Histopathological Characteristics with Emphasis on Connective Tissue Disorder in Intracranial Aneurysm Patients: Comparison of Intracranial and Extracranial Vessels

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ABSTRACT

Background

Changes in different layers of blood vessels histopathologically and alterations in collagen and elastin levels weaken the vessel walls which eventually leads to out-pouching from the weakened area of the vessels leading to the formation of an aneurysm. Similar conditions occur in connective tissue disorders where there are issues with the production or maturation of collagen.

Methods

Biopsy of aneurysmal dome and the superficial temporal artery (STA) were done in cases however biopsy of STA taken from those who have undergone surgical procedure like trauma and other cranial pathology. All specimens were obtained during craniotomy. Parameters in vessels biopsy were seen as per histopathological parameters that involves Eccentric fibrointimal thickening, Luminal narrowing, Myxoid degeneration, disruption of internal elastic lamina, medial fibrosis, loss of smooth muscle in media, loss of elastic fibres.

Results

The study was conducted in 27 patients to find out the histopathological parameters of vessels as mentioned above in relation to Connective Tissue Disorder. This was compared with 20 patients with non-aneurysmal pathology. Changes observed in the superficial temporal artery and the dome of the aneurysm in cases of intracranial aneurysms indicate weak connective tissue characteristics.

Conclusions

The histologic changes seen in dome of aneurysm and STA in cases of intra cranial aneurysm a weak connective tissue. In patients with intracranial aneurysms, these changes resemble those seen in CTD. We found notable differences between the two groups in terms of histopathological findings. eccentric thickening of the fibrous inner layer, myxoid degeneration, reduced muscle in the middle layer of the vessel wall, and loss of elastic fibers compared to the control group.

Keywords: Biopsy; Connective tissue disorders; Ehlers-Danlos syndrome; Marfan syndrome; Neurofibromatosis type 1, Internal elastic lamina; Intracranial aneurysms

Introduction

Prevalence of Intracranial Aneurysms (IA) in the population ranges from 0.4 to 6.0 percentage[1]. Intracranial Aneurysm formation and rupture is a multifactorial disease and is a mix of genetic and environmental factors[2-5]. Factors that increase the risk include female gender, age over 60 years, certain conditions such as polycystic kidney disease, Marfan's syndrome, hypertension, smoking, etc.[6-8].

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Weakness of vessel wall has been consistently observed as a risk factor for IA formation [9]. A weak vessel wall over time leads to local outpouching which eventually develops into an aneurysm. Connective tissue disorders such as Marfan's syndrome and Ehlers Danlos lead to weakening of vessel wall[10,11] and thus are definite risk factors for development of IAs[12].

But many patients of IA don't have a known connective tissue disorder. These patients also have a weak vessel wall. But do the histopathological characteristics of this weak vessel wall mimic the one with known connective tissue disorder? The present study is an attempt to histologically evaluate the connective tissue changes in IA.

Materials and Methods

This was a prospective case control study involving 27 patients and 20 control subjects in the department of neurosurgery over a period of 1.5 years from Jan 2023 to June 2024. Patients of all ages, both sexes who had ruptured IA and underwent clipping were included as cases and patient who underwent craniotomy for non-aneurysmal pathology were included as control subjects. This study was approved by the institutional ethics committee and consent was taken from all the subjects.

At the time of surgery, tissue was taken from the aneurysm wall and segment of STA (or its branches). For IA cases, both the samples – STA and aneurysm wall were taken. For controls, only STA was taken.

Specimens were fixed in Bouin's fluid and embedded in paraffin. Then1-5 µm sections were prepared. For light microscopic examination, routine Hematoxylin and Eosin stain (H and E stain) along with special stains were performed (Masson's trichome, PAS-AB, EVG stains) to look for extracellular mucin deposition.

These specimens were then examined by a team of expert neuro pathologists.

Following features were noted in STA wall and aneurysm. Each feature was assigned a Histopathological Scoring (Table 1) adapted by Kendziora et al, 2023 which was widely accepted parameters to analyze the histopathological parameters of the vessels. This was done for both cases and controls. Cases were further subdivided into those with single aneurysm and the ones with multiple aneurysms. Thus three groups were created:

1. cases with single aneurysm,
2. cases with multiple aneurysms, and
3. controls.

The features studied were:

A. Eccentric fibrointimal thickening
B. Luminal narrowing
C. Myxoid degeneration
D. Disruption of internal elastic lamina.
E. Medial fibrosis
F. Loss of smooth muscle in media
G. Loss of elastic fibres

The data was then analyzed for each group with respect to grading of connective tissue disorder.

Results

There were 47 patients in our study (27 cases and 20 controls). The mean ages for the cases were 51.74 years and controls were 48.20 years. Majority of the patient of the aneurysm (cases) were operated in GCS 15, accounting for 96.3%.

All the patients (cases) undergoing surgery had anterior circulation aneurysms. Hypertension and smoking were found to be associated with causal for aneurysm in this study. 59.3% were hypertensive only and 11.1% were associated with HTN and smoking in combination and 7.4% with smoking. 22.2% were not associated with any of these. Prominent findings on STA wall biopsy (Figure 1) in patient with IA were eccentric fibrointimal thickening, luminal narrowing, myxoid degeneration, disruption of the IEL (Figure 2) and loss of smooth muscle in media with loss of

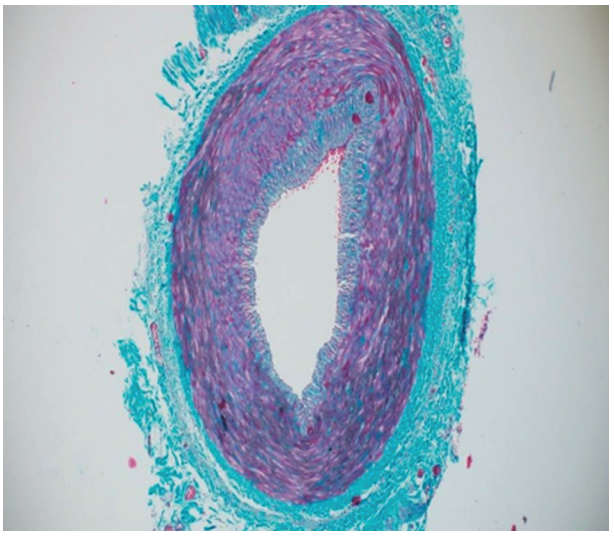

Figure 1: Histology (LPF-MT stain) of STA vessel wall

elastic fibres. These findings are also compared with control subjects and found to be statistically significant (<0.05).

Findings in both single and multiple IA were limited to the fibrointimal (Figure 3) luminal narrowing (Figure 4), myxoid degeneration (Figure 5) and loss of smooth muscle in media (Figure 6) and elastic fibres.

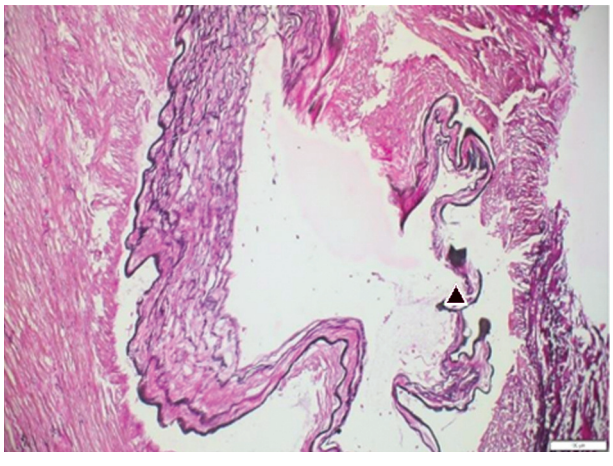

Figure 2: Histology (EVG Stain) highlighting fragmentation of internal elastic lamina, reduplication and breaks (arrow heads)

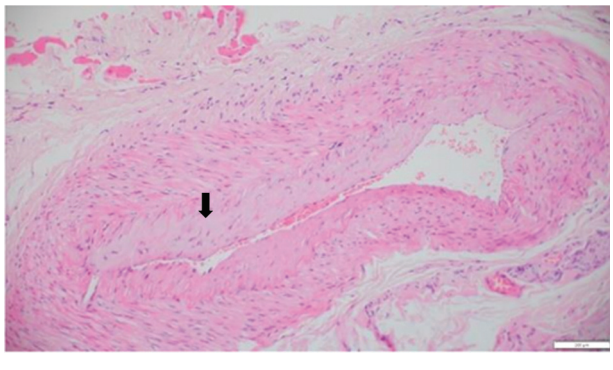

Figure 3: Histology (Hematoxylin and Eosin stain with magnification 100x) showing fibrointimal thickening (shown with thick arrow) with partial occlusion of lumen(shown with thin arrow) in cases.

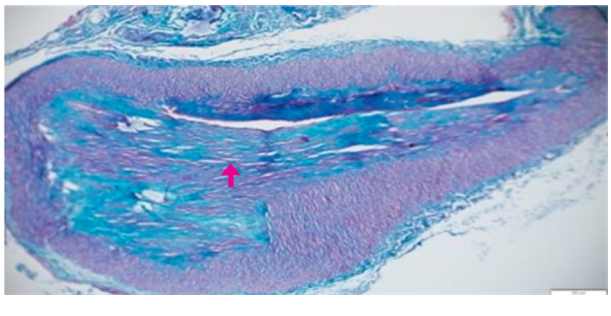

Figure 4: Histology (Masson's Trichome stain) highlights the fibrointimal thickening and fibrosis (arrowhead).

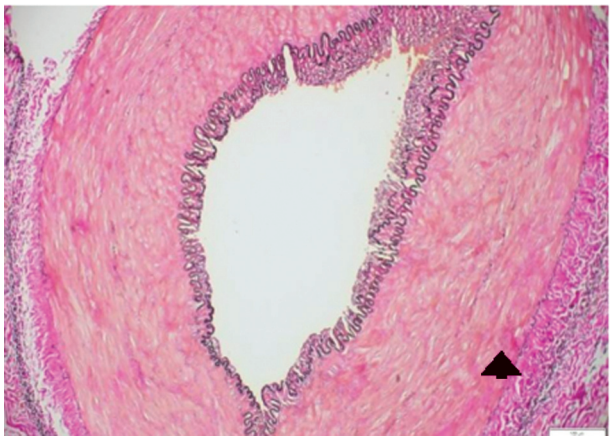

Figure 5: Histology (PAS-AB stain) highlights myxoid degeneration of the tunica media (arrow head)

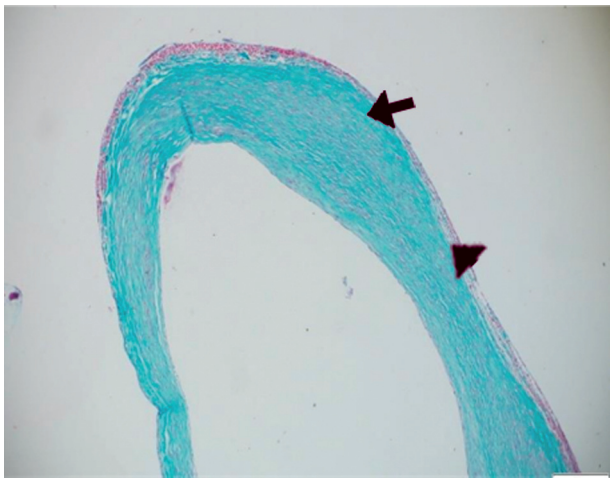

Figure 6: Histology (MT stain) highlighting dilated and ectatic aneurysmal wall (arrow head) with complete loss of smooth muscle in the wall with hyalinised stroma and fibrosis (triangle)

Table 1: Histopathological Scoring: Adapted from Kendziora et al, 2023

S.N.	Parameter	Score	Criteria
1	Eccentric intimal fibroplasia	0	Not present
		1	< 10%
		2	10–50%
		3	> 50%
2	Luminal occlusion	0	0%
		1	25–50%
		2	50–75%
		3	> 75%
3	Myxoid degeneration	0	Absent
		1	Present
4	Disruption of Internal Elastic Lamina (IEL) – circumferential involvement	0	Absent
		1	Present

S.N.	Parameter	Score	Criteria
5	Medial fibrosis	0	Absent
		1	< 10%
		2	10–50%
		3	> 50%
6	Smooth muscle loss	0	Absent
		1	< 10%
		2	10–50%
		3	> 50%
7	Elastic fibre loss	0	Absent
		1	< 10%
		2	10–50%
		3	>50%

We found that patients harboring IA had more frequent and more severe connective tissue abnormalities when compared with controls (Table 2). This was statistically significant across most of the parameters.

A further sub group analysis was done between those IA patients having single aneurysm and those having multiple aneurysms. Changes in aneurysm wall were seen in both the subgroups but there was no inter group difference. (Table 3)

Discussion

Despite rapid advances in diagnosis, advanced micro-neurosurgery techniques, endovascular methods and improved ICU care over last 4-5 decades, the mortality and morbidity associated with ruptured intracranial aneurysm continues to be high. Naturally for a disease with high mortality and morbidity, it is imperative to study the risk factors associated with the disease[13,14]. A weak vessel wall has been consistently associated with aneurysm formation and rupture. Patients having known connective tissue disorder like Marfan's and Ehlers Danlos disease indeed have increased incidence of IA when compared to normal population. But

Table 2: Histopathologic changes in STA

		Total Patients	Single Aneurysm	Multiple Aneurysm	Controls	p-value	p value Single vs control	Multiple vs control
Histopathologic changes	HP grading		No of patients	No of patients	No of patients			
ECCENTRIC FIBROINTIMAL THICKENING	1	14	0	0	14	0.001	0.001	0.001
	2	14	8	5	1			
	3	3	3	0	0			
LUMINAL NARROWING	0	2	0	0	2	0.013	0.001	0.158
	1	21	4	4	13			
	2	8	7	1	0			
MYXOID DEGENERATION	0	20	6	0	14	0.006	0.063	0.001
	1	10	4	5	1			
	2	1	1	0	0			
DISRUPTION OF IEL	0	8	2	0	6	0.161	0.395	0.26
	1	23	9	5	9			
MEDIAL FIBROSIS	0	1	0	0	1	0.007	0.032	0.001
	1	22	7	1	14			
	2	8	4	4	0			
LOSS OF SMOOTH MUSCLE IN MEDIA	0	6	0	0	6	0.018	0.023	0.018
	1	21	9	3	9			
	2	4	2	2	0			
LOSS OF ELASTIC FIBRES	0	6	0	0	6	0.003	0.023	0.004
	1	20	9	2	9			
	2	5	2	3	0			

Table 3: Histopathologic changes in Aneurysmal wall seen in single vs multiple IA

Histopathologic changes	HP grading		Single Aneurysm	Multiple aneurysms	P value
		Total patients	Number of patients	Number of patients	
ECCENTRIC FIBROINTIMAL THICKENING	0	11	7	4	0.217
	1	0	0	0	
	2	3	3	0	
LUMINAL NARROWING	0	11	7	4	0.466
	1	2	2	0	
	2	1	1	0	
MYXOID DEGENERATION	0	3	3	0	0.497
	1	10	6	4	
DISRUPTION OF IEL	0	2	2	0	0.334
	1	12	8	4	
MEDIAL FIBROSIS	1	3	3	0	0.326
	2	1	1	0	
	3	10	6	4	
LOSS OF SMOOTH MUSCLE IN MEDIA	1	2	2	0	0.466
	2	1	1	0	
	3	11	7	4	
LOSS OF ELASTIC FIBRES	1	2	2	0	0.211
	2	3	1	2	
	3	9	7	2	

what about patients who do not have known connective tissue disorder but still suffer from IA? Do these patients also have weak vessel wall? We studied histopathological parameters of vessels including STA[15] and aneurysmal dome in patients of IA and compared it with controls. This was done with an aim to study the vessel wall abnormalities and compare these abnormalities with known connective tissue disorders.

We found that patients with IAs showed more frequent abnormalities in their connective tissue compared to the control group. These abnormalities suggest a weaker connective tissue structure, possibly due to an inherited / acquired defect in the connective tissue itself.

In a study by Yurt et al.[16] consistent changes were found in the blood vessel tissue of their subjects. These changes included fragmentation of the inner elastic layer (IEL), color changes in the inner lining and layers just beneath it (metachromasia), and growth of tissue in the inner layer (intimal proliferation). Our study observed similar changes, such as reduced elastic fibers in the inner lining, tissue growth in the inner layer, loss of smooth muscle in the middle layer, and narrowing of the vessel opening in segments of the superficial temporal artery (STA) or its branches. These changes in the connective tissue of blood vessels are thought to be involved in the development of aneurysms. When the vessel wall is weakened and combined with changes in blood flow dynamics—like those seen at points where vessels branch, in conditions like high blood pressure, or due to other developmental issues—the vessel can bulge or dilate abnormally[17].

Miyazawa N et al[18] conducted a study where they found that patients with intracranial aneurysms, multiple or large intracranial aneurysms were mostly older males with current smokers.

In a study "Factors associated with aneurysm size in patients with subarachnoid hemorrhage: effect of smoking and aneurysm location" done by Qureshi AI et al[19], smoking and having an aneurysm in the middle cerebral artery increase the likelihood of developing large aneurysms and rupture and they concluded that there is a connection between smoking cigarettes and the development of intracranial aneurysms.

Silvia et al.[20] conducted a study where they found that hypertension (HTN) affects elastic fibers in arteries by causing them to become tired and break down faster. This leads to a loss of flexibility in the arterial walls. The breakdown products from these elastic fibers can signal cells to grow, move, change their characteristics, and break down the extracellular matrix (ECM). These processes contribute to the formation and rupture of aneurysms.

The abnormal alignment of elastic and collagen fibers contributes to reduced strength in blood vessels, which is linked to the formation of aneurysms[21]. Similar changes in the inner layer of the artery (intima) suggest that there are underlying degenerative processes and increased growth of tissue, which could help explain the risk factors for developing aneurysms in patients without a history of connective tissue disorders. So, the weaknesses observed in the blood vessel walls where intracranial aneurysms formation and rupture are likely due not only to local blood flow dynamics but also to broader issues with the body's connective tissue. If such a link between connective tissue disorder with aneurysm formation are found out and this can be used in categorizing low risk vs high risk, therefore intracranial aneurysm can be diagnosed before rupture and if feasible for targeted therapy for connective tissue disorders[22].

Therefore, patients with intracranial aneurysms, whether they have one or multiple aneurysms, show similar changes in the structure of blood vessel walls (specifically in the superficial temporal artery) and in the dome of the aneurysm as those observed in classical connective tissue disorders as shown in this study too.

The limitations of the study is the small sample size. The control group were relatively younger. Since the study was conducted in a single centre, that introduces biases. So, need for further multicentric studies are required.

The study supports the idea that the tendency to develop intracranial aneurysms might be caused by changes in how connective tissue components are structured, oriented, and function at a very detailed level. Larger prospective trials in the future that analyze the molecules and genes in connective tissues, along with detailed structural examinations, can help establish connections. These studies could also make it possible to use biopsies of the superficial temporal artery and aneurysm dome as a screening tool, especially when there's a family history suggesting a higher risk for developing intracranial aneurysms.

Conclusion

The present findings suggest that patients with intracranial aneurysms exhibit distinct histopathological features consistent with an intrinsic weakness of the vessel wall, resembling changes seen in connective tissue disorders. These changes include eccentric fibro-intimal thickening, myxoid degeneration, loss of smooth muscle cells in the tunica media, and marked reduction in elastin fibers. Although clinical manifestations of connective tissue disorders were absent, the microscopic vascular changes indicate a subclinical or inherent connective tissue abnormality that may be congenital in nature. The significant differences observed between aneurysm and control groups underscore the role of underlying connective tissu like pathology in the development of intracranial aneurysms, highlighting the importance of histopathological evaluation in understanding aneurysm pathogenesis and identifying individuals at potential risk.

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