 µL of 20× miR-Amp Primer Mix and 17.5 µL of RNase-free water were added into a 1.5-mL centrifuge tube for each sample and then vortexed. Further, 45 µL of the miR-Amp Primer Mix and 5 µL of the RT reaction product were added into each plate. The plate was placed in a thermal cycler and kept for 5 min for 1 cycle at 95°C, 3 min for 14 cycles at 95°C, 30 min for 14 cycles at 60°C and 10 min for 1 cycle at 99°C. The resulting cDNA product was diluted with 0.1× TE buffer in a 1:10 ratio. To prepare the PCR mixture, 10 µL of TaqMan® Fast Advanced Master Mix (2×), 1 µL of TaqMan® Advanced miRNA Assay (20×) and 4 µL of RNase-free water were added to a 1.5-mL centrifuge tube for each sample and vortexed. Then, 15 µL of the PCR mixture and 5 µL of the cDNA reaction product were added into each plate. The plate was placed in a thermal cycler and kept for 20 min for 1 cycle at 95°C, 3 min for 40 cycles at 95°C and 30 min for 40 cycles at 60°C. U6snRNA was used as the internal control for miRNAs. All samples were normalised to internal controls. All results were expressed as cycle threshold (Ct) values and normalised to the calculated reference Ct of each sample (Δct). The relative expression was calculated using the comparative Ct method ($2^{-\Delta\Delta Ct}$). miRNAs with a fold change of <0.5 or >2 were determined to be differentially expressed. The coefficient of variation between the assays performed for all biomarkers was <15% for all miRNAs.

Statistical Analysis

Statistical evaluation was conducted using the Statistical Package for Social Sciences (SPSS) for Windows 20 (IBM SPSS Inc., Chicago, IL) software. The normality of data distribution was evaluated using the Kolmogorov–Smirnov test. Numerical variables are presented as mean ± standard deviation and categorical variables as numbers and percentages. Student's t-test and Mann–Whitney U test were used to compare numerical variables between the two groups according to the normality of distribution, and analysis of variance or Kruskal–Wallis H test was used in comparison in substitution for aneurysm; $p<0.05$ (*) was considered significant in all statistical analyses. The false discovery rate test correction was used for miRNA analysis.

RESULTS

The study group consisted of 56 female patients and 44 male patients. The mean age of the study group was 53.4 ± 11.7 years. Age and gender distributions were similar for the aneurysm and control groups ($p>0.05$). Additionally, 34% of the patients with aneurysm (n=17) had ruptures. With respect to the location of the aneurysm, 40% (n=20) of the patients had aneurysms located in the anterior communicating artery, 26% (n=13) had aneurysms in the middle cerebral artery and 12% (n=6) had aneurysms in the internal carotid artery. The aneurysms were located at multiple sites in 22% (n=11) of the patients (Table I).

Table I: Demographic Features

Variable	Total n=100	Aneurysm n=50	STA n=50	p
Age, years	53.4 ± 11.7	54.3 ± 8.5	52.1 ± 14.5	0.348
Gender, n (%)				
Female	56 (56.0)	33 (66.0)	23 (46.0)	0.069
Male	44 (44.0)	17 (34.0)	27 (54.0)	
Bleeding				
Yes	17 (17.0)	17 (34.0)	-	-
No	33 (33.0)	33 (66.0)	-	
Aneurysm location				
ACOM	20 (20.0)	20 (40.0)	-	-
ICA	6 (6.0)	6 (12.0)	-	
MCA	13 (13.0)	13 (26.0)	-	
Multiple	11 (11.0)	11 (22.0)	-	

Numerical variables are shown as mean ± standard deviation. Categorical variables are shown as numbers (%).

In terms of miRNA expression levels, miR-26a, miR-29a and miR-448-3p showed an 8-fold (p=0.001), 3.73-fold (p=0.003) and 3.25-fold (p=0.035), respectively, increased upregulation in the aneurysm group compared with the control group (Figure 1). A significant correlation was found between miRNA expressions in patients with aneurysm (Figure 2). Comparison of miR-26a, miR-448-3p and miR-29a expression levels according to aneurysm location showed no significant differences (Figure 3). In the aneurysm group, the expression levels of miR-26a, miR-448-3p and miR-29a were comparable in patients with and those without ruptured aneurysms (Figure 4).

DISCUSSION

In this study, we compared the expression levels of miR-26a, miR-29a and miR-448-3p in the tissue samples obtained from 50 patients with cerebral aneurysm and in STA tissue samples obtained from 50 other patients who underwent surgery for other vascular pathologies. All three miRNAs were significantly overexpressed in patients with aneurysm compared with control patients. In the aneurysm group, when the miRNA expression levels were compared between patients with and those without ruptured aneurysm, the expression levels were found to be similar in both groups. When the patients in the aneurysm group were classified according to aneurysm location and miRNA expressions were compared,

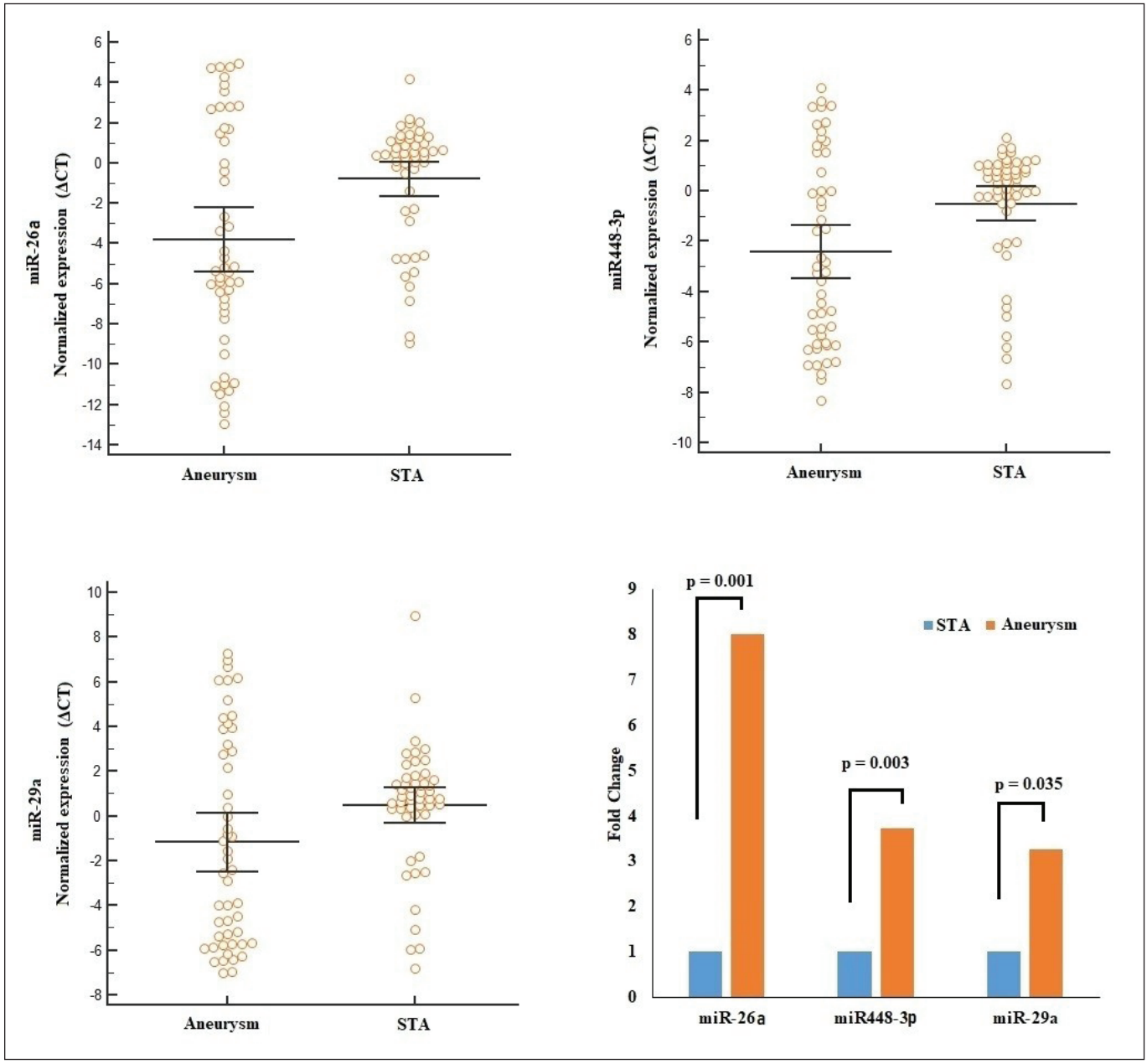

Figure 1: miRNA expressions in the aneurysm and control groups.

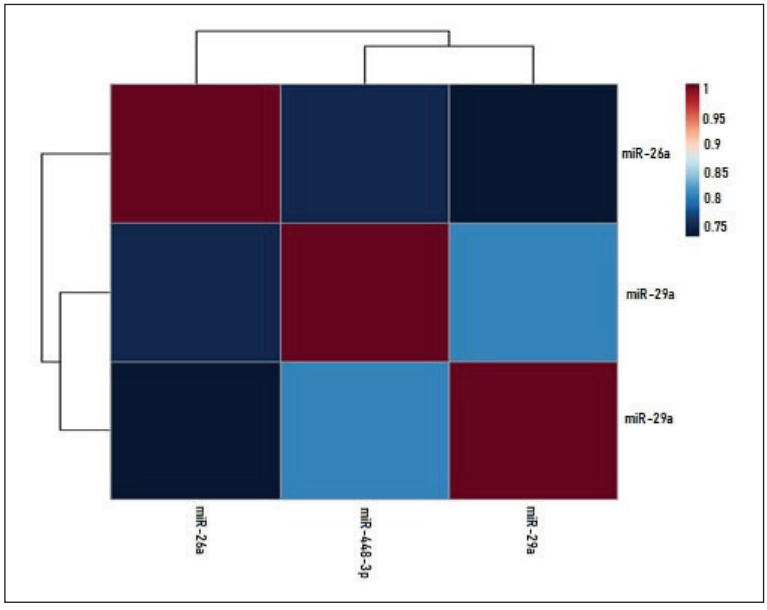

Figure 2: Correlation matrix graph.

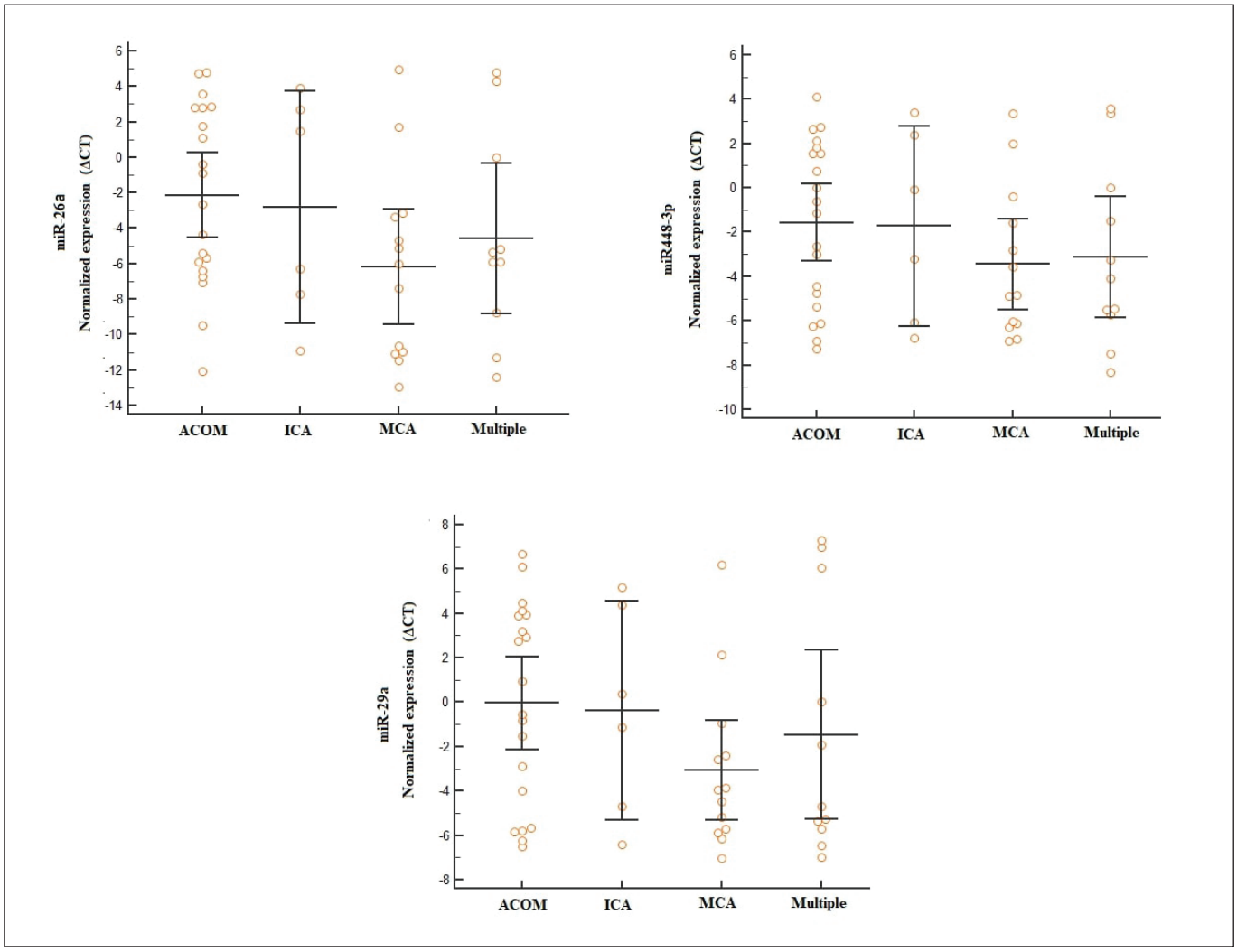

Figure 3: miRNA expression levels according to aneurysm location.

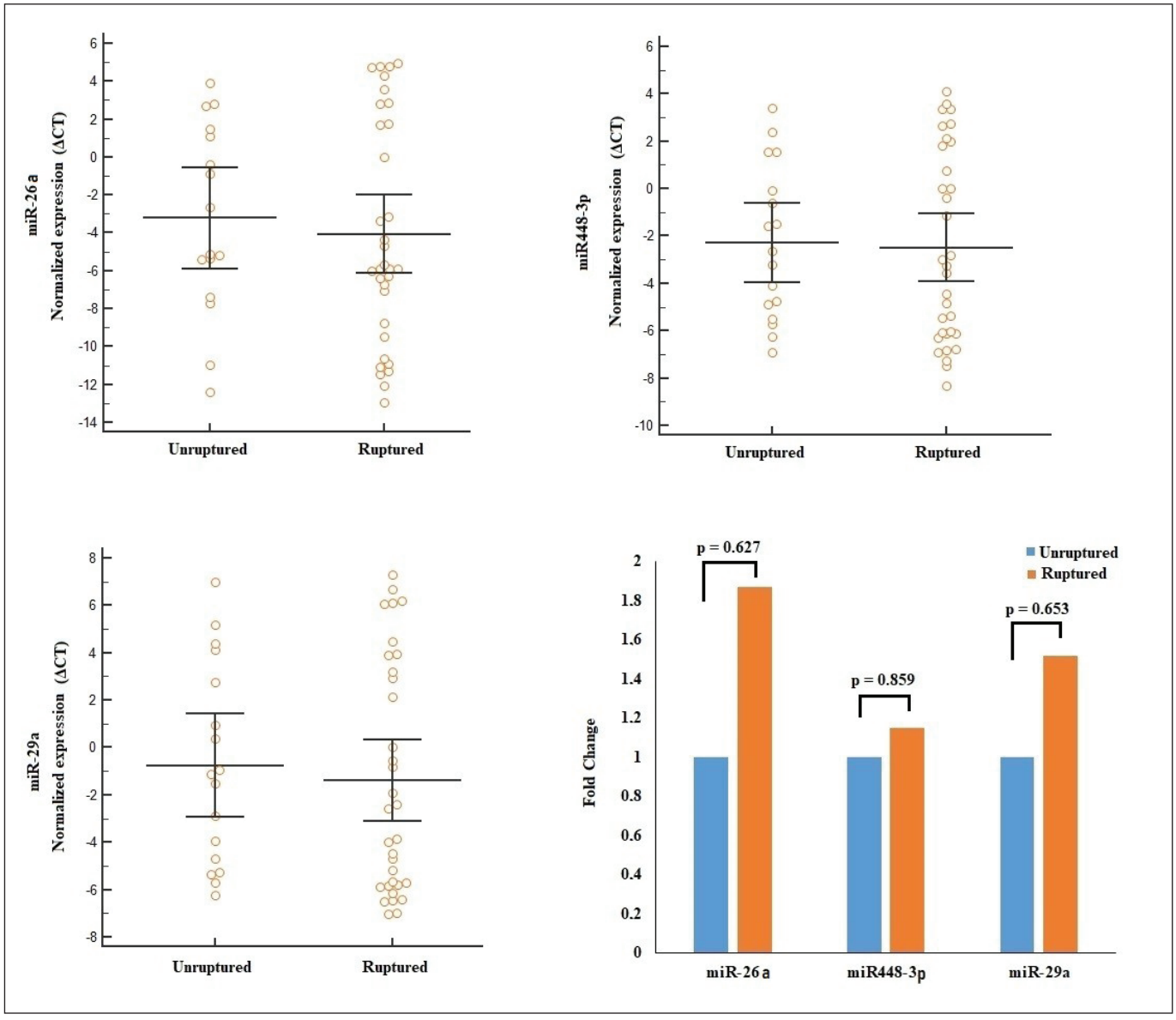

Figure 4: Comparison of miRNA expression levels between patients with and those without ruptured aneurysms.

no significant difference was found in the expression levels according to the aneurysm location.

Previous studies have tried to clarify the role of miRNAs in IA development. Wang et al. found that miR-29a expression was higher in the peripheral blood of patients with aneurysm than those in control individuals. Moreover, they found no significant correlation between increased expression and age, gender and aneurysm size and location but found a significant association between aneurysm rupture rate and increased miR-29a expression. Based on these data, they hypothesised that miR-29a might play a role in IA development and hence could serve as an effective biological marker for IA diagnosis (18). Liao et al. found increased miR-29a expression in the plasma exosomes of patients with aneurysm compared with healthy controls. They could not detect significant differences between ruptured and unruptured aneurysms in terms of miR-29a expression levels. No correlation was found between the expression levels and sex, presence of diabetes mellitus, alcohol consumption and smoking status. In this case, they suggested that miR-29a is an independent noninvasive biomarker of IA development (11). Lee et al. conducted a study in which they experimentally induced cerebral aneurysm in mice and found that miR-29a expression increased in these mice. In all samples, cerebral aneurysm was at an advanced stage, and they hypothesised that miR-29a could play a role in the progression of cerebral aneurysm (9). In an *in vitro* study, Zhao et al. found that miR-29a downregulation reduced the apoptosis of vascular smooth muscle cells of the human brain (24), whereas miR-29a overexpression increased the apoptosis of these smooth muscle cells. They confirmed using *in vivo* IA models that miR-29a overexpression could induce apoptosis

through mitochondrial pathways and concluded that miR-29a could contribute to IA progression by regulating mitochondrial apoptotic pathways. As a result, they suggested that miR-29a could be a potential therapeutic target in IA (24). The results of our study showed that miR-29a expression levels were higher in the aneurysm group than in the control group regardless of the aneurysm location. These findings suggest that miR-29a overexpression could play an important role in IA development regardless of the location. In addition, no difference was found between patients with and those without ruptured aneurysms in terms of miR-29a expression levels. These findings suggest that miR-29a might be a potential therapeutic target in IA.

miR-26a is an miRNA involved in modulating the proliferation and apoptosis of vascular smooth muscle cells. The dysregulation of miR-26a may be associated with various vascular pathologies. In our study, miR-26a expression levels were higher in the tissue samples collected from patients with aneurysm than in those collected from patients of the control group. In a previous study, Peng et al. found that miR-26a expression was low in the peripheral blood of patients with abdominal aortic aneurysm (AAA). They also found that miR-26a was overexpressed in an H_2O_2-mediated vascular smooth muscle cell damage model and suggested that miR-26a could protect vascular smooth muscle cells from H_2O_2-mediated accumulation of free oxygen radicals and apoptosis through the phosphatase and tensin homolog (PTEN)/AKT/mTOR signalling pathway. Based on these results, they hypothesised that miR-26a could be a prognostic biomarker and a therapeutic target in patients with AAA (13). Leeper et al. found that miR-26a overexpression in human aortic smooth muscle cell cultures inhibited vascular smooth muscle cell differentiation and apoptosis and simultaneously induced vascular smooth muscle cell proliferation by modulating the tissue growth factor beta (TGF-β) signalling pathway. As a result, they suggested that miR-26a could be a new regulator of smooth muscle cell biology and a potential therapeutic target in AAA (10). Elfaki et al. reported that miR-26a decreased the inflammation rate in rat aortic smooth muscle cells through matrix metalloproteinase downregulation, matrix metalloproteinase inhibition and TGF-β upregulation, inhibited the angiogenesis of rat aortic endothelial cells and targeted connective tissue growth factor and PTEN, which are key proteins directly involved in AAA pathogenesis. In line with these data, they concluded that miR-26a might be an appropriate therapeutic target in AAA (3). Liu et al. found that miR-26a expression in six IA tissue samples was lower than that in six normal STA samples. They suggested that miR-26a is involved in biological processes such as apoptosis through certain target genes, oxidative stress response, TGF-β signalling pathway, smooth muscle cell proliferation and aortic dissection (12). Unlike the literature, the present results revealed that regardless of the aneurysm location, miR-26a expression levels were higher in the aneurysm group than in the control group. These findings suggest that miR-26a overexpression could play an important role in IA development regardless of its location. Moreover, no difference was found between patients with and those without ruptured aneurysm in terms of miR-26a expression levels. These findings suggest that miR-26a might be a potential therapeutic target in IA.

miR-448-3p is an inhibitory miRNA whose expression decreases during the development of several cancers. It has a tumour-suppressive role (23) and is involved in the development of heart diseases caused by oxidative stress (7). Zhang et al. found that miR-448-3p levels in the arterial wall samples of rats with surgically induced IA were downregulated as compared with those in the arterial wall samples of normal rats; additionally, the *KLF5* gene was upregulated in the arterial wall samples of rats with surgically induced IA compared with those in the arterial wall samples of normal rats. They observed that in the miR-448-3p-treated group, the aneurysm size and lumen area decreased, arterial wall thickness was preserved and macrophage infiltration was suppressed. Based on these, they suggested that miR-448-3p inhibited IA growth through its anti-inflammatory effects on macrophages, and this effect is partly linked to the suppression of *KLF5* expression (22). Unlike the literature, we found higher miR-448-3p expression levels in the aneurysm group than in the control group regardless of the aneurysm location. These findings suggest that miR-448-3p overexpression could play an important role in IA development regardless of its location. In addition, no difference was found in the miR-448-3p expression levels between patients with and those without ruptured aneurysm. These findings suggest that miR-448-3p might be a potential therapeutic target in IA.

CONCLUSION

Epigenetics and miRNA have been shown to play an important role in the development of several cancers and vascular diseases. This study aimed to clarify the role of miRNA in IA development, IA location and aneurysm rupture. The findings of this study revealed that the overexpression of miR-26a, miR-29a and miR-448-3p could play an important role in IA development regardless of the location and rupture status of the aneurysm. miR-26a, miR-29a and miR-448-3p could acts as potential therapeutic targets in IA. Further studies are needed on this issue.

AUTHORSHIP CONTRIBUTION

Study conception and design: VA, OO
Data collection: YG, ZB
Analysis and interpretation of results: OA, ZB
Draft manuscript preparation: OO, OA
Critical revision of the article: OO, VA
Other (study supervision, fundings, materials, etc...): VA, ZB
All authors (ZB, OA, VA, OO, YG) reviewed the results and approved the final version of the manuscript.

REFERENCES

1. Caranci F, Briganti F, Cirillo L, Leonardi M, Muto M: Epidemiology and genetics of intracranial aneurysms. Eur J Radiol 82(10):1598-1605, 2013
2. Chalouhi N, Ali MS, Jabbour PM, Tjoumakaris SI, Gonzalez LF, Rosenwasser RH, Koch WJ, Dumont AS: Biology of intracranial aneurysms: Role of inflammation. J Cereb Blood Flow Metab 32(9):1659-1676, 2012
3. Elfaki L, Pirani R, Afrasiabi Z, Matkar P, Chen H, Kuliszewski M, Leong-Poi H: Elucidating the molecular mechansims of mir-26a on the pathogenesis of abdominal aortic aneurysm - in vitro studies. Can J Cardiol 34(10):S69-S70, 2018
4. Gao J, Liu QG: The role of miR-26 in tumors and normal tissues (Review). Oncol Lett 2:1019-1023, 2011
5. Huang F, Yi J, Zhou T, Gong X, Jiang H, Yao X: Toward understanding non-coding RNA roles in intracranial aneurysms and subarachnoid hemorrhage. Transl Neurosci 8:54-64, 2017
6. Jin H, Li C, Ge H, Jiang Y, Li Y: Circulating microRNA: A novel potential biomarker for early diagnosis of Intracranial Aneurysm Rupture a case control study. J Transl Med 11: 296, 2013
7. Kyrychenko S, Kyrychenko V, Badr MA, Ikeda Y, Sadoshima J, Shirokova N: Pivotal role of miR-448 in the development of ROS-induced cardiomyopathy. Cardiovasc Res 108(3):324-334, 2015
8. Lee D, Ahn SJ, Cho ES, Kim YB, Song SW, Jung WS and Suh SH: High prevalence of intracranial aneurysms in patients with aortic dissection or aneurysm: Feasibility of extended aorta CT angiography with involvement of intracranial arteries. J Neurointerv Surg 9:1017-1021, 2017
9. Lee HJ, Yi JS, Lee HJ, Lee IW, Park KC, Yang JH: Dysregulated expression profiles of microRNAs of experimentally induced cerebral aneurysms in rats. J Korean Neurosurg Soc 53(2):72-76, 2013
10. Leeper NJ, Raiesdana A, Kojima Y, Chun HJ, Azuma J, Maegdefessel L, Kundu RK, Quertermous T, Tsao PS, Spin JM: MicroRNA-26a is a novel regulator of vascular smooth muscle cell function. J Cell Physiol 226(4):1035-1043,2011
11. Liao B, Zhou MX, Zhou FK, Luo XM, Zhong SX, Zhou YF, Qin YS, Li PP, Qin C: Exosome-derived MiRNAs as biomarkers of the development and progression of intracranial aneurysms. J Atheroscler Thromb 27(6):545-610, 2020
12. Liu D, Han L, Wu X, Yang X, Zhang Q, Jiang F: Genome-wide microRNA changes in human intracranial aneurysms. BMC Neurol 14:188, 2014
13. Peng J, He X, Zhang L, Liu P: MicroRNA 26a protects vascular smooth muscle cells against H2O2 induced injury through activation of the PTEN/AKT/mTOR pathway. Int J Mol Med 42(3):1367-1378, 2018
14. Schievink WI: Intracranial aneurysms. N Engl J Med 336:28-40, 1997
15. Shan C, Fei F, Li F, Zhuang B, Zheng Y, Wan Y, Chen J: miR-448 is a novel prognostic factor of lung squamous cell carcinoma and regulates cells growth and metastasis by targeting DCLK1. Biomed Pharmacother 89:1227-1234, 2017
16. Suh JH, Choi E, Cha MJ, Song BW, Ham O, Lee SY, Yoon C, Lee CY, Park JH, Lee SH, Hwang KC: Up-regulation of miR-26a promotes apoptosis of hypoxic rat neonatal cardiomyocytes by repressing GSK-3β protein expression. Biochem Biophys Res Commun 423(2):404-410, 2012
17. Unda SR: MicroRNAs potential biomarkers and therapeutic targets for intracranial aneurysm. J Clin Epigenet 4: 1158-2472, 2019
18. Wang WH, WangYH, Zheng LL, Li XW, Hao F, Guo D: MicroRNA-29a: A potential biomarker in the development of intracranial aneurysm. J Neurol Sci 364:84-89, 2016
19. Wiebers DO, Whisnant JP, Huston J . Meissner I, Brown RD Jr, Piepgras DG, Forbes GS, Thielen K, Nichols D, O'Fallon WM, Peacock J, Jaeger L, Kassell NF, Kongable-Beckman GL, Torner JC: Unruptured intracranial aneurysms: Natural history, clinical outcome, and risks of surgical and endovascular treatment. Lancet 362:103-110, 2003
20. van Rooij E, Sutherland LB, Thatcher JE, DiMaio JM, Naseem RH, Marshall WS, Hill JA, Olson EN: Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. Proc Natl Acad Sci USA 105(35):13027-13032, 2008
21. Villarreal G Jr, Oh DJ, Kang MH, Rhee DJ: Coordinated regulation of extracellular matrix synthesis by the microRNA-29 family in the trabecular meshwork. Invest Ophthalmol Vis Sci 52:3391-3397, 2011
22. Zhang JZ, Chen D, Lv LQ, Xu Z, Li YM, Wang JY, Han KW, Yu MK, Huang CG, Hou LJ: miR-448-3p controls intracranial aneurysm by regulating KLF5 expression. Biochem Biophys Res Commun 505(4):1211-1215, 2018
23. Zhang R, Sui L, Hong X, Yang M, Li W: MiR-448 promotes vascular smooth muscle cell proliferation and migration in through directly targeting MEF2C. Environ Sci Pollut Res Int 24(28):22294-22300, 2017
24. Zhao W, Zhang H, Su JY: MicroRNA 29a contributes to intracranial aneurysm by regulating the mitochondrial apoptotic pathway. Mol Med Rep 18(3):2945-2954, 2018