RESEARCH ARTICLE OPEN ACCESS

The Relationship Between ADAMTS-4 and ADAMTS-5 Enzyme Levels in Patients With Degenerative Disc Disease: A Prospective Biochemical Study

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Received: 18 September 2024 | **Revised:** 24 October 2024 | **Accepted:** 12 December 2024

Funding: This work was supported by Manisa Celal Bayar Üniversitesi.

Keywords: ADAMTS-4 | ADAMTS-5 | biomarkers | lumbar disc degeneration

ABSTRACT

Study Design: Prospective biochemical study of comparison of A Disintegrin and Metalloproteinase with Thrombospondin motifs-4 (ADAMTS-4) and A Disintegrin and Metalloproteinase with Thrombospondin motifs 5 (ADAMTS5) levels in preoperative and postoperative venous blood, as well as in disc tissue obtained during surgery, in patients undergoing surgery for intervertebral disc disease, with enzyme levels in venous blood from a control group.

Objective: To compare the levels of ADAMTS-4 and ADAMTS-5 between patients with degenerative intervertebral discs and a healthy control group, aiming to identify biomarkers associated with intervertebral disc degeneration.

Literature: Although numerous studies have investigated the relationship between ADAMTS-4 and ADAMTS-5 enzymes and degeneration in experimental rat models and human tissues, no study has correlated their serum levels with intervertebral disc degeneration.

Method and Materials: Venous blood samples were obtained preoperatively and postoperatively from 41 patients (age: 42 ± 9.7 years, range 20–63) diagnosed with intervertebral disc disease. The affected disc levels were L4-L5 in 22 patients and L5-S1 in 19 patients. These patients were selected based on surgical indications due to radicular pain that persisted after an adequate course of conservative management, without any non-neurological deficit. Disc tissue samples were also obtained during surgery. Additionally, venous blood samples were collected from a control group with no diagnosed diseases, and lumbar MRIs of the control group showed no significant signs of degeneration. ADAMTS-4 and ADAMTS-5 levels were measured using the ELISA method on samples obtained after centrifugation of the collected blood and tissue specimens.

Results: The level of ADAMTS-4 in patient serum was found to be lower compared to the control group, while the level of ADAMTS-5 was higher in the patient serum and lower in the control group.

Conclusion: Elevated levels of ADAMTS-5 in the blood may be associated with intervertebral disc degeneration.

1 | Introduction

Intervertebral disc degeneration is a significant health issue affecting millions worldwide. It is a leading cause of lower back pain, impacting approximately 700 million people globally [1, 2]. This condition not only impairs the quality of life but also leads to substantial economic burdens due to increased healthcare costs and productivity loss [3]. Lower back pain, often resulting from degenerative disc disease, affects individuals across various age groups and socioeconomic backgrounds. Lower back pain has a multifactorial etiology, with various factors contributing to its occurrence. However, it is widely accepted that degenerative disc disease is considered one of the leading causes of lower back pain [4].

The intervertebral disc (IVD) is composed of three main components: the two cartilaginous endplates adjacent to the vertebrae, the gel-like nucleus pulposus rich in hyaluronic acid, and the annulus fibrosus surrounding the nucleus pulposus. The disc's avascular nature necessitates nutrient diffusion from the endplates. Damage to these endplates disrupts nutrient flow and initiates degenerative changes, including the degradation of proteoglycans such as aggrecan [5–7].

Numerous studies have demonstrated that enzymes called "aggrecanases" are responsible for aggrecan degradation in diseases affecting cartilage, such as osteoarthritis and rheumatoid arthritis. These enzymes are members of the a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) family. In human arthritis, in the context of intervertebral disc degeneration, A Disintegrin and Metalloproteinase with Thrombospondin motifs-4 (ADAMTS-4) and A Disintegrin and Metalloproteinase with Thrombospondin motifs 5 (ADAMTS5) are recognized as significant aggrecanases [8–11]. Despite extensive research on these enzymes in cartilage-related diseases, there is limited information regarding their serum levels and association with intervertebral disc degeneration. This study aims to explore the relationship between ADAMTS-4 and ADAMTS-5 levels in serum and disc tissue and their potential as biomarkers for disc degeneration.

2 | Materials and Methods

2.1 | Study Design

The study involved correlating and comparing ADAMTS-4 and ADAMTS-5 levels in venous blood samples obtained preoperatively and postoperatively, as well as in disc tissue collected during surgery, with enzyme levels in a control group.

2.2 | Participants

A total of 41 subjects, for whom surgery was decided due to the diagnosis of Carragee type 2 and type 3 intervertebral disc herniation with radicular symptoms but no neurological deficit, were examined. The average age of this group was 42 ± 9.7 years (range 20–63), with a gender distribution of 20 males and 21 females. Herniated disc levels were L4-L5 (10 right, 10 left, or 2 bilateral) in 22 patients and L5-S1 (10 right, 9 left) in 19 patients, all of whom experienced radicular pain persisting after adequate conservative management without neurological deficits. Venous blood samples (10 cc) were collected from these subjects both preoperatively and postoperatively. Additionally, disc tissue obtained during surgery was collected for analysis.

The control group consisted of 41 individuals with an average age of 36.5 ± 4.5 years (range 30–46) and a gender distribution of 19 males and 22 females. Individuals in the control group had not been diagnosed with any autoimmune disease, did not have complaints of back or leg pain, and lumbar MRIs did not reveal any significant signs of degeneration. Similar to the study group, 10 cc venous blood samples were collected from the control group.

ADAMTS are associated with various diseases, particularly osteoarthritis. Therefore, both groups were selected to exclude individuals with any inflammatory diseases such as cartilage degeneration (rheumatoid arthritis, osteoarthritis), heart, or skin diseases. This decision was based on anamnesis.

2.3 | Sample Collection and Storage

Samples were stored at the Celal Bayar University Hospital Biochemistry Laboratory during the collection phase. Tissues were kept in sterile containers, and venous blood samples were centrifuged at 3000 rpm for 15 min in clot activator tubes without anticoagulant. The resulting sera were separated and stored at −80°C until further analysis.

2.4 | Processing of Samples

After reaching a sufficient number of samples, the disc tissues obtained during surgery were washed with cold phosphate-buffered saline (PBS) and mechanically dissected. The weights of tissue fragments were measured with a precision balance. Subsequently, the tissue fragments were homogenized in PBS (tissue weight (g): PBS (mL) = 1:9) using a tissue homogenizer (IKA Labortechnik, Staufen, Germany) on ice. The homogenates were then centrifuged at 5000 rpm for 10 min, and the supernatants were separated.

The concentrations of ADAMTS-4 and ADAMTS-5 in serum and tissue supernatant samples were analyzed using the Enzyme-Linked Immunoassay (ELISA) method. ELISA washing procedures were performed with an automated washing device (BioTek ELx50, BioTek Instruments Inc. Highland Park, Winooski, VT, USA), and absorbance readings were taken with an ELISA plate reader (BioTek Epoch BioTek Instruments Inc. Highland Park, Winooski, VT, USA).

2.5 | ADAMTS-4 and ADAMTS-5 Analysis

The levels of human ADAMTS4 in serum and tissue supernatants were analyzed using ELISA kits (Elabscience Biotechnology, Wuhan, China) according to the manufacturer's instructions. The kit has a sensitivity of 37.5 pg/mL with a

measurement range of 62.5–4000 pg/mL. Intra-assay precision CV values for the kit were calculated as 5.39% for a concentration of 194.77 pg/mL, 5.87% for 637.05 pg/mL, and 3.49% for 1878.94 pg/mL. Inter-assay precision CV values were calculated as 6.57% for a concentration of 194.68 pg/mL, 5.35% for 684.14 pg/mL, and 3.91% for 1824.55 pg/mL. The positive internal quality control range was 825–1375 pg/mL, while the negative internal quality control range was ≤ 57.50 pg/mL. Both positive and negative internal quality control samples were detected within the specified ranges.

The levels of Human ADAMTS5 in serum and tissue supernatants were analyzed using ELISA kits (Elabscience Biotechnology, Wuhan, China) following the manufacturer's instructions. The kit has a sensitivity of 0.47 ng/mL with a measurement range of 0.78–50 ng/mL. Intra-assay precision CV values for the kit were calculated as 6.61% for a concentration of 2.57 ng/mL, 4.4% for 7.27 ng/mL, and 4.27% for 23.18 ng/mL. Inter-assay precision CV values were calculated as 6.23% for a concentration of 2.57 ng/mL, 5.75% for 7.13 ng/mL, and 3.29% for 24.01 ng/mL. The positive internal quality control range was 18.83–31.38 ng/mL, while the negative internal quality control range was ≤ 0.72 ng/mL. Both positive and negative internal quality control samples were detected within the specified ranges.

The concentrations of ADAMTS4 and ADAMTS5 are reported in pg/mL and ng/mL units for serum samples, respectively. For tissue supernatants, the dilution factor was calculated based on the measured tissue weights and prepared suspension amounts. The concentrations are reported as pg/g and ng/g, respectively.

2.6 | Statistical Analysis

The data were analyzed using SPSS 23.0 statistical software. Descriptive statistics were used for the evaluation, including counts, percentages, mean ± standard deviation, and median (min-max). The student *t*-test was employed for univariate continuous data analysis. The critical type 1 error value was considered < 0.05.

3 | Results

Age, gender, and enzyme values of the study and control groups are summarized in Table 1 and Table 2.

3.1 | ADAMTS-4 and ADAMTS-5 Levels in Tissue

In the analysis of disc tissues obtained during surgery, the median ADAMTS-4 level was found to be 62.5 pg/mL (range 62.5–31065.45 pg/mL), and the median ADAMTS-5 level was determined to be 12.27 ng/mL (range 0.78–82.11 ng/mL).

3.2 | ADAMTS-4 Levels in Serum

The average ADAMTS-4 level in the patient serum was 2442.73 ± 896.35 pg/mL (range 2159.81–2725.65 pg/mL). In the control group serum, the average ADAMTS-4 level was 3112.75 ± 1692.49 pg/mL (range 2578.53–3646.97 pg/mL).

3.3 | ADAMTS-5 Levels in Serum

The average ADAMTS-5 level in the patient serum was 3.1 ± 1.1 ng/mL (range 2.9–3.6 ng/mL). In the control group serum, the average ADAMTS-5 level was 1.1 ± 0.5 ng/mL (range 0.9–1.3 ng/mL).

3.4 | Preoperative and Postoperative Levels of ADAMTS-4 and ADAMTS-5

A statistically significant increase in ADAMTS-4 and ADAMTS-5 enzyme levels was observed in postoperative serum samples compared to preoperative samples.

3.5 | Comparison of ADAMTS-4 and ADAMTS-5 Levels

Univariate analysis revealed that the control group was younger on average than the patient group, a difference that was statistically significant. Gender distribution was balanced in both groups with no significant difference.

No correlation was found between ADAMTS-4 levels and age in serum. However, a statistically significant moderate positive relationship was observed between ADAMTS-5 levels and age in both serum and disc tissue.

A statistically significant strong positive relationship was observed between ADAMTS-4 and ADAMTS-5 levels in disc tissue.

In the control group serum, ADAMTS-4 levels were higher than in the patient group, and this difference was statistically significant. Conversely, in the patient group serum, ADAMTS-5 levels were higher than in the control group, and this difference was statistically significant.

4 | Discussion

The pathogenesis of intervertebral disc degeneration involves multiple factors, including age, gender, environmental influences, and genetic predispositions. It is well known that these factors contribute to disc degeneration. Central to this process is the initiation of inflammation due to insufficient diffusion in the avascular disc. Evidence has shown that key proinflammatory cytokines, especially tumor necrosis factors-α (TNF-α), interlukin-1 (IL-1), and interleukin-6 (IL-6), increase destruction and decrease synthesis in disc degeneration [12].

In intervertebral disc degeneration, changes in collagen molecules are accompanied by a decrease in proteoglycan content. It has been reported that the increased production of matrix metalloproteinases (MMP) accelerate the breakdown of proteoglycans, contributing to disc degeneration. ADAMTS

TABLE 1 | Data of the study group.

		ADAMTS-4 pg/mL values			ADAMTS-5 ng/mL values		
Patient	Age/ Gender	Pre-operative serum levels	Post-operative serum levels	Disc tissue levels	Pre-operative serum levels	Post-operative serum levels	Disc tissue levels
1.	33/F	430,07	1245,05	7142,19	3,02	4,02	16,20
2.	63/F	1082,56	714,71	< 62.5	8,88	8,23	16,12
3.	32/M	2396,38	1731,13	7893,43	< 0.78	< 0.78	12,27
4.	52/F	1587,28	1543,74	< 62.5	2,86	2,78	10,68
5.	50/F	3125,38	2991,28	< 62.5	3,94	4,05	27,87
6.	41/M	2373,93	2492,64	< 62.5	1,79	3,00	< 0.78
7.	42/M	2698,65	2799,26	2119,90	0,83	0,80	8,69
8.	34/M	2157,61	2287,97	5696,99	3,36	3,52	< 0.78
9.	42/F	2883,09	3044,64	1500,92	2,81	2,97	< 0.78
10.	51/M	3057,50	3210,33	31 065,45	1,22	1,71	82,11
11.	53/F	3156,37	3957,18	< 62.5	3,45	4,27	21,66
12.	40/F	2184,65	1098,04	< 62.5	1,35	< 0.78	< 0.78
13.	38/F	3170,35	2721,18	3201,80	2,42	2,40	19,29
14.	39/F	2306,43	2370,36	< 62.5	1,84	1,85	7,42
15.	59/M	3041,60	3375,83	< 62.5	6,97	7,26	22,28
16.	27/M	2962,31	3319,53	652,75	1,52	1,63	7,26
17.	29/M	3489,10	3581,86	< 62.5	2,99	2,72	13,65
18.	40/M	1856,78	2513,59	12 561,17	2,58	3,43	30,93
19.	30/F	1866,00	1614,44	19 162,21	1,84	1,54	16,15
20.	26/F	1632,13	1986,46	< 62.5	1,47	1,57	10,66
21.	46/M	2730,79	1937,32	3846,49	3,59	6,20	< 0.78
22.	20/M	1063,27	2142,17	7830,47	2,53	1,02	< 0.78
23.	40/F	3143,08	2658,31	738,81	2,34	1,92	31,07
24.	45/M	3174,11	2955,91	< 62.5	2,23	2,09	27,83
25.	41/F	2522,44	3827,09	< 62.5	3,19	3,90	18,96
26.	41/F	2607,85	3342,64	< 62.5	4,07	2,80	18,86
27.	42/F	2529,00	2365,16	1140,56	4,57	2,64	8,76
28.	48/F	1672,27	1664,68	2747,04	3,43	4,21	22,59
29.	34/F	3030,44	3752,56	1933,65	1,52	1,54	7,73
30.	41/M	3405,28	1997,96	< 62.5	2,20	2,47	< 0.78
31.	34/M	3797,44	2964,33	< 62.5	2,13	2,24	8,51
32.	43/M	2565,75	2745,06	< 62.5	4,78	4,86	< 0.78
33.	57/F	2107,80	1745,07	< 62.5	3,52	3,92	< 0.78
34.	44/F	2251,75	2421,78	< 62.5	4,96	4,43	34,34
35.	56/M	770,52	1227,72	4336,66	2,35	3,65	60,28

(Continues)

TABLE 1 | (Continued)

		ADAMTS-4 pg/mL values			ADAMTS-5 ng/mL values		
Patient	Age/ Gender	Pre-operative serum levels	Post-operative serum levels	Disc tissue levels	Pre-operative serum levels	Post-operative serum levels	Disc tissue levels
36.	49/M	3016,25	2357,36	< 62.5	2,28	2,43	< 0.78
37.	30/F	4417,02	4608,96	< 62.5	2,91	2,71	< 0.78
38.	55/M	2376,53	1856,14	< 62.5	4,96	6,86	25,43
39.	56/M	3151,60	2727,47	< 62.5	4,79	4,19	12,38
40.	40/M	< 62.5	1835,48	< 62.5	2,62	1,72	17,52
41.	40/F	2298,33	1662,15	9941,84	6,77	5,15	43,26

Abbreviations: ADAMTS-4: A Disintegrin and Metalloproteinase with Thrombospondin motifs-4; ADAMTS5: A Disintegrin and Metalloproteinase with Thrombospondin motifs 5.

exhibit more potent proteolytic properties than the MMP family, with its subtypes having more specific working mechanisms. Numerous studies on the ADAMTS family indicate that ADAMTS-1, ADAMTS-4, ADAMTS-5, ADAMTS-8, ADAMTS-9, and ADAMTS-15 function as aggrecanases [12]. Particularly in osteoarthritis pathology, the ability of ADAMTS-4 and ADAMTS-5 to cleave aggrecan, a proteoglycan, has been demonstrated in numerous studies [13–15]. Suppression of ADAMTS-4 and ADAMTS-5 has been found to reduce aggrecan degradation in cartilage tissue [16].

Studies aimed at understanding cartilage tissue diseases and exploring treatment methods have focused on the aggrecanase properties of ADAMTS-4 and ADAMTS-5. The primary objective of our study was to identify which of these enzymes is primarily responsible for degeneration in disc tissues. Additionally, we aimed to elucidate the relationship between these enzymes in disc tissue and blood, investigating their potential as biochemical markers in venous blood.

The decrease in proteoglycan leads to impaired cell membrane permeability, allowing large molecules such as cytokines to enter the cell. Cytokines involved in signal transmission in disc cells, such as IL-1, suppress proteoglycan production through nitric oxide, accelerating the degeneration process [17].

Interleukin-1 β (IL-1β) is a major cytokine in disc degeneration [18]. Catabolic cytokines like IL-1β and TNF-α induce ADAMTS-4 from chondrocytes, with ADAMTS-5 also being structurally produced [13, 19]. However, some mouse experiments have shown that ADAMTS-5 induction is supported by IL-1 [20, 21]. This discrepancy may be attributed to differences between human and mouse chondrocytes. A recent study demonstrated that IL-1β induces ADAMTS-5 expression in a human osteoarthritis model [20]. IL-1β increases ADAMTS-4 expression in nucleus pulposus cells through the activation of the nuclear factor-kappa B (NF-κB) pathway and facilitates ADAMTS-5 synthesis [22].

In a study by Chen et al. the induction of ADAMTS-5 by cytokines was examined in endplate tissues of patients undergoing surgery for intervertebral disc degeneration [7]. Histopathological and molecular examinations showed increased expression of both ADAMTS-5 and TNFα genes in the degenerated group compared to the control group, revealing a statistically significant relationship between the two genes. Bovine endplate chondrocytes cultured with TNFα showed increased ADAMTS-5 in response to TNFα stimulation via the NF-κB pathway, while ADAMTS-4 remained unchanged [7].

In studies investigating disc degeneration mechanisms, Patel et al. observed an increase in ADAMTS-4 levels in human degenerated disc tissue as degeneration progressed, but found no significant difference for ADAMTS-5, associating IVD degeneration with ADAMTS-4 [11, 23]. Conversely, Maitre et al. detected ADAMTS-5 gene expression in degenerated discs but not in non-degenerate discs, linking IVD degeneration with ADAMTS-5 [24]. Given these contrasting findings, further studies are needed to identify the main aggrecanase responsible for intervertebral disc degeneration. Our study found a coordinated increase in the levels of both enzymes.

Aggrecan fragments detected in synovial fluid have served as markers of aggrecanase-mediated catabolic activity since metabolites within the joint often clear through synovial fluid [25]. However, in IVD, metabolite clearance occurs through diffusion in the cartilaginous endplate, making the detection of aggrecan degradation products in serum more challenging [26]. Nevertheless, damage to vascular permeability during degeneration can increase aggrecan degradation products and major aggrecanase levels in the bloodstream. This led us to design our experiment to detect elevated levels of these products in serum resulting from vascular permeability impairment due to mechanical disc damage. Our study observed increased enzyme levels in postoperative venous blood in the discectomy patient group, suggesting that mechanical disc damage impairs vascular permeability, increasing enzyme quantities in the bloodstream.

Identifying the main enzyme responsible for aggrecanase in IVD can lead to targeted local treatments for specific aggrecanases, such as those with Carrage Type 1 and Type 4, where surgery is not the primary consideration. This targeted treatment

TABLE 2 | Data of the control group.

Control	Age/ Gender	ADAMTS-4 pg/ mL levels in serum	ADAMTS-5 ng/ mL levels in serum
1.	42/M	4224,18	1,51
2.	40/M	339,02	1,84
3.	35/F	3985,91	0,79
4.	43/M	4939,77	< 0.78
5.	46/F	1112,06	1,10
6.	33/F	4334,83	1,92
7.	37/M	609,47	< 0.78
8.	30/M	2809,82	1,44
9.	41/M	3105,60	< 0.78
10.	36/M	3594,79	2,36
11.	33/M	1489,81	1,39
12.	32/M	3876,82	< 0.78
13.	34/M	3141,80	< 0.78
14.	34/M	2113,84	1,04
15.	38/M	3714,37	1,91
16.	35/F	5129,56	2,36
17.	41/F	2192,91	< 0.78
18.	34/F	4481,06	< 0.78
19.	43/F	4391,63	< 0.78
20.	30/M	3055,11	< 0.78
21.	41/F	5301,64	1,93
22.	30/F	3320,92	1,30
23.	32/M	2653,85	0,93
24.	38/F	5071,72	0,90
25.	30/F	3699,85	< 0.78
26.	34/F	3347,15	< 0.78
27.	34/M	2513,71	< 0.78
28.	37/M	5685,85	< 0.78
29.	46/M	3779,70	1,76
30.	38/F	3949,10	< 0.78
31.	43/F	2252,81	< 0.78
32.	33/F	3001,77	1,11
33.	37/F	3723,56	1,35
34.	36/M	2757,30	1,02
35.	32/M	3722,03	0,87
36.	35/F	2410,25	2,40
37.	33/F	2505,76	< 0.78
38.	39/F	3762,74	0,96
39.	44/M	2365,44	1,11
40.	32/M	2725,74	< 0.78
41.	38/F	3297,31	0,87

Abbreviations: ADAMTS-4: A Disintegrin and Metalloproteinase with Thrombospondin motifs-4; ADAMTS5: A Disintegrin and Metalloproteinase with Thrombospondin motifs 5.

could facilitate reabsorption of herniated disc tissue without surgery. Alternatively, inhibiting the aggrecanase responsible for high blood levels may prevent degeneration progression. We also believe that widespread disc degeneration can be biochemically monitored.

In our study, ADAMTS-5 levels in serum were higher in the patient group, while ADAMTS-4 levels were higher in the healthy group. This suggests that ADAMTS-5 may be the responsible aggrecanase in intervertebral disc degeneration.

A positive correlation was observed between age and ADAMTS-5 levels in both serum and disc tissue. Although the close relationship between ADAMTS-5 and aging is assumed, the statistical difference in average age between the patient and control groups in our study may have influenced the results [10]. Additionally, the current disc pathologies of the operated patients were not radiologically classified. Further studies can be planned to identify the main aggrecanase responsible for intervertebral disc degeneration.

5 | Conclusion

Monitoring ADAMTS enzyme levels might help in assessing the progression of disc degeneration. Future research should focus on understanding the specific roles of ADAMTS-4 and ADAMTS-5 in disc degeneration and their potential as biomarkers or therapeutic targets. Elevated serum ADAMTS-5 levels may be linked to disc degeneration. Targeting specific aggrecanases could lead to non-surgical treatments for disc degeneration.

Disclosure

The authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this article.

Ethics Statement

This study was approved by the local institutional review board (IRB).

Conflicts of Interest

The authors declare no conflitcs of interests.

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