Original

**Pathophysiology and Maturation of the Transverse Carpal
Ligament in Idiopathic Carpal Tunnel Syndrome
— Clinical, Pathological and Immunohistochemical Study —**

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Abstract: The etiology of idiopathic carpal tunnel syndrome remains poorly understood. This study investigated the relationship between idiopathic carpal tunnel syndrome and transverse carpal tunnel ligament histology, especially clinical severity of this disease and immunohistochemistry of the ligament¹⁾. Ligament biopsy specimens were obtained from eleven wrists during operation for carpal tunnel release, and one control specimen was obtained. After staining of different slices from each specimen with HE and antibodies to collagen types I to VI, each slice was classified into 3 grades based on pathology, where grade 1 was the least and 3 the greatest pathological progression. Out of eight specimens from mild carpal tunnel syndrome patients, 4 were grade H-1, and the other 4 were grade H-2 based on HE staining. Out of 4 specimens from moderate to severe carpal tunnel syndrome patients, 1 was grade H-2 and the other 3 were grade H-3 with HE. Grades for anti-type I collagen were C-1 in 6 out of 8 mild CTS patients, whereas the grades were C-2 or C-3 in 3 out of 4 moderate to severe patients. Grades for anti-type III collagen were C-1 in 4 out of 4 mild patients whereas the grades were C-2 or C-3 in all 4 moderate to severe. The sum of type I and type III grades were 2 in control patients, 2-3 in the mild CTS patients, 4 in the moderate patients and 6 in the severe patients.

There was a correlation between the severity of this disease and the degree of transverse ligament pathology stained with HE, anti-type I and III collagen antibodies. These suggest that disturbance of the maturation of collagen fibers in the transverse carpal ligament is an important factor in idiopathic carpal tunnel syndrome.

Key words: carpal tunnel syndrome, transverse carpal ligament, anti-collagen immuno-histochemistry, pathophysiology, maturation of collagen fiber

Introduction

Idiopathic carpal tunnel syndrome is the most common entrapment neuropathy of the median nerve at the transverse carpal ligament. This syndrome frequently occurs in women

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aged from 40–60 years. The transverse carpal ligament between the carpal bones is the floor of the carpal tunnel. This ligament is a thick fibrous band attached on the radial side to the tuberosity of the scaphoid and a portion of the trapezium and on the ulnar aspect to the pisiform and hook of the hamate. The median nerve lies superficial directly beneath this ligament. Although the disease is very common, in many patients little specific cause can be found and its pathophysiology is still not clearly understood. To date, many researchers have investigated the pathoanatomy of the flexor synovium or synovial sheath, and speculated that idiopathic carpal tunnel syndrome is caused by mechanical and chronic tenosynovitis and increase in volume of the tunnel. The purpose of this study was to investigate the histology of the transverse carpal ligament in patients with idiopathic carpal tunnel syndrome, and to analyze expression of collagen subtypes I–VI, and clarify the relationship between the degree of maturation of the collagen fibers and the severity of the median neuropathy. Our hypothesis was that disturbance of the maturation of collagen fibers in the transverse carpal ligament may be an important factor in idiopathic carpal tunnel syndrome.

Materials and Methods

Materials

Twelve transverse carpal ligaments were obtained from 11 patients diagnosed with idiopathic carpal tunnel syndrome despite amyloidosis who underwent open surgery at our department, and one ligament was obtained from an injured patient as a control. The patients were 1 male and 10 females with a mean age of 51.4 years, and 6 ligaments were from the right hand and 6 from the left. All patients favored the right hand. All females were housewives and one male was a desk worker. The diagnosis of idiopathic carpal tunnel syndrome was established by patient interview, physical examination and electrophysiological study (Table 1). The electrophysiological diagnosis criteria were a terminal latency

Table 1. Clinical symptoms and neural findings in each carpal tunnel syndrome patient

Cases	Age	Sex	Diagnosis	Op. Side	Chief Complaint	Phalen Test	Tinel Sign	MMT** (APB)	APB*** Atrophy
1	56	F	Bil-CTS	Lt	Numbness	+	+	2	+
2	57	M	Lt-CTS	Lt	Numbness	2+	1+	5	—
3	27	F	Bil-CTS	Lt	Numbness	—	—	5—	—
4	56	F	Bil-CTS	Rt	Numbness, Night pain	—	+ / —	4	+
5	41	F	Bil-CTS	Lt	Numbness, Night pain	—	+	4	+
6	78	F	Rt-CTS	Rt	Numbness	+	2+	3	2+
7	28	F	Rt-CTS	Rt	Numbness	+	+	5	—
8	46	F	Rt-CTS	Rt	Numbness	—	—	5—	—
9*	56	F	Bil-CTS	Rt	Numbness	+	+	4	+
10*	57	F	Bil-CTS	Lt	Numbness	+	+ / —	4	+ / —
11	54	F	Rt-CTS	Rt	Numbness	+	+	4—	+ / —
12	47	F	Lt-CTS	Lt	Numbness	—	—	4	+
13	29	F	Control	Rt					

Case 9, 10* : same patient

MMT** : manual muscle test

APB*** : abductor pollicis brevis

Fig. 1. Resection of a transverse carpal ligament with carpal tunnel syndrome

We dissected the ligament as far as possible on the ulnar side and the median nerve was decompressed. Also, before sectioning a 4-0 nylon suture was tied at the proximal part of the transverse carpal ligament on the palmar side.

(TL) of the abductor pollicis brevis (APB) muscle of more than 4.2 msec and a sensory conduction velocity (SCV) of less than 45 m/sec.

Dissection of the Transverse Carpal Ligaments

Open wrist surgery was performed for 12 hands diagnosed with idiopathic carpal tunnel syndrome, in which the patients requested surgical treatment. The standard method in which the skin incision does not exceed the wrist skin line, or a skip incision²⁾, which is frequently performed at our department, was employed in the operations. After confirming the transverse carpal ligament by direct vision, we dissected the ligament as far as possible on the ulnar side and the median nerve was decompressed. Then, the ligament was sectioned and a specimen of about 5 to 6 mm was taken from the radial side. Also, before sectioning, a 4-0 nylon suture was tied at the proximal part of the transverse carpal ligament on the palmar side, which was subsequently used as a mark for sectioning and follow-up (Fig. 1). In the single control case, the median nerve was ruptured in an injury, and a specimen of the ligament was sectioned during the nerve suture. Before taking the tissue specimens, all patients were given a full explanation and informed consent was obtained.

Methods

The 13 transverse carpal ligaments extirpated during surgery were formalin-fixed, paraffin-embedded, and sections were made. HE staining, and the distribution of collagen types I to VI was investigated using the unlabelled antibody technique (LSAB-POD method) of the enzyme antibody technique. The relationship between the histological picture and the severity of the neurological severity of the manifestations was investigated. The neurological severity in the 12 hands with idiopathic carpal tunnel syndrome was comprehensively evaluated, mainly on the basis of the clinical findings and the

Table 2. Neurological severity in all subjects

Case	Age	Sex	Diagnosis	Op. Side	T.L.*		SCV**		SNAP***		Severity
					Rt.	Lt.	Rt.	Lt.	Rt.	Lt.	
2	57	M	Lt-CTS	Lt	3.4	3.8	45.8	50.6	1	2	Mild
3	27	F	Bil-CTS	Lt	6.6	5.9	50.6	38.9	2	2	Mild
4	56	F	Bil-CTS	Rt	5.1	5.2	38.9	40.7	2	1	Mild
5	41	F	Bil-CTS	Lt	6.7	5.9	38.1	34.6	1	2	Mild
7	28	F	Rt-CTS	Rt	4.2	3.5	45.4	54.6	1	1	Mild
8	46	F	Rt-CTS	Rt	5.6	3.1	32.8	50.3	2	1	Mild
9	56	F	Bil-CTS	Rt	10.6	10	26.5	17.7	2	3	Mild
12	47	F	Lt-CTS	Lt	3	3.9	57.4	36.5	1	2	Mild
10	57	F	Bil-CTS	Lt	10.6	10	26.5	17.7	2	3	Moderate
11	54	F	Rt-CTS	Rt	8.9	4.5	20.2	40.5	3	2	Moderate
1	56	F	Bil-CTS	Lt	5.6	7.3	39.2	NR	2	3	Severe
6	78	F	Rt-CTS	Rt	9.9	4.8	NR	40.8	3	2	Severe
13	29	F	Control	Rt							

T.L.* : terminal latency

SCV** : sensory conduction velocity

SNAP*** : sensory nerve action potential

electrophysiological assessment. The clinical assessment was made by a surgeon masked to the histological evaluation, and the microscopic assessment was made by a pathologist masked to the clinical evaluation.

1) Electrophysiological Evaluation and Severity of Neurological Manifestations

The electrophysiological evaluation was classified into three types based on analysis of the waveform of the sensory nerve action potential (SNAP) by Inagaki *et al.*³⁾; type 1, reduction in the amplitude or extension of the latency period; type 2, temporal dispersion; and type 3, remarkable reduction of the amplitude and multiple phases.

The clinical findings are shown in Table 1, and the severity of the neurological manifestations was classified into three groups based on the clinical course; mild type, moderate type, and severe type. Mild carpal tunnel syndrome (CTS) includes SNAP Types 1 and 2 with or without muscular atrophy, moderate CTS includes SNAP Type 3 without muscular atrophy, and severe CTS includes SNAP Type 3 with muscular atrophy (Table 2). In the present study, cases with complete degeneration of the abductor pollicis brevis (APB) with muscle power of [0] or [1] by manual muscle testing (MMT) were excluded.

2) HE Staining and Classification

According to the conventional method, the specimens were stained with HE. The results of HE staining were classified into Grades H-1 to H-3. In grade H-1, the fibers of the ligament were not disordered. In grade H-2, the principal fascicles of the ligament were reduced with little new fiber formation. In grade H-3, the principal fascicles of the ligament were reduced and loosened with abundant new fiber formation among them. All cases were negative for Congo red stain.

3) Anticollagen Staining^{4,5)} (Labeled Avidin Biotin Peroxidase) and Classification

The procedure for treatment of ligament and anticollagen staining is shown in Table 3. Polyclonal antibody (Japan Tanner Corporation) was used as the primary antibody for

Table 3. Technique of Collagen Staining

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- 1 The extirpated ligament is fixed in 10% formalin.
 - 2 Embedded in paraffin.
 - 3 Cut into 3 μ m-thick sections and dried at 60°C for 48 hours.
 - 4 Extraction of paraffin; shake with xylol for 5 minutes twice, then with 100% alcohol degraded to 70% alcohol several times.
 - 5 Wash with water.
 - 6 Circle with PAP-pen and wash with PBS (Phosphate buffer solution).
 - 7 Treat with protease K (DAKO Ltd.) at 4°C for 1 minutes.
 - 8 Wash with water for 5 minutes.
 - 9 Peroxidase reaction with 3% hydrogen peroxide for 20 minutes.
 - 10 Wash with water for 5 minutes and then with distilled water.
 - 11 Serum blocking with 5% pig serum for 30 minutes.
 - 12 Primary antibody reaction: the primary antibody is reacted at 4°C overnight with dilution with PBS.
Dilution is; Type I : 100 times, Type II : 80 times, Type III : 80 times, Type IV to VI : 200 times
 - 13 Wash with PBS for 5 minutes 3 times.
 - 14 Secondary antibody reaction: secondary antibody diluted with PBS is reacted at 37°C for 30 minutes.
Multi-type (IMMUNON Ltd.) diluted 100 times was used as a secondary antibody.
 - 15 Wash with PBS for 5 minutes 3 times.
 - 16 Tertiary antibody reaction: streptavidin solution (DAKO Ltd.) diluted 600 times was reacted at 37°C for 30 minutes.
 - 17 Wash with PBS for 5 minutes 3 times.
 - 18 Visualization: 3.3 mg DAB (3,3'-Diaminobenzidine Tetrahydrochloride) diluted with 20 ml PBS is reacted by adding 30% hydrogen peroxide.
The coloring status is observed under the microscope.
 - 19 Stop the color development by washing out with running water for 5 minutes.
 - 20 Wash with water sufficiently after nuclear staining with hematoxylin.
 - 21 Immerse in xylol after dehydration with 70%-100% alcohol, and then mount (on glass slides).
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anticollagen staining. The results of staining for each collagen were classified into Grades C-1 to C-3. In staining for collagen type, the principal fascicles of the ligaments were stained darkly in Grade C-1, less darkly stained in Grade C-2, and moderately stained in Grade C-3. In staining for collagen type II, the specimens were classified by the appearance of chondrocytes. No chondrocytes were seen in Grade C-1, were suspected in Grade C-2, and were manifest in Grade C-3. In staining for collagen type III, the classification was based on staining of newly-formed fibers. The newly-formed fibers were stained faintly in Grade C-1, stained moderately in Grade C-2, and stained intensely in Grade C-3. The staining for collagen types IV, V and VI was classified into Grades C-1 to C-3, the same as collagen type III on the basis of staining for newly formed fibers.

4) Statistical analysis

We used Fishers method for statistical analysis.

Results

Relationship between electrophysiological severity and clinical stages

Eight hands were classified based on clinical findings as mild type (66.7%), two hands

Table 4. Staining grade for HE and antibodies to types I to VI collagen

Case	Severity	HE	Collagen types					
			I	II	III	IV	V	VI
2	Mild	H-1	C-1	C-1	C-2	C-1	C-1	C-1
3	Mild	H-1	C-1	C-1	C-1	C-2	C-1	C-1
4	Mild	H-1	C-1	C-1	C-1	C-3	C-1	C-2
5	Mild	H-2	C-1	C-1	C-1	C-2	C-1	C-2
7	Mild	H-2	C-2	C-1	C-1	C-1	C-1	C-2
8	Mild	H-2	C-1	C-1	C-2	C-1	C-1	C-3
9	Mild	H-2	C-2	C-1	C-2	C-1	C-1	C-3
12	Mild	H-1	C-1	C-1	C-2	C-1	C-2	C-2
10	Moderate	H-3	C-2	C-1	C-2	C-3	C-2	C-3
11	Moderate	H-2	C-1	C-1	C-3	C-3	C-2	C-3
1	Severe	H-3	C-3	C-2	C-3	C-3	C-2	C-3
6	Severe	H-3	C-3	C-2	C-3	C-3	C-2	C-3
13	Control	H-1	C-1	C-1	C-1	C-1	C-1	C-1

were moderate type (16.7%) and two were severe type (16.7%). According to the electrophysiological classification for carpal tunnel syndrome, one was classified as Type 1 (8.3%), seven were Type 2 (58.3%) and four were Type 3 (33.3%). As the electrophysiologic classification was determined in an early stage of carpal tunnel syndrome when symptoms are usually mild, there was no relationship.

Relationship between pathology of the transverse carpal ligament and clinical severity

Table 4 shows the relationship between the results of pathology of the transverse carpal ligament and clinical severity. Out of eight cases with mild CTS, there were 4 cases of grade H-1 (almost normal ligament without new fiber formation) and 4 cases of grade H-2 (principal fascicles of the ligament were reduced with little new fiber formation). In two cases with moderate CTS, one was grade H-2 and the other was grade H-3 (marked reducedly and loosened fascicles of the ligament with abundant new fiber formation). Two severe CTS were H-3. There was a tendency for severe type carpal tunnel syndrome to have a higher grade of HE staining. In all 12 cases, there was no evidence of inflammatory cellular infiltration or vascularization.

In the collagen staining, we focused on collagen types I and III, and analyzed the relationship between the staining grade and clinical severity. For type I collagen staining, out of 8 patients with mild CTS, 6 cases were stained as grade C-1 (75%), and the other 2 cases were stained as grade C-2 (25%). In two cases with moderate CTS, one was grade C-1 and the other was grade C-2. In two cases with severe CTS, both were graded C-3 (Table 5).

For type III collagen staining, out of eight patients with mild CTS, four were grade C-1 (50%), and four were grade C-2 (50%). In two cases with moderate CTS, one was grade C-2 and another was grade C-3. Both cases with severe CTS were graded C-3 (Table 5).

Staining score and severity of median neuropathy

The sum of the grades for type I and type III collagen for each patient was defined as

Table 5. Staining grade of HE, type I and III collagen in mild, moderate and severe carpal tunnel syndrome

Severity		Mild	Moderate	Severe
HE	Grade H-1	4		
	Grade H-2	4	1	
	Grade H-3		1	2
Collage type I	Grade C-1	6	1	
	Grade C-2	2	1	
	Grade C-3			2
Collagen type III	Grade C-1	4		
	Grade C-2	4	1	
	Grade C-3		1	2

Between severity and collagen type I, differences were considered significant at $p < 0.005$ (mild-severe), $p < 0.05$ (moderate-severe); mild-moderate was not significant.

Between severity and collagen type III, differences were considered significant at $p < 0.05$ (mild-moderate), $p < 0.01$ (mild-severe); moderate-severe was not significant.

Table 6. Relationship between staining score and neural severity in each specimen

Case	Severity	Collagen		Score (I + III)
		I	III	
3	Mild	C-1	C-1	2
4	Mild	C-1	C-1	2
5	Mild	C-1	C-1	2
2	Mild	C-1	C-2	3
7	Mild	C-2	C-1	3
8	Mild	C-1	C-2	3
12	Mild	C-1	C-2	3
9	Mild	C-2	C-2	4
10	Moderate	C-2	C-2	4
11	Moderate	C-1	C-3	4
1	Severe	C-3	C-3	6
6	Severe	C-3	C-3	6
13	Control	C-1	C-1	2

Differences between severity and score were considered significant at $p < 0.05$ (mild-moderate), $p < 0.001$ (mild-severe), $p < 0.01$ (moderate-severe).

the 'staining score', and this was correlated with clinical severity. The average score was 2 in controls, 2.8 in mild stage, 4 in moderate and 6 in severe stage. There was a strong correlation between this point system and clinical severity (Table 6).

Other collagen subtypes

In type II collagen staining, all eight mild cases were grade C-1 (100%). There were

two moderate cases graded C-1 (100%). Both severe-type specimens were stained grade C-2.

Type V collagen fibers had similar pathological characteristics to type III collagen; the regenerating fibers were well stained in the more severe type of carpal tunnel syndrome. We could not find any correlation between types IV and VI and severity of neuropathy.

Discussion

This study investigated the pathophysiology of the transverse carpal ligament using HE staining and specific antibodies to collagen types I to VI. Results suggested that as neural severity increased, there was derangement of the primary collagen fiber bundle and reduction in thick fiber bundles. The surrounding regenerating neofibers appeared as irregular constricted fibrous bands. Previous anti-collagen antibody immunohistochemistry studies using LSAB-POD reported that collagen type I was the mature collagen fiber, type II was fibrils of hyaline cartilage, type III was fine reticular fibers, type IV was vascular basement membrane, and type V was exocytoskeletal and pericellular connecting elements⁶⁻⁸⁾. Collagen type VI has not been investigated yet. Our results suggested that as the severity of carpal tunnel syndrome advanced, the fibrosis and degeneration of the collagen bundles and the formation of regenerating fibers in the surroundings increased. Idiopathic carpal tunnel syndrome therefore originates from thickening of the transverse carpal ligament with increased fibrosis. In the ligament maturation process, there may be some transformation of the fiber due to disturbance in the normal maturation process of collagen. In the first stage, the turn-over rate in maturation of the collagen fibers might be blocked, and in the second stage irreversible and fibrotic changes to the transverse carpal ligament could happen. The changes in collagen type III histology indicated the second process. After the disturbance of the maturation of the collagen fibers, the secondary changes of the fibers, detachment and irregularity of the bundles, and interposed newborn regenerating small fibers may appear in response to the degeneration (Fig. 2).

Taniguchi *et al.*⁹⁾ reported that chondrocyte-like cells which are positive for PAS staining analysis are commonly observed in the transverse carpal ligament of idiopathic carpal tunnel syndrome patients, and that all of the cells were positive for S-100 protein. In contrast, Kobayashi *et al.*¹⁰⁾ reported that all cases they tested were negative for PAS staining analysis, and that there were no significant findings in the ligaments. In the present study, type II collagen, which is a marker of chondrocytes, was possibly stained in chondrocyte-like cells in the neurologically severe cases, but these results were not clear. However, chondrocyte-like cells positive for type II collagen were observed in the transverse carpal ligament of patients with carpal tunnel syndrome receiving dialysis¹¹⁾. Therefore, chondrocytes may be observed as the ligaments further degenerate.

Congestion or ischemia of the median nerve has been reported as one possible etiology of this syndrome. In 1976, Sunderland¹²⁾ reported venous congestion from hypotonia as a precursor to anoxic capillary endothelial damage and edema in carpal tunnel syndrome. This concept might explain the correlation between edema and vascular sclerosis. We agree with this hypothesis, however, these are the findings of circulation in the median nerve, and may represent a secondary change after fibrosis and thickening of the ligament and compression of the nerve¹³⁾. The nerve compression caused by the thickening of the ligament should be the first pathological change in idiopathic carpal tunnel syndrome.

Phalen¹⁴⁾ proposed that tenosynoviocyte fibrosis and chronic inflammatory cell invasion

Fig. 2A Case 13, 29 y.o., Female, Control
(upper) staining of Collagen types I ($\times 20$)
(lower) staining of Collagen types III ($\times 20$)
The fibers of the ligament were not disordered and were stained darkly for collagen I. There was no new fiber formation. Collagen III stain was grade C-1.

Fig. 2B Case 1, 56 y.o., Female, Severe type Idiopathic C.T.S.
(upper) staining of Collagen types I ($\times 20$)
(lower) staining of Collagen types III ($\times 20$)
The principal fascicles of the ligament were reduced and lost with abundant new fiber formation among them. The principal fascicles of the ligaments in grade C-3 were mildly stained, and new fibers were intensely stained in grade C-3.

are involved in the etiology of idiopathic carpal tunnel syndrome. In addition, Taniguchi *et al.*¹⁵⁾ reported that the presence of metaplastic chondrocytes plays a role in the hyperplasia and sclerosis of the transverse carpal ligament. Kobayashi *et al.* showed that hyperplasia of the synovial membrane surrounding the flexor digitorum profundus muscle (FDP) is responsible for idiopathic carpal tunnel syndrome.

There have been four major histological analyses of flexor tenosynovitis. Kerr *et al.*¹⁶⁾ reported that 96% of 625 cases showed chronic inflammation of the flexor tenosynovium. Fuchs *et al.*¹⁷⁾ noticed vascular sclerosis in 98% and edema in 85% of 177 cases. Scelsi *et al.*¹⁸⁾ identified collagen fibrosis in 80% of 50 cases. Schuind *et al.*¹⁹⁾ found no noteworthy abnormal findings other than hyperplasia. In the present study, no histological analysis of flexor tenosynovitis was performed, but the presence of synovitis as well as degeneration of the transverse carpal ligaments are considered to be responsible for the idiopathic carpal tunnel syndrome. Currently, endoscopic open carpal tunnel release has proven a very effective treatment for idiopathic carpal tunnel. However, in the future, new chemical

treatments using extracts from type I and type III anticollagen are expected to be developed.

This study investigated the ligament named “transverse carpal ligament”, but this name is not described in the PNA or the Japanese Society for Surgery of the Hand glossary. The PNA used the name “flexor retinaculum” to include both “volar carpal ligament” and “transverse carpal ligament”. However, we described it as the “transverse carpal ligament” by BNA and JNA, because the specimen in this study was just “transverse carpal ligament” and did not include “volar carpal ligament”²⁰⁾.

In conclusion, we investigated the clinical severity of idiopathic carpal tunnel syndrome and examined collagen expression in the transverse carpal ligament. The results indicated that as the severity of carpal tunnel syndrome advanced, the irregularity and degeneration of the collagen bundles increased in type I collagen and the formation of surrounding regenerating fibers also increased in type III collagen. These results suggest that disturbance of the maturation of collagen fibers in the transverse carpal ligament is an essential cause of idiopathic carpal tunnel syndrome.

Acknowledgments

The authors thank Ms. Yoshiko Sasaki from the Second Department of Pathology, and the staff of Orthopedic Surgery, Showa University School of Medicine, for their help in preparing the manuscript.

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[Received December 3, 1999 : Accepted December 20, 1999]