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ORIGINAL ARTICLE

Investigation of cartilage degradation in patients with spinal cord injury by CTX-II

G Findikoglu[1], B Gunduz[2], H Uzun[1], B Erhan[2], S Rota[3] and F Ardic[1]

Study design: Clinical cross-sectional study.
Objectives: To investigate the cartilage degradation by turnover of C-telopeptide fragments of collagen type-II (CTX-II), a molecule specific for articular cartilage in spinal cord injured patients with respect to clinical functional status.
Setting: Physical Medicine and Rehabilitation Clinics, hospital settings.
Methods: In 68 patients with spinal cord injury (SCI) level and severity of lesion, duration of disease, American Spinal Injury Association Impairment Scale (AIS), motor and sensory score, presence of spasticity, functional ambulation score (FAS) and duration of daily ambulation were evaluated. Cartilage degradation was demonstrated by urinary CTX-II (uCTX-II) measured by enzyme-linked immunosorbent assay. *T* test, analysis of variance and Pearson correlation analysis were used for statistical calculations.
Results: uCTX-II level was significantly higher in patients with AIS grade A, non-functional ambulators or in patients who did not ambulate at all ($P<0.05$). Although AIS grade, lower extremity motor score, FAS score and duration of daily ambulation were found to be correlated ($P<0.05$) with uCTX-II, duration of disease, level of neurological lesion, presence of spasticity were not.
Conclusion: This is the first study providing evidence that cartilage degradation is associated with elevated uCTX-II levels in non-ambulating or non-functional ambulating SCI patients. AIS grade A, FAS zero score and no time for daily ambulation were found to cause significant differences in CTX-II level. It may be important to initiate therapeutic programs as soon as possible after SCI to prevent cartilage atrophy.
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Keywords: spinal cord injury; immobilization; cartilage; osteoarthritis; biomarkers; CTX-II

INTRODUCTION

In the patients with spinal cord injury (SCI), neurological severity depending on the level and the extent of the injury determines the functional status of the extremities, which may remain partially or totally unloaded and restricted in movement. Most of the literature studying the effect of unloading or immobilization on articular cartilage is animal studies, which demonstrate morphologic, biochemical and biomechanical adaptations.[1]

Mechanical loading is known to influence the development, maintenance and aging of skeletal tissues including articular cartilage. External stimulus is supposed to effect cell differentiation.[1] It has been proposed that intermittent hydrostatic pressure promotes cartilage biosynthesis and maintains its structural and functional competence, and that shear stress, prolonged static loading or absence of loading promote cartilage degradation and ossification.[2]

Joint unweighting is proposed to promote tidemark advancement and thinning of the uncalcified cartilage layer.[1] Decrease in sound speed by scanning acoustic microscopy was observed in the transitional area, indicating the softening of the articular cartilage as early as 1 week after immobilization in rats.[3] A decrease in canine tibial cartilage thickness after rigid immobilization was observed in contrast with the findings after non-rigid immobilization.[4] Enneking and Horowitz[5] found normal cartilage in human knee joints with SCI of 3 and 1.5 years of duration.

Research on effect of immobilization on articular cartilage in human relies on non-invasive radiological studies. Narrowing of the hip joint space by >50% in patients with flaccid paralysis was identified by radiography. However, radiography does not allow direct visualization of cartilage. Significant differences in patellar and tibial cartilage thickness of SCI patients compared with healthy volunteers were shown by magnetic resonance imaging (MRI).[4,6] Furthermore, a longitudinal MRI study showed progressive loss of thickness 12 and 24 months after SCI in patellar, tibial cartilage compared with thickness in 6 months and of healthy controls.[6] Local areas of thinning was shown for the cartilage of the patella 6 and 12 months after injury by thickness maps and morphological parameters.[7]

Progressive loss of articular cartilage is the hallmark of osteoarthritis (OA). Change in joint space width detected by radiography is the gold standard. On the other hand, it does not allow the early detection of cartilage damage or the efficient monitorization of treatment due to its poor sensitivity and relatively large precision error.[8] Biochemical markers that reflect variations in joint tissue remodeling have been proposed as a tool for early detection of cartilage damage. Analysis of body fluids can provide information regarding the turnover of the cartilage before the development of gross pathology and can help to follow changes attributable to the treatment.[9]

Type II collagen is cartilage specific and forms the basic fibrillary structure of the extracellular matrix of hyaline cartilage, which is

[1]Department of Physical Medicine and Rehabilitation, University of Pamukkale, Denizli, Turkey; [2]1st Physical Medicine and Rehabilitation Clinic, Istanbul Physical Medicine and Rehabilitation Research and Training Hospital, Istanbul, Turkey and [3]Department of Medical Biochemistry, University of Pamukkale, Denizli, Turkey
Correspondence: Assistant Professor G Findikoglu, Department of Physical Medicine and Rehabilitation, University of Pamukkale, Denizli 20100, Turkey.
E-mail: gulin_f@yahoo.com
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broken down by matrix metalloproteinases. The degradation of mature matrix type II collagen can be assessed by measuring the urinary excretion of crosslinked C-telopeptide fragments (uCTX-II) by monoclonal antibodies. Concentrations of uCTX-II were found to be higher in patients with OA or rheumatoid arthritis.[9] In patients with OA, CTX-II is associated with the burden of the disease within one or multiple joints and higher levels are associated with more rapid radiological progression.[8]

In tail suspended rats, it was demonstrated that skeletal unloading increased systemic type-II collagen degradation that correlated significantly with tidemark advancement and increased the alkaline phosphatase expression at the deep zone.[10] However, to our knowledge, there is no report on the effects of unloading on turnover of type II collagen in SCI patients, as well as on functional status of the patients related with level of injury, severity of injury, duration of disease, type and duration of ambulation, and spasticity of joints. Therefore, we aimed to demonstrate cartilage degradation associated with biochemical changes measured by uCTX-II in SCI patients with respect to functional status for the first time and explain the relationship between joint use or disuse and cartilage adaptation as a critical step in the process of developing strategies to prevent and treat cartilage disorders in SCI patients.

MATERIALS AND METHODS

A total of 68 SCI patients (20 women and 48 men) between 20–79 years of age who were followed in the Physical Medicine and Rehabilitation Clinics departments of two centers between March and June 2010 and fulfilled the inclusion-exclusion criteria were included in the study (Table 1).

Patients were examined according to the International Standards for Neurologic Classification of Spinal Cord Injury by doctors of medicine in Physical Medicine and Rehabilitation. All patients were evaluated for duration of disease, level and severity of neurological lesion, duration of daily ambulation, and upper and lower extremity motor and sensory score by the American Spinal Injury Association (ASIA) Impairment Scale (AIS),[11] presence of spasticity by Ashworth Scale[12] and ambulatory status by functional ambulation score (FAS).[13] None of the patients were taking a physical exercise program at the time of the study. Functional status of patients was evaluated as 'able to do', 'able to do with assistance', or 'not able to do'.

Second void morning urine samples after an overnight fasting were collected to avoid a possible diurnal variability of the CTX-II and kept at $-20\,^{\circ}C$ until analyzed. If patients were using a clean intermittent catheter, second sample of urine was taken. CTX-II was measured in the same lab using a competitive enzyme-linked immunosorbent assay (CartiLaps; IDS, Boldon, UK) based on a mouse monoclonal antibody raised against the EKGPDP sequence of human type II collagen C-telopeptide according to the manufacturer's instructions. The uCTX-II level ($\mu g\,l^{-1}$) was corrected by the urinary creatinine concentration (CTX-II ng per creatinine mmol). Creatinine measurement was performed by Architect ci8200 (Abbott Laboratories, Abbott Park, IL, USA) analyzer.

This study was planned as a cross-sectional clinical study of two centers. The research protocol was approved by the Ethical committee. We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during the course of this research.

As uCTX-II values were not normally distributed, a logarithmic transformation was applied (log uCTX-II). *T* test and analysis of variance were used for comparison of groups. Pearson correlation analysis and linear regression were used to find the relation between the factors under investigation. $P<0.05$ was accepted as significant. SPSS 17.0 program (SPSS Inc., Chicago, IL, USA) was used for statistical calculations.

RESULTS

In all, 20 (24. 9%) female and 48 (70.6%) male SCI patients with a mean age of 39.88 ± 14.01 years and a mean body mass index of $24.12 \pm 5.13\,kg\,m^{-2}$ were included in the study (Table 2). Range of duration of disease was between 4–64 months (mean: 62.49 ± 62.66 months). A total of 20 patients (24.9%) were tetraplegic and 48 (70.6%) were paraplegic. Mean ASIA total motor score was 53.48 ± 28.33. Mean motor score of lower and upper extremities were 7.61 ± 12.81 and 45.87 ± 25.60, respectively. In all, 36 (52.9%) patients had spasticity (Table 2). Functional and ambulatory status of patients evaluated by FAS are shown in Table 3. Average duration of daily ambulation on the condition that joints were loaded was found to be 100.92 ± 123.27 min.

As level of log uCTX-II was not found to be correlated with factors that were supposed to effect level of CTX-II such as age, gender, weight and body mass index, a correction was not applied.

Level of log uCTX-II was not significantly different among AIS grades and between males and females (Table 4). As distribution of number of patients in each AIS grade, in each FAS score group and in

Table 1 Inclusion exclusion criteria for admission

Inclusion criteria
Patients with SCI for at least 3 months
Postmenopausal, if women
Exclusion criteria
Taking drugs for osteoporosis (estrogens, bisphosphonates, selective estrogen receptor modulators)
Taking drugs interfering with bone metabolism (prednisolone, anabolic androgens, teriparatide)
Having metabolic disorder of bone (hyperthyroidism, hyperparathyroidism)
Having urinary tract infection
Liver dysfunction
Kidney dysfunction
History of osteoarthritis
Intra-articular steroid or hyaluronic acid injection within last 3 months

Abbreviation: SCI, spinal cord injury.

Table 2 Demographic characteristics and neurological findings of the patients

Age (years)	39.88 ± 14.01
Gender (female/male) *n*	20/48
BMI ($kg\,m^{-2}$)	24.12 ± 5.13
Duration of disease (months)	62.49 ± 62.66 (range: 4–64)
Level of injury	
C1–C4	5 (7.4%)
C5–C8	15 (22.1%)
T1–T12	43 (63.2%)
L1–L3	5 (7.4%)
AIS grade	
A	37 (54.4%)
B	12 (17.6%)
C	8 (11.8%)
D	11 (16.2%)
ASIA total motor score	53.48 ± 28.33
ASIA upper extremity motor score	45.87 ± 25.60
ASIA lower extremity motor score	7.61 ± 12.81
ASIA total pin prick score	56.94 ± 26.07
ASIA total light touch score	62.13 ± 26.57
Presence of spasticity	36 (52.9%)

Abbreviations: AIS, American Spinal Injury Association Impairment Scale; ASIA, American Spinal Injury Association; BMI, body mass index.
Values are represented as mean ± s.d. or *n* (%).

Table 3 Functional status, functional ambulatory status of patients and duration of ambulation

	Able to do, n *(%)*	*Able to do with assistance,* n *(%)*	*Not able to do,* n *(%)*
Functional status			
Turning in bed	51 (14.7)	7 (10.3)	10 (14.7)
Sitting after lying in bed	48 (70.6)	5 (7.4)	15 (22.1)
Sitting (knee bended)	48 (70.6)	8 (11.8)	12 (17.6)
Sitting (knee extended)	41 (60.3)	10 (14.7)	17 (25)
Standing from sitting position	28 (41.2)	17 (25)	23 (33.8)
Maintain standing balance	20 (29.4)	18 (26.4)	30 (44.1)
Walking	17 (25)	9 (13.2)	42 (61.8)
Climbing stairs	8 (11.8)	6 (8.8)	54 (79.4)
Functional ambulation			
Non-functional ambulator (score 0)			49 (72.1)
Ambulator-dependent for physical assistance-level 2 (score 1)			2 (2.9)
Ambulator-dependent for physical assistance-level 1 (score 2)			4 (5.9)
Ambulator-dependent for supervision (score 3)			1 (1.5)
Ambulator-independent-level surfaces only (score 4)			6 (8.8)
Ambulator-independent (score 5)			6 (8.8)
Duration of ambulation/day, mean (range)			102.92 ± 123.27 (0–720)

Table 4 Comparison of characteristics of SCI patients with respect to log CTX-II level

Factor	*Log uCTX-II (μg ml^{-1}), mean ± s.d.*	P
Gender		
Female (n=20)	2.84 ± 0.35	>0.05
Male (n=48)	2.76 ± 0.36	
Level of injury		
C1–C4 (n=5)	2.86 ± 0.26	>0.05
C5–C8 (n=15)	2.74 ± 0.52	
T1–T12 (n=43)	2.80 ± 0.33	
L1–L3 (n=5)	2.79 ± 0.36	
AIS scale		
A (n=37)	2.85 ± 0.34	**<0.05**
Non-A (B, C, D) (n=31)	2.63 ± 0.35	
FAS		
Non-functional ambulator (score 0; n=49)	2.85 ± 0.33	**<0.05**
Non-zero (score 1, 2, 3, 4, 5; n=19)	2.60 ± 0.35	
Duration of ambulation		
0 min (n=21)	2.92 ± 0.34	**<0.05**
>0 min (n=47)	2.72 ± 0.35	
Walking		
Able to do (n=17)	2.58 ± 0.36	**<0.05**
Not able to do (n=42)	2.85 ± 0.32	
Presence of spasticity		
No (n=32)	2.85 ± 0.35	>0.05
Yes (n=36)	2.72 ± 0.36	

Abbreviations: AIS, American Spinal Injury Association Impairment Scale; FAS, functional ambulation score; SCI, spinal cord injury; uCTX-II, urinary collagen type-II.
Statistically significant values are shown in bold (t test, analysis of variance).

groups of daily ambulation time were low in number for comparison, patients were grouped as AIS grade A and others (non-A), FAS score zero and others (non-zero) and patients who did not ambulate vs patients who spend time for daily ambulation. Log uCTX-II level was found to be significantly different in grade A patients than non-A patients ($P<0.05$) and patients with FAS zero score than others (non-zero; $P<0.05$). Patients who did not spend time for daily ambulation had a significantly higher log uCTX-II level compared with ambulating patients. This finding was supported by the answer to a simple question asked to the patient: 'Can you walk?' Patients who described themselves to walk had significantly lower log uCTX-II level than patients who did not ($P<0.05$). Patients with and without spasticity in the joints did not have statistical difference in levels of log uCTX-II ($P>0.05$).

Duration of disease, level of neurological lesion, ASIA total or upper extremity motor score and presence of spasticity were not correlated with CTX-II level ($P>0.05$; Table 5). On the other hand, AIS grade, FAS score, duration of daily ambulation and ASIA lower extremity motor score were found to be correlated with level of log uCTX-II. FAS score was also correlated with level of injury (r=0.377, $P<0.05$). Among these factors, FAS score was found as the most effective determinant on CTX-II level by regression analysis.

DISCUSSION

Experimental evidence indicated that the biosynthetic response of chondrocytes was sensitive to the frequency and amplitude of loading. Excessive, repetitive loading may induce cell death, and cause morphological and cellular damage as seen in degenerative joint disease. It was demonstrated that continuous cyclic hydrostatic pressure (5 MPa, 1 Hz for 4 h) induced apoptosis in human chondrocytes derived from osteoarthritic cartilage *in vitro*. In contrast, cyclic, physiological-like loading was found to trigger a partial recovery of morphological and ultrastructural aspects in osteoarthritic human articular chondrocytes.[8] Although overloading or continuous loading have severe effects on the cartilage, it is interesting to note that unloading or restricted movement appear to cause degenerative changes.

It was shown that cartilage formation was induced by intermittent compression and tension administered to chick embryonic tissue. However, immobilization of the cell cultures caused transformation of this cartilage into a bone-like structure. This finding implies that once developed, cartilage tissue requires mechanical stimulation to be maintained as cartilage and not undergo endochondral ossification.[14] Decreased stiffness of articular cartilage was shown after a period of joint immobilization based on the instantaneous shear modulus and equilibrium shear modules of canine cartilage. A decrease in sound speed in the transitional area of both the femoral and tibial cartilage, indicating the softening of cartilage as early as 1 week after immobilization in rat knee was observed.[3] In an experimental study of Tomiya *et al.*, it was demonstrated that skeletal unloading temporarily accelerated subchondral ossification and induced a full-thickness patellar cartilage defect without any fibrillation, and alkaline phosphatase activity was increased at the deep zone in hindlimb-suspended rats. In addition, a significant correlation between the uCTX-II levels and tidemark advancement was observed.[10]

Type II collagen is localized almost exclusively in articular cartilage, where it is a major structural component of the tissue. Hence,

Table 5 Correlations between log CTX-II and factors related with ambulation

CTX-II	*Factors related with ambulation*	r	P
Log CTX-II ($\mu g\,ml^{-1}$)	Duration of disease (months)	−0.131	>0.05
	Neurological lesion level	−0.33	>0.05
	AIS grade (A, B, C, D)	−0.251	**<0.05**
	AIS grade (A and others)	−0.270	**<0.05**
	ASIA upper extremity motor score	−0.60	>0.05
	ASIA lower extremity motor score	−0.262	**<0.05**
	ASIA total motor score	−0.175	>0.05
	Presence of spasticity	−0.172	>0.05
	Walking	−0.134	>0.05
	FAS score (0, 1, 2, 3, 4, 5)	−0.360	**<0.05**
	FAS score (score zero and others)	−0.325	**<0.05**
	Time for ambulation (0 min, 0–60 min, >60 min)	−0.243	**<0.05**
	Time for ambulation (0 min, >0 min)	−0.250	**<0.055**

Abbreviations: AIS, American Spinal Injury Association Impairment Scale; ASIA, American Spinal Injury Association; FAS, functional ambulation score; uCTX-II, urinary collagen type-II. Statistically significant values are shown in bold (Pearson's correlation analysis).

measurements of fragments derived from this protein may potentially be a specific marker for cartilage degradation.[9]

Our results support the findings of the other reports related with the effects of immobilization. Patients with a complete lesion of the spinal cord, or who were non-ambulators or non-functional ambulators were found to have a higher risk of cartilage degradation. It might be expected that as the level of injury gets higher, uCTX-II level is more elevated. In contrast, we did not find any correlation between uCTX-II and level of injury or any difference among groups of injury levels. As the FAS score is found to be correlated with the level of injury, this finding might be partially because of the distribution of small number of patients in injury groups. On the other hand, severity of injury is another factor determining the functions of extremities. We demonstrated that patients with complete lesion had higher uCTX-II levels. Partially preserved muscle function could allow better mobilization of joints and thus better ambulation. Spasticity was not found to be effective on level of CTX-II, indicating that muscular contractions might not provide sufficient loading for articular cartilage.

Vanwanseele *et al.*,[6] showed a progressive thinning in knee cartilage of SCI patients by MRI. Decrease in mean cartilage thickness in 12 months varied between 9–13% in the four compartments of tibiofemoral joint and the patella. Follow-up of SCI patients 12 and 24 months after injury demonstrated 14–16% and 19–25% of reduction in patellar and tibial cartilage thickness, respectively, compared with data in 6 months.[4] Additionally, in 43% of the patients, the individual thickness maps and morphological parameters revealed local areas of atrophy in patellar cartilage 6 and 12 months after injury.[7] In contrast to these, we did not find any correlation between duration of disease and level of uCTX-II. Since cases we included were heterogenous in terms of duration of disease, which might have affected uCTX-II level. This finding might further imply that ambulatory status could be more important than the duration of disease in degradation of articular cartilage.

Level of uCTX-II usually reflects systemic disorders, as opposed to a single joint disorder. uCTX-II implied the involvement of multiple joints in our patients who were expected not to be able to use or to disuse the joints below the level of the lesion. The presence of intervertebral disk disease or degenerative involvement of the non-paralytic joints may seem as a confounding factor.[15,16] In a study on cartilage of shoulder joints that take part in ambulation and transfers of complete paraplegic patients, no significant changes was observed in MRI findings.[7]

Although we did not provide any radiographical evidence for the degenerative findings, an association between uCTX-II and the severity, prevalence and the progression of radiographic OA at the knee and hip was observed.[1,16–18] Imaging modalities yield results only when gross damage to the cartilage has already occurred.[9] On the other hand, one must consider the difficulties of optimal positioning of the patients with paralysis, which requires loading in flexed position. Furthermore, uCTX-II was shown to have a relatively strong association with longitudinal loss of cartilage shown by MRI, which is more sensitive than radiography in detecting cartilage loss.[19,20]

One of the limitations of our study is the relatively small number of patients. In addition, as this study was designed as a cross-sectional study, it does not reflect alterations in cartilage over the time. Radiological demonstration of cartilage thinning would be considered for the further support of biochemical findings.

The findings of our study have some clinical implications. If patients are immobilized for substantial periods of time, stress distribution may change and degradation of the cartilage may occur that predispose the joint to osteoarthritic degeneration. Therefore, it may be important to initiate therapeutic programs as soon as possible after SCI. This is particularly important, as some animal studies indicated that changes in the cartilage during immobilization were not fully reversible after remobilization.[4] The extent to which the changes can be decelerated or prevented by therapeutic interventions is also not known. It needs to be determined further weather CTX-II could monitor the effectiveness of exercise programs.

CONCLUSION

As a conclusion, non-ambulatory or non-functional ambulatory SCI patients or patients with complete injury of spinal cord were found to be associated with increased level of a cartilage-specific biological marker, CTX-II. Duration of disease, AIS A grade, FAS zero score and daily ambulation time might cause significant differences in the level of CTX-II. It could be proposed that this increase might reflect degradation of cartilage and, thus, development of degenerative diseases due to the unloading or abnormal stress distribution. It may be important to initiate therapeutic programs as soon as possible after SCI to prevent cartilage degradation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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