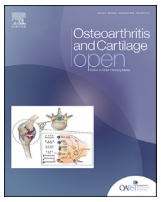
Osteoarthritis and Cartilage Open 4 (2022) 100323

Contents lists available at ScienceDirect

Osteoarthritis and Cartilage Open

journal homepage: www.elsevier.com/journals/osteoarthritis-and-cartilage-open/2665-9131



The search for systemic biomarkers for monitoring degenerative lumbar spinal disorders

Nader Tarabeih [a,b], Adel Shalata [c], Orabi Higla [d], Alexander Kalinkovich [a], Gregory Livshits [e,*]

[a] Department of Anatomy and Anthropology, Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel
[b] Maale HaCarmel Mental Health Center, Affiliated to Rappaport Faculty of Medicine Technion, Israel Institute of Technology, Haifa, Israel
[c] The Simon Winter Institute for Human Genetics, Bnai Zion Medical Center, The Ruth and Bruce Rappaport Faculty of Medicine, Technion, Haifa, Israel
[d] Department of Orthopedic Surgery, Sourasky Medical Center, Tel Aviv, Israel
[e] Deparment of Morphological Sciences, Adelson School of Medicine, Ariel University, Ariel, Israel

ARTICLE INFO

Keywords:
Low back pain
Facet joint osteoarthritis
Lumbar intervertebral disk
GDF-15
Vaspin
Body composition

ABSTRACT

Objectives: In our previous study, we reported that low back pain (LBP) severity and disability significantly correlate with body composition and several blood biochemical factors. Herein, we tested the hypothesis that these covariates are associated with anatomical deformations of the lumbar spine, in particular, radiographic facet joint osteoarthritis (FJOA) and lumbar disc degeneration (LDD) features important contributors to LBP.
Methods: CT and MRI images of the lumbar spine were obtained from 200 individuals suffering from LBP-sciatica. We examined the FJOA and total LDD score - the sum of the scores of the three radiographic features (intervertebral disc herniation, osteophythosis and spondylolisthesis) at the L1 - S1 vertebral levels. By implementing a bioelectrical impedance analysis, we assessed the participants for body composition, specifically, extracellular water (ECW). Plasma levels of growth and differentiation factor 15 (GDF-15) and visceral adipose tissue-derived serine protease inhibitor (vaspin), were detected by ELISA.
Results: By conducting a series of multivariable regression analyses, we report that the circulating levels of GDF-15, vaspin, and ECW are significantly and independently associated with FJOA scores [$\beta_{GDF15} = 0.38 \pm 0.08$, $p = 0.0001$; $\beta_{VASPIN} = 0.36 \pm 0.07$, $p = 0.000004$; $\beta_{ECW} = 0.24 \pm 0.07$, $p = 0.002$]. The levels of GDF-15 ($\beta = 0.30 \pm 0.10$, $p = 0.007$) and ECW ($\beta = 0.20 \pm 0.09$, $p = 0.03$) were also found significantly associated with the LDD scores.
Conclusion: The obtained new data suggest that GDF-15, vaspin and ECW may serve as biomarkers for FJOA and LDD phenotypes.

1. Introduction

Musculoskeletal pain disorders, specifically, low back pain (LBP) are the leading cause of years lived with disability, affecting 70%–85% of the population worldwide [1,2]. However, the pathogenesis of this multifactorial condition is still not fully understood. We recently reported that complex, age-associated and most probably, hierarchical relationships exist between LBP disability, inflammation-related soluble markers, body composition parameters, and the sideways curve of the spine – scoliosis [3,4]. However, it is unclear whether these factors affect LBP via spine-morphological changes, such as spinal osteoarthritis, specifically, facet joint osteoarthritis (FJOA) and lumbar disc degeneration (LDD), acknowledged as the major contributors to LBP [5].

FJOA is strongly associated with lumbar disc herniation [6]. The prevalence of FJOA-related spinal pain in the general population increases with age [7]. Similar to other osteoarthritis phenotypes, the radiographic characteristics of FJOA include joint space narrowing, subchondral bone erosions, osteophyte formation, etc. [8] Nevertheless, as of today, FJOA has been less studied compared to other osteoarthritis phenotypes, and other pathological changes in spine anatomy, in particular LDD [8]. LDD, defined as the wear and tear of the lumbar disc that acts as a cushion for the spine, can occur at any level, but most often at the L3-L4 and L5-S1 vertebrae [9]. Lumbar intervertebral disk (IVD) is composed of an annulus fibrosus (AF) and a nucleus pulposus (NP) [10], both comprised primarily of water, reaching 80–85% of the healthy NP, and up to ~65% in the outer AF [11]. Whilst LDD progresses, the IVD loses water content and height, leading to a loss of flexibility, elasticity, shock absorption and segmental instability, causing degenerative

*Corresponding author. Department of Morphological Sciences, Adelson School of Medicine, Ariel University, Ariel 4077625, Israel.
E-mail address: zviliv@ariel.ac.il (G. Livshits).

https://doi.org/10.1016/j.ocarto.2022.100323
Received 8 August 2022; Received in revised form 7 November 2022; Accepted 19 November 2022

spondylosis, affecting the facet joints and surrounding soft tissue, thus resulting in canal narrowing also known as degenerative stenosis [12].

Extracellular water (ECW) content serves as an important predictor of the health/disease status in a variety of diseases, e.g. by predicting mortality in hemodialysis patients [13]. ECW has been found to be associated with LBP severity [3,14]. Chronic, systemic inflammation manifested by the enhanced production of diverse pro-inflammatory cytokines, may be involved in the pathogenesis of spinal degenerative disorders [15]. Several cytokines have been found to correlate with LDD progression, accompanied by a reduction in NP cell numbers, a deterioration of the IVD microenvironment, herniation and radicular pain [16].

We have recently reported that plasma levels of GDF-15, and adipokine vaspin, are significantly and independently associated with LBP severity [3,14,17]. Interestingly, circulating levels of these blood factors were found to be associated with several body composition parameters [14,18], and both factors were found to be associated with the inflammation process [18,19]. However, the associations of GDF-15 and vaspin with degenerative lumbar vertebrae disorders have as yet not been assessed.

Therefore, the major aim of the present study was to evaluate the extent to which the FJOA and LDD radiological manifestations are associated with the plasma levels of GDF-15, vaspin, and body composition characteristics.

2. Materials and methods

2.1. Study design

This is a case-control, community-based, cross-sectional study, comprised of families with a relatively high prevalence of LBP [20].

2.2. Sample

The data were collected from 1078 individuals in the city of Sakhnin (in the Northern District of Israel) from 1/2014-1/2021, and focused on an ethnically homogeneous Arab population in Israel. The family-based design was required to both diminish the genetic heterogeneity and enrich the sample for familial LBP cases. In such a design, LBP-affected cases (N = 447) and non-affected controls (N = 522) were the members of the same 98 nuclear and more complex three-generation families. The inclusion criteria included: the families were selected via a proband (<45 years of age), previously diagnosed with LBP by a physician, confirmed by an orthopedist, and had at least one first-degree relative diagnosed with a similar LBP condition. The exclusion criteria were as follows: (1) a spinal fracture or surgery within the past 2 years; (2) congenital anomalies; (2) traumatic or tumorous disorders; (3) severe heart problems; (4) and/or <18 years of age. All individuals in the study sample, regardless of their LBP status (both cases and LBP negative controls), were assessed by certified and experienced nurses, and demographic data, anthropometric, body composition measurements, and blood samples were collected from each individual. Blood samples were used to assay plasma concentrations of biochemical factors relevant to this study. Further details have been reported in our recent studies on this subject [3,21].

This research was approved by the IRB-Helsinki Committee (Number: 042/2013K, Date: November 04, 2013) of the Meir Medical Center, Kfar Saba, Israel, and the Ethics Committee of Tel Aviv University, Tel Aviv, Israel. Written informed consent was obtained from all participants prior to their inclusion.

2.3. Low back pain evaluation

LBP was assessed by an orthopedic physician. Two self-reported questionnaires were employed: the Medical Research Council Nurses' Study questionnaire (MRCQ) [22] and the Rolland-Morris Disability questionnaire (RMDQ) [23]. A detailed description of the corresponding methods and obtained results have been recently described elsewhere [14].

2.4. Imaging parameters

Of the 447 participants afflicted with LBP-sciatica, 200 individuals, aged 18 to 75, had undergone MRI or CT imaging of the lumbar spine in local hospitals between January 01, 2016 and December 31, 2020. An experienced orthopedic surgeon masked to any clinical and prior imaging data, reviewed all images. The scored FJOA and LDD-related phenotypes were assessed over the five lumbar discs (L1 to L5) and the sacral base S1. For MRI/CT reading, transverse plane images as well as sagittal and coronal reconstruction were used, where needed. To evaluate the validity of the FJOA and LDD features' assessment, an intra-observer reliability test was undertaken. The 20 MRI/CT images were examined twice prior to the present study and assessed over a two-week interval between the two measurements. Validity was evaluated by the intraclass correlation coefficient (ICC) [24]. The ICC estimates were all >0.9, i.e., 0.975 (95% CI, 0.93–0.99), between the measurements of FJOA and summary LDD phenotype LSUM (described below).

2.5. Assessing FJOA and LDD related features

LDD-related phenotypes of each IVD (between L1 and S1) were assessed as to the extent of IVD herniation, osteophytosis, and spondylolisthesis/lysis, as follows: FJOA: four grades of FJOA were defined using criteria similar to those published by Pathria et al. [25]: 0-normal facets, 1-narrowing of the facet joint, 2-narrowing plus sclerosis or hypertrophy, 3-severe osteoarthritis with narrowing, sclerosis, and osteophytes.

Disc herniation (DSH) was assessed according to the Michigan State University (MSU) classification [26], dividing four grades according to the size of disc herniation: 0- no disc herniation; 1- the disc herniation extends up to or less than 50% of the distance from the non-herniated posterior aspects of the disc to the intra-facet line; 2-the disc herniation extends up to or more than 50% of the distance from the non-herniated posterior aspects of the disc to the intra-facet line; 3-the herniation extends completely beyond the intra-facet line.

Spondylolisthesis/lysis (SPL): the lumbar spine was reviewed for each case using bone window. Spondylolysis and spondylolisthesis were defined as present or absent (dichotomous indices) for each subject.

Osteophytosis (OSP): Radiographic features related to lumbar OSP at each study vertebral level were classified into four groups: grade 0, grade 1-minor appearance of the single osteophytes, grade 2-mild change and grade 3-severe change.

The combined grades of each of the radiographic features (FJOA, DSH, SPL and OSP) of the entire lumbar area (from L1/L2 to L5/S1), were subsequently computed. Herein, we examined the summary scores obtained for FJOA and the sum of the combined scores for the remaining three LDD phenotypes. This phenotype was defined as LSUM.

2.6. Anthropometric and body composition assessment

Demographic, anthropometric and body composition data have been recently described in detail elsewhere [17]. Briefly, we used an anthropometrically measured body mass index (BMI) in kg/m^2 and waist-to-hip ratio (WHR) in mm/mm. Body composition measures have been assessed by the bioimpedance (BIA) method using the BIA101 device (Akern Bioresearch, Italy) [27]. BIA is a safe, reliable, simple, accurate, and inexpensive method employed to assess a variety of body composition parameters [28]. In our study, this device evaluated the following body composition parameters: fat mass (FM), skeletal muscle mass (SMM) in kilograms, ECW and total body water (TBW). ECW was chosen due to its fundamental physiological significance [29], and examined as the ECW-to-TBW ratio (ECW/TBW). As body mass components are strongly inter-correlated and depend on body weight, they were examined as

ratios to body weight, i.e., FM/W and SMM/W.

2.7. Soluble biomarker analysis

Venous blood samples obtained by venipuncture following overnight fasting underwent centrifugation at 1800 g for 15 min at 4 °C within 1 h after collection. Plasma samples were separated and stored in aliquots at −80 °C until usage. Circulating levels of GDF-15, and vaspin were detected by ELISA using DuoSet kits (R&D Systems, Minneapolis, MN, USA), according to the manufacturer's protocols. All the measurements in the sample were above the minimal detection level: 7.8 pg/ml for GDF-15 and 49.6 pg/ml for vaspin. The intra- and inter-assay coefficients of variation were between 2.3% and 6.3%, indicating good reliability of the assessment. Due to a significant deviation of the respective distributions of the biomarker's circulating levels from the normality assumptions, the original measurements of these factors were subjected to a log-normal transformation and standardized prior to analysis.

2.8. Statistical analysis

Statistical analysis of the data was conducted using Statistica 64 (TIBCO Software, Version 13.5). All the measurements in the sample were compared between sexes by the *t*-test. Descriptive statistics were reported as mean and standard error based on the data distribution for continuous variables. Data analysis included identification of the major potential predictors from each group of variables, body composition and soluble markers using the linear regression and correlation analysis with simultaneous adjustment for age and sex, with FJOA and LDD-phenotypes as dependent variables. Subsequently, the best potential predictors were simultaneously examined by multiple logistic regression analyses.

3. Results

The basic descriptive statistics, according to sex of all the study variables, are presented in Table 1. FJOA, osteophytosis and spondylolisthesis scores tended to be higher in females, even though osteophytosis values did not reach statistical significance. Several body compositions variables, such as BMI, FM/WT and ECW were significantly higher in women in comparison with men, whereas, SMM/WT was higher in the men. Both biochemical factors (GDF-15 and vaspin) displayed no significant difference between males and females. Since all the variables were significantly age-dependent (Table S1, supplementary material), age effect was taken into account in all the following analyses.

FJOA and each of the LDD phenotypes correlated significantly with most of the studied covariates, independently of age and sex (Table 2), but also correlated significantly with one another (Table S2). To avoid redundancy, we opted to focus on FJOA and LSUM, which showed most significant and consistent correlations with the studied covariates (Table 2). Since body composition variables were also significantly intercorrelated (Table S1), we implemented the same approach by selecting the most independent parameters, namely, the WHR, SMM/W and ECW in order to test their association with the FJOA and LSUM.

Preliminary comparison of FJOA and LSUM in individuals with low vs high severity scores of the disease based on the RMDQ scores (1st quarter of the distribution vs 4th quarter) of our data showed that they are highly significantly different ($p < 0.00001$), by both parametric and nonparametric tests. Thus, FJOA scores were: 0.07 ± 0.05 (95%CI: 0.03–0.17) vs 4.00 ± 0.65, (95%CI: 2.58–5.41), and for LSUM: 1.92 ± 0.23 (95%CI: 1.44, 2.40) vs 6.46 ± 0.91 (95%CI: 4.47, 8.44).

To simultaneously test the extent of the associations between the selected covariates on the one hand, and FJOA and LSUM on the other hand, a multiple logistic regression analysis was applied in two stages. First, we tested which of the selected body composition variables would significantly and independently associate with FJOA and LSUM (Table 3). Next, all the covariates were simultaneously examined in similar design analyses. The results were virtually the same, and indicated that the ECW consistently, significantly and independently associated with both FJOA and LSUM, regardless of other covariates (Table 4). Of interest, in both analyses, the associations of the FJOA and LSUM with WHR and SMM/W were completely removed by the ECW. GDF-15 levels were highly significantly associated with FJOA and LSUM, regardless of the adjustment. Vaspin levels were highly significantly ($p = 0.000004$) and independently associated with FJOA. However, it was found not associated independently and significantly with LSUM.

Interestingly, the FJOA did not show independent association with age, while being significantly associated with GDF-15, vaspin and ECW, as shown in Table 4, for the entire sample. The example of the FJOA manifestations (CT scans) are given in Fig. 1. The figure also provides their scores and corresponding plasma levels of the GDF-15, vaspin, and ECW. The figure compares two LBP- affected individuals with clearly observed FJOA manifestation with non-LBP individual having healthy facet joints.

Table 1
Descriptive statistics of the study variables. **A:** Summary scores of FJOA- and LDD-related phenotypes. Each score summed over scores of the five lumbar discs L1/L2 - L5/S1. **B:** Body composition and circulating factor measurements.

	Males (N = 110)		Females (N = 90)		
Measurement	Mean ± SE	95% CI	Mean ± SE	95% CI	P*
A. FJOA and LDD phenotypes					
FJOA	0.50 ± 0.13	(0.23, 0.76)	1.04 ± 0.23	(0.57, 1.51)	0.03#
Disc herniation	2.95 ± 0.26	(2.41, 3.48)	2.46 ± 0.24	(1.97, 2.95)	0.18#
Osteophytosis	0.83 ± 0.17	(0.47, 1.19)	1.45 ± 0.28	(0.88, 2.02)	0.07#
Spondylolisthesis	0.01 ± 0.01	(-0.01, 0.05)	0.17 ± 0.04	(0.07, 0.26)	0.003#
LSUM	3.80 ± 0.37	(3.04, 4.55)	4.09 ± 0.44	(3.21, 4.97)	0.61#
B. Potential Covariates					
Age (y)	45.97 ± 1.55	(45.85, 49.08)	50.08 ± 1.50	(47.08, 53.09)	0.05
BMI (kg/m^2)	26.89 ± 0.50	(25.88, 27.90)	30.79 ± 0.67	(29.43, 32.14)	0.00001
WHR	0.92 ± 0.008	(0.91, 0.94)	0.91 ± 0.01	(0.89, 0.93)	0.34
FM/WT	0.25 ± 0.006	(0.24, 0.26)	0.38 ± 0.009	(0.37, 0.40)	0.001
SMM/WT	0.37 ± 0.005	(0.36, 0.38)	0.27 ± 0.004	(0.26, 0.28)	0.001
ECW/TBW	0.46 ± 0.005	(0.45, 0.47)	0.50 ± 0.006	(0.48, 0.51)	0.00001
GDF-15 (pg/ml)	1.82 ± 0.01	(1.80, 1.84)	1.83 ± 0.01	(1.80, 1.85)	0.70
Vaspin (pg/ml)	5.87 ± 0.12	(5.63, 6.12)	6.22 ± 0.16	(5.89, 6.56)	0.09

Data are presented as mean±standard errors with 95% confidence intervals; FJOA, facet joint osteoarthritis, LDD, lumbar disc degeneration; LSUM, summary score for three LDD phenotypes (disc herniation, osteophytosis and spondylolisthesis); BMI, body mass index; WHR, waist-to-hip ratio; FM/WT, fat mass/weight ratio; SMM/WT, skeletal muscle mass/weight ratio; ECW/TBW, extracellular water-to-total body water ratio; GDF-15, growth and differentiation factor 15. *Independent sample *t*-test for mean value comparison between males and females. # These variables were also compared by the Mann-Whitney test: for the FJOA and spondylolisthesis the P-values were: $p = 0.03$, $p = 0.003$ respectively; the P-values were >0.05 for all the rest.

4. Discussion

Atkinson et al. [30] defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a

Table 2
Spearman correlations between the scores of FJOA and LDD phenotypes and their potential covariates by sex.

Covariate	Gender	FJOA	Disc herniation	Osteophytosis	Spondylolisthesis	LSUM
Age (y)	Males	0.39, $p < 0.001$	0.36, $p = 0.002$	0.53, $p < 0.001$	−0.25, $p = 0.04$	0.50, $p < 0.001$
	Females	0.44, $p < 0.001$	0.19, $p = 0.158$	0.44, $p = 0.007$	0.13, $p = 0.338$	0.40, $p = 0.008$
BMI (kg/m^2)	Males	0.21, $p = 0.247$	0.17, $p = 0.09$	0.13, $p = 0.337$	−0.18, $p = 0.168$	0.17, $p = 0.08$
	Females	0.38, $p = 0.001$	0.11, $p = 0.680$	0.40, $p < 0.001$	0.11, $p = 0.387$	0.35, $p = 0.009$
WHR	Males	0.09, $p = 0.454$	0.07, $p = 0.678$	0.09, $p = 0.781$	0.47, $p < 0.001$	0.13, $p = 0.490$
	Females	0.27, $p = 0.04$	0.22, $p = 0.07$	0.21, $p = 0.114$	0.12, $p = 0.312$	0.29, $p = 0.01$
FM/WT	Males	0.31, $p = 0.01$	0.14, $p = 0.181$	0.17, $p = 0.257$	−0.24, $p = 0.06$	0.16, $p = 0.156$
	Females	0.34, $p = 0.004$	0.12, $p = 0.569$	0.29, $p = 0.008$	0.08, $p = 0.502$	0.31, $p < 0.01$
SMM/WT	Males	−0.35, $p = 0.003$	−0.26, $p = 0.03$	−0.28, $p = 0.04$	0.29, $p = 0.02$	−0.31, $p = 0.01$
	Females	−0.30, $p = 0.001$	−0.26, $p = 0.03$	−0.24, $p = 0.05$	−0.07, $p = 0.580$	−0.37, $p < 0.01$
ECW/TBW	Males	0.44, $p = 0.002$	0.19, $p = 0.015$	0.33, $p = 0.01$	−0.20, $p = 0.131$	0.30, $p = 0.03$
	Females	0.36, $p = 0.004$	0.28, $p = 0.03$	0.28, $p = 0.03$	0.18, $p = 0.163$	0.37, $p = 0.004$
GDF-15 (pg/ml)	Males	0.35, $p = 0.003$	0.33, $p = 0.02$	0.11, $p = 0.364$	0.01, $p = 0.99$	0.28, $p = 0.001$
	Females	0.46, $p < 0.001$	0.41, $p = 0.005$	0.42, $p < 0.001$	0.35, $p = 0.01$	0.54, $p < 0.001$
Vaspin (pg/ml)	Males	0.15, $p = 0.08$	−0.17, $p = 0.246$	−0.09, $p = 0.90$	−0.05, $p = 0.673$	−0.16, $p = 0.360$
	Females	0.40, $p = 0.001$	0.02, $p = 0.661$	0.11, $p = 0.929$	0.32, $p = 0.01$	0.08, $p = 0.527$

Spearman correlations and corresponding p-values for all the tests are shown after adjustment for age; FJOA, facet joint osteoarthritis; LDD, lumbar disk degeneration; LSUM, summary score for three LDD phenotypes (disc herniation, osteophytosis and spondylolisthesis); BMI, body mass index; WHR, waist-to-hip ratio; FM/WT, fat mass/weight ratio; SMM/WT, skeletal muscle mass/weight ratio; ECW/TBW, extracellular water-to-total body water ratio.

Table 3
Multiple regression analysis: associations of the FJOA and LSUM scores with the body composition measurements.

Dependent Variable: FJOA					Dependent Variable: LSUM			
Independent	Beta	SE of Beta	t	P	Beta	SE of Beta	t	P
Age	0.29	0.10	2.82	0.005	0.45	0.10	4.13	0.00007
WHR	0.04	0.09	0.48	0.629	−0.08	0.09	−0.84	0.40
SMM/WT	−0.09	0.09	−0.99	0.322	0.07	0.09	0.81	0.41
ECW/TBW	0.24	0.09	2.64	0.009	0.20	0.09	2.04	0.03

Beta represents standardized coefficients; SE, standard error; FJOA, facet joint osteoarthritis; LSUM, summary score for three LDD phenotypes (disc herniation, osteophytosis and spondylolisthesis); WHR, waist-to-hip ratio; SMM/WT, skeletal muscle mass/weight ratio; ECW/TBW, extracellular water-to-total body water ratio.

Table 4
Multiple regression analysis: associations of FJOA and LSUM scores with the plasma levels of the soluble biomarkers and body composition measurements.

Dependent Variable: FJOA					Dependent Variable: LSUM			
Independent	Beta	SE of Beta	t	P	Beta	SE of Beta	t	P
Age	0.13	0.09	1.46	0.146	0.19	0.11	1.70	0.09
ECW/TBW	0.24	0.07	3.14	0.002	0.20	0.09	2.04	0.03
GDF-15 (pg/ml)	0.38	0.08	4.26	0.0001	0.30	0.10	2.90	0.004
Vaspin (pg/ml)	0.36	0.07	4.90	0.000004	−0.01	0.08	−0.13	0.89

Beta represents standardized coefficients; SE, standard error; FJOA, facet joint osteoarthritis; LSUM, summary score for three LDD phenotypes (disc herniation, osteophytosis and spondylolisthesis). ECW/TBW, extracellular water-to-total body water ratio.

therapeutic intervention". However, taking into account the poor understanding of the mechanisms underlying the pathogenesis of FJOA and LDD, considered as one of the main factors in the development of LBP [31], meeting such a requirement is not a simple task. Nevertheless, in our study, we report that elevated GDF-15 plasma levels and ECW are highly significantly and independently associated with FJOA and LSUM scores assessed by MRI or CT imaging. The vaspin plasma levels displayed a highly significant correlation with the FJOA scores. These observations suggest that GDF-15, vaspin and ECW levels may serve as systematic biomarkers of FJOA and/or LDD.

These findings raise the question as to the role of GDF-15, vaspin and ECW in the development of LDD and FJOA. GDF-15 belongs to the transforming growth factor beta (TGFβ) superfamily of cytokines. In inflammatory conditions, multiple cell types have been shown to release GDF-15, including epithelial cells, vascular smooth muscle, macrophages and adipocytes [32,33]. Secretory GDF-15 is released into the bloodstream or slowly released into the extracellular medium [34]. Elevated circulating levels of GDF-15 have been observed in patients suffering from LBP [3,17], OA [35], rheumatoid arthritis (RA) [36] and various types of skeletal muscle pathology [37]. A close correlation of elevated plasma levels of GDF-15 with FJOA and LDD (current study) as well as with LBP severity and disability [3,17] suggests its involvement in the pathogenic FJOA/LDD/LBP link. However, the source of enhanced GDF-15 production in spinal degenerative disorders as well as its functional role, as mentioned, remains unknown. A recent study reported that GDF-15 is secreted by the skeletal muscle during exercise, promoting lipolysis, thus suggesting that elevated circulating levels of GDF-15 may provide a beneficial rather than detrimental effect [37]. Accordingly, elevated plasma levels of GDF-15 found herein and in our previous studies may indicate its beneficial (probably protective) rather than detrimental role in the FJOA/LDD/LBP link [3,17]. However, the issue of "beneficial vs harmful" effects of GDF-15, at least in skeletal muscular disorders, is still challenging [38]. The role of GDF-15 in FJOA/LDD-associated LBP should be verified further in controlled, longitudinal studies.

Our study also revealed that elevated vaspin plasma levels significantly and independently correlate with FJOA. We recently found a statistically significant association of vaspin plasma levels with several manifestations of LBP severity [3,14]. Vaspin is expressed in a variety of tissues including adipose, skin, stomach and skeletal muscle, but is released into circulation mainly by visceral adipose tissue [39,40]. As to joint pathology, vaspin serum levels did not significantly differ in

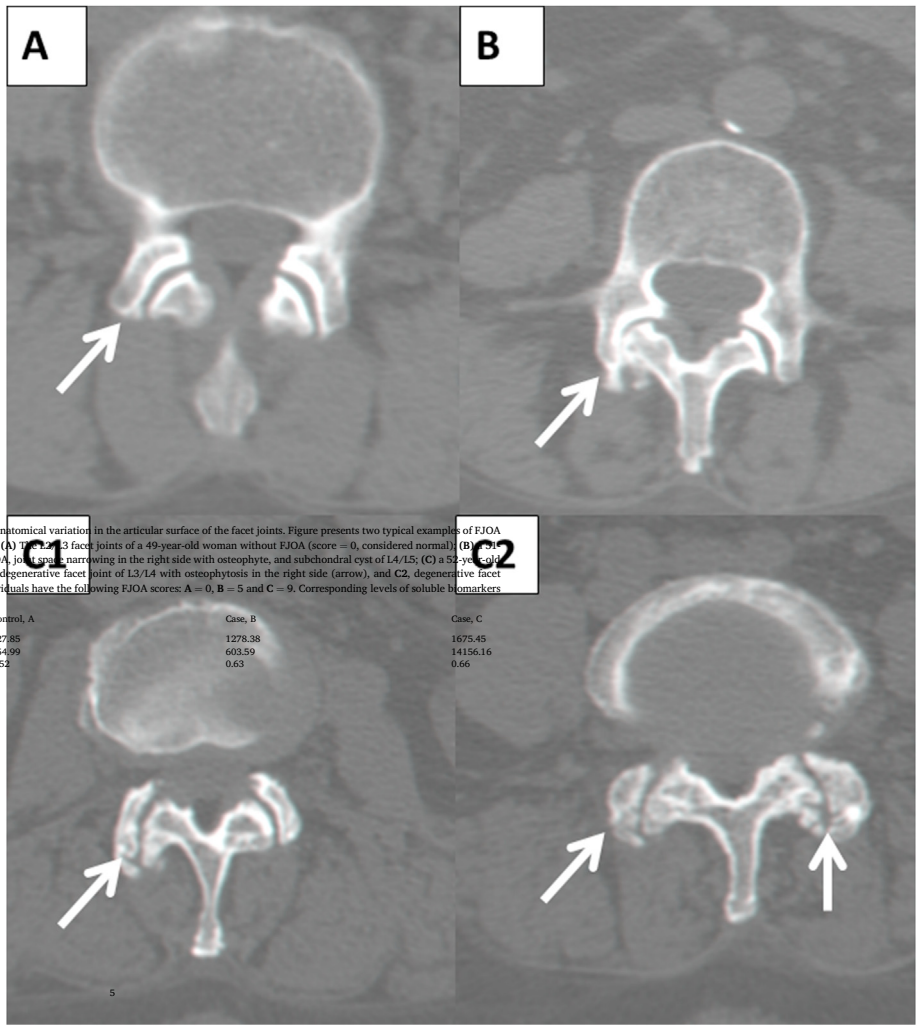


Fig. 1. Axial computed tomography images depicting the anatomical variation in the articular surface of the facet joints. Figure presents two typical examples of FJOA appearance in two LBP individuals vs a healthy individual. **(A)** The L3/L4 facet joints of a 49-year-old woman without FJOA (score = 0, considered normal); **(B)** a 51-year-old woman with LBP accompanied by sciatica and FJOA, joint space narrowing in the right side with osteophyte, and subchondral cyst of L4/L5; **(C)** a 52-year-old woman with LBP accompanied by sciatica and FJOA: **C1**, degenerative facet joint of L3/L4 with osteophytosis in the right side (arrow), and **C2**, degenerative facet joint of L4-5 with the osteophytosis in both sides. The individuals have the following FJOA scores: **A** = 0, **B** = 5 and **C** = 9. Corresponding levels of soluble biomarkers and ECW are presented in the table.

Covariate	Control, A	Case, B	Case, C
GDF-15 (pg/ml)	427.85	1278.38	1675.45
Vaspin (pg/ml)	254.99	603.59	14156.16
ECW/TBW	0.52	0.63	0.66

patients afflicted with juvenile idiopathic arthritis compared to healthy controls. No association was found between disease activity and vaspin levels [41]. In patients with ankylosing spondylitis, however, low vaspin levels were found associated with endothelial dysfunction [42]. In RA patients, elevated serum levels of vaspin were found associated with inflammation and the development of clinical manifestations [43].

In our previous study, we found that vaspin plasma levels highly significantly associate with several LBP phenotypes, with the odds ratios ranging between 1.24 (95%CI = 1.03–1.50) and 1.33 (95%CI = 1.07–1.64) [14]. Herein, we found a reliable association between the vaspin levels and radiographic FJOA. However, it did not correlate with any of the LDD phenotypes. Whether this observation reflects a different involvement of vaspin in the development of diverse spinal pathologies remains to be studied. It has been demonstrated that vaspin protects human osteoblasts from apoptosis [44], suppresses osteoclastogenesis in the pre-osteoblast cell line [45], and inhibits the IL-1β and the leptin-induced production of catabolic and pro-inflammatory mediators in chondrocytes [46]. These observations clearly emphasize the involvement of vaspin in the pathogenesis of various arthritidies [47]. However, again the question whether the circulating vaspin levels play a protective vs a detrimental role in these processes remains unclear. Several studies have suggested that vaspin plays a protective role in the course of the harmful, mainly inflammatory conditions [48]. If this is confirmed, we may assume that elevated levels of vaspin found in our current and previous studies [3,14] reflect vaspin's protective role in the FJOA/LDD/LBP axis. Thus, although the results are encouraging, they still require further confirmation and replication.

In the present study, we also observed a significant and independent association of ECW levels with FJOA and all the LDD phenotypes. Previously, we observed a clear association of ECW with the severity of LBP

manifestations [3,14]. These associations could probably be explained in changes that occur in the IVD and vertebrae due to water loss. The water leakage from the NP of the IVD is associated with the enhanced production of several pro-inflammatory cytokines and the activation of proteases, which in turn leads to extracellular matrix damage and pain sensitization which characterize LDD and LBP [49]. The available data demonstrate that the water content changes after dynamic loading by physical lumbar exercise are involved in the maintenance of the interstitial matrix in the NP and cartilage in the facet joint [50]. These data probably provide physiological explanations of our findings on the consistent associations of ECW levels with LDD and LBP-related phenotypes, thus implying that it might serve as an important biomarker for these disorders. This belief, however, has to be verified in future longitudinal studies.

There are several limitations to our study, primarily, the case control, cross-sectional design, thus, we could not address the temporal relationship between the onset of biomarker abnormalities and the onset of spine degeneration. Furthermore, this also restricts drawing the appropriate conclusions regarding the causality of the associations found. IVD specimens used to measure the LDD and LBP biomarkers would be preferable, but implausible in general population studies. However, determination of serum/plasma concentrations of several cytokines and other soluble molecules has been successfully used for monitoring the initiation, intensity, and progression of human LBP [3,14], and LDD [51]. These obviously could also be done with respect to GDF15 and vaspin. It should be, however, underlined that despite these encouraging and promising observations, it is too early to make a clinically- oriented conclusions regarding the usefulness of these blood markers as biomarkers of LBP-related conditions. Obviously, it is necessary to replicate the present results in the independent samples, and, if confirmed, to clarify whether the observed associations biomarker → anatomical change (e.g. FJOA) →LBP are causally related. It is of great importance to clarify whether these factors are markers or factors of the process, and therefore whether or not they could be used as a therapeutic target.

5. Conclusions

This is the first study providing evidence that FJOA and LDD are significantly and independently associated with elevated circulating levels of GDF-15 and ECW, a body composition parameter. FJOA also correlates significantly with circulating vaspin levels. We suggest that these factors could serve as systemic biomarkers for spinal degenerative disorders, providing valuable information as to the degenerative state of IVD, and the structural/morphological findings observed on imaging. Moreover, these findings further contribute to our understanding of LDD, and may eventually lead to new diagnostic and therapeutic options. Future longitudinal studies will be required to assess the validity of these factors for identifying discogenic-related LBP, monitoring disease progression and/or treatment effects, as well as for replication in other populations.

Ethical approval information

This research was approved by the IRB-Helsinki Committee.

Author contributions

G.L., N.T. planned and designed the project; G.L. supervised the entire study and the data analysis; N.T. collected the data and conducted analyses; A.S. organized and supervised the data collection; N.T. and A.K. conducted laboratory analyses; O.H. assessed CT/MRI scans. N.T., A.K., and G.L collaborated in the data analysis and prepared the first draft of the manuscript. All authors approved the final manuscript. G.L. is the guarantor of the study. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

All authors declare that they have no conflict of interest.

Role of the funding source

This research was funded by the Israel Science Foundation; grant number 2054/19.

Acknowledgements

Research submitted in partial fulfillment of the requirements for a PhD degree in Anatomy and Anthropology of Nader Tarabeih, Sackler Faculty of Medicine, Tel Aviv University.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ocarto.2022.100323.

References

[1] J. Hartvigsen, M.J. Hancock, A. Kongsted, et al., What low back pain is and why we need to pay attention, Lancet 391 (10137) (2018) 2356–2367, https://doi.org/10.1016/S0140-6736(18)30480-X.
[2] E.L. Hurwitz, K. Randhawa, P. Torres, et al., The Global Spine Care Initiative: a systematic review of individual and community-based burden of spinal disorders in rural populations in low- and middle-income communities, Eur. Spine J. 27 (Suppl 6) (2018) 802–815, https://doi.org/10.1007/s00586-017-5393-z.
[3] N. Tarabeih, A. Kalinkovich, A. Shalata, S.S. Cherny, G. Livshits, Deciphering the causal relationships between low back pain complications, metabolic factors, and comorbidities, J. Pain Res. 15 (2022) 215–227, https://doi.org/10.2147/JPR.S349251.
[4] Tarabeih N, Masharawi Y, Shalata A, Higla O, Kalinkovich A, Livshits G. Scoliosis and skeletal muscle mass are strongly associated with low back pain-related disability in humans: an evolutionary anthropology point of view. Am J Hum Biol. Published online May 9, 2022. doi:10.1002/AJHB.23757.
[5] D. Borenstein, Does osteoarthritis of the lumbar spine cause chronic low back pain? Curr. Rheumatol. Rep. 6 (1) (2004) 14–19, https://doi.org/10.1007/S11926-004-0079-Z.
[6] K. Zhu, Q. Su, T. Chen, et al., Association between lumbar disc herniation and facet joint osteoarthritis, BMC Muscoskel. Disord. 21 (1) (2020), https://doi.org/10.1186/S12891-020-3070-6.
[7] P. Tiwari, H. Kaur, H. Kaur, V. Jha, N. Singh, A. Ashraf, Prevalence of facet joint arthritis and its association with spinal pain in mountain population - a cross-sectional study, J. Craniovertebral Junction Spine 11 (1) (2020) 36–45, https://doi.org/10.4103/JCVJS.JCVJS_121_19.
[8] A.C. Gellhorn, J.N. Katz, P. Suri, Osteoarthritis of the spine: the facet joints, Nat. Rev. Rheumatol. 9 (4) (2013) 216–224, https://doi.org/10.1038/NRRHEUM.2012.199.
[9] T.J. Errico, Lumbar disc arthroplasty, Clin. Orthop. Relat. Res. 435 (435) (2005) 106–117, https://doi.org/10.1097/01.BLO.0000165718.22159.D9.
[10] Q. Wei, X. Zhang, C. Zhou, Q. Ren, Y. Zhang, Roles of large aggregating proteoglycans in human intervertebral disc degeneration, Connect. Tissue Res. 60 (3) (2019) 209–218, https://doi.org/10.1080/03008207.2018.1499731.
[11] J.C. Iatridis, J.J. MacLean, M. O'Brien, I.A.F. Stokes, Measurements of proteoglycan and water content distribution in human lumbar intervertebral discs, Spine (Phila Pa 32 (14) (2007) 1493–1497, https://doi.org/10.1097/BRS.0B013E318067DD3F, 1976.
[12] M.T. Modic, J.S. Ross, Lumbar degenerative disk disease, Radiology 245 (1) (2007) 43–61, https://doi.org/10.1148/RADIOL.2451051706.
[13] R. Pérez-Morales, J. Donate-Correa, E. Martín-Núñez, et al., Extracellular water/total body water ratio as predictor of mortality in hemodialysis patients, Ren. Fail. 43 (1) (2021) 821–829, https://doi.org/10.1080/0886022X.2021.1922442.
[14] N. Tarabeih, A. Kalinkovich, A. Shalata, G. Livshits, Circulating levels of visceral adipose tissue-derived serine protease inhibitor (vaspin) appear as a marker of musculoskeletal pain disability, Diagnostics 10 (10) (2020), https://doi.org/10.3390/diagnostics10100797.
[15] C. Cunha, A.J. Silva, P. Pereira, R. Vaz, R.M. Gonçalves, M.A. Barbosa, The inflammatory response in the regression of lumbar disc herniation, Arthritis Res. Ther. 20 (1) (2018), https://doi.org/10.1186/S13075-018-1743-4.
[16] F.J. Lyu, H. Cui, H. Pan, et al., Painful intervertebral disc degeneration and inflammation: from laboratory evidence to clinical interventions, Bone Res. 9 (1) (2021), https://doi.org/10.1038/S41413-020-00125-X.
[17] N. Tarabeih, A. Shalata, S. Trofimov, A. Kalinkovich, G. Livshits, Growth and differentiation factor 15 is a biomarker for low back pain-associated disability, Cytokine 117 (2019) 8–14, https://doi.org/10.1016/j.cyto.2019.01.011.
[18] S.H. Lee, J.Y. Lee, K.H. Lim, Y.S. Lee, J.M. Koh, Associations between plasma growth and differentiation factor-15 with aging phenotypes in muscle, adipose

tissue, and bone, Calcif. Tissue Int. 110 (2) (2022) 236–243, https://doi.org/10.1007/S00223-021-00912-6.
[19] I.M.P. Dwipayana, I.M.S. Semadi, W. Gotera, M.R. Saraswati, K. Suastika, Vaspin in Developing obesity (Vande-Ob); the correlation of waist circumference and visceral fat percentage with vaspin levels in patients with type II diabetes mellitus, Open Access. Maced. J. Med. Sci. 7 (1) (2019) 50–52, https://doi.org/10.3889/OAMJMS.2019.011.
[20] G. Livshits, Z. Cohen, O. Higla, K. Yakovenko, Familial history, age and smoking are important risk factors for disc degeneration disease in Arabic pedigrees, Eur. J. Epidemiol. 17 (7) (2001) 643–651, https://doi.org/10.1023/A:1015503329989.
[21] N. Tarabeih, A. Shalata, S. Trofimov, A. Kalinkovich, G. Livshits, Growth and differentiation factor 15 is a biomarker for low back pain-associated disability, Cytokine 117 (2019), https://doi.org/10.1016/j.cyto.2019.01.011.
[22] J. Smedley, H. Inskip, C. Cooper, D. Coggon, Natural history of low back pain. A longitudinal study in nurses, Spine 23 (22) (1998) 2422–2426. http://www.ncbi.nlm.nih.gov/pubmed/9836356. (Accessed 8 January 2019). Accessed.
[23] A. Chiarotto, L.J. Maxwell, C.B. Terwee, G.A. Wells, P. Tugwell, R.W. Ostelo, Roland-Morris Disability Questionnaire and Oswestry Disability Index: which has better measurement properties for measuring physical functioning in nonspecific low back pain? systematic review and meta-analysis, Phys. Ther. 96 (10) (2016) 1620–1637, https://doi.org/10.2522/ptj.20150420.
[24] P.E. Shrout, J.L. Fleiss, Intraclass correlations: uses in assessing rater reliability, Psychol. Bull. 86 (2) (1979) 420–428, https://doi.org/10.1037//0033-2909.86.2.420.
[25] M. Pathria, D.J. Sartoris, D. Resnick, Osteoarthritis of the facet joints: accuracy of oblique radiographic assessment, Radiology 164 (1) (1987) 227–230, https://doi.org/10.1148/RADIOLOGY.164.1.3588910.
[26] L.W. Mysliwiec, J. Cholewicki, M.D. Winkelpleck, G.P. Eis, MSU classification for herniated lumbar discs on MRI: toward developing objective criteria for surgical selection, Eur. Spine J. 19 (7) (2010) 1087–1093, https://doi.org/10.1007/S00586-009-1274-4.
[27] N. Achamrah, G. Colange, J. Delay, et al., Comparison of body composition assessment by DXA and BIA according to the body mass index: a retrospective study on 3655 measures, PLoS One 13 (7) (2018), https://doi.org/10.1371/journal.pone.0200465.
[28] Y. Iizuka, H. Iizuka, T. Mieda, et al., Association between neck and shoulder pain, back pain, low back pain and body composition parameters among the Japanese general population, BMC Muscoskel. Disord. 16 (1) (2015) 333, https://doi.org/10.1186/s12891-015-0759-z.
[29] M.L. McManus, K.B. Churchwell, K. Strange, Regulation of cell volume in health and disease, in: F.H. Epstein (Ed.), N. Engl. J. Med. 333 (19) (1995) 1260–1266, doi: 10.1056/NEJM 199511093331906.
[30] A.J. Atkinson, W.A. Colburn, V.G. DeGruttola, et al., Biomarkers and surrogate endpoints: preferred definitions and conceptual framework, Clin. Pharmacol. Ther. 69 (3) (2001) 89–95, https://doi.org/10.1067/MCP.2001.113989.
[31] W. Brinjikji, F.E. Diehn, J.G. Jarvik, et al., MRI Findings of disc degeneration are more prevalent in adults with low back pain than in asymptomatic controls: a systematic review and meta-analysis, AJNR Am. J. Neuroradiol. 36 (12) (2015) 2394–2399, https://doi.org/10.3174/AJNR.A4498.
[32] V.W.W. Tsai, S. Lin, D.A. Brown, A. Salis, S.N. Breit, Anorexia-cachexia and obesity treatment may be two sides of the same coin: role of the TGF-b superfamily cytokine MIC-1/GDF15, Int. J. Obes. 40 (2) (2016) 193–197, https://doi.org/10.1038/IJO.2015.242.
[33] S. O'Rahilly, GDF15-From biomarker to allostatic hormone, Cell Metabol. 26 (6) (2017) 807–808, https://doi.org/10.1016/J.CMET.2017.10.017.
[34] J.J. Li, J. Liu, K. Lupino, X. Liu, L. Zhang, L. Pei, Growth differentiation Factor 15 maturation requires proteolytic cleavage by PCSK3, -5, and -6, Mol. Cell Biol. 38 (21) (2018), https://doi.org/10.1128/MCB.00249-18.
[35] N. Arnold, M. Rehm, G. Büchele, et al., Growth differentiation Factor-15 as a potent predictor of long-term mortality among subjects with osteoarthritis, J. Clin. Med. 9 (10) (2020) 1–14, https://doi.org/10.3390/JCM9103107.
[36] Y.W. He, C.S. He, Association of growth and differentiation Factor 15 in rheumatoid arthritis, J. Inflamm. Res. 15 (2022) 1173–1181, https://doi.org/10.2147/JIR.S350281.
[37] C. Laurens, A. Parmar, E. Murphy, et al., Growth and differentiation factor 15 is secreted by skeletal muscle during exercise and promotes lipolysis in humans, JCI Insight 5 (6) (2020), https://doi.org/10.1172/JCI.INSIGHT.131870.
[38] K. Johann, M. Kleinert, S. Klaus, The role of GDF15 as a myomitokine, Cells 10 (11) (2021), https://doi.org/10.3390/CELLS10112990.
[39] T. Nicholson, C. Church, D.J. Baker, S.W. Jones, The role of adipokines in skeletal muscle inflammation and insulin sensitivity, J. Inflamm. 15 (1) (2018), https://doi.org/10.1186/S12950-018-0185-8.
[40] J. Weiner, K. Zieger, J. Pippel, J.T. Heiker, Molecular mechanisms of vaspin action – from adipose tissue to skin and bone, from blood vessels to the brain, Adv. Exp. Med. Biol. 1111 (2019) 159–188, https://doi.org/10.1007/5584_2018_241. Springer.
[41] L. Cantarini, G. Simonini, A. Fioravanti, et al., Circulating levels of the adipokines vaspin and omentin in patients with juvenile idiopathic arthritis, and relation to disease activity, Clin. Exp. Rheumatol. 29 (6) (2011) 1044–1048. https://pubmed.ncbi.nlm.nih.gov/22032341/. (Accessed 22 April 2022). Accessed.
[42] H.H. Wang, Q.F. Wang, Low vaspin levels are related to endothelial dysfunction in patients with ankylosing spondylitis, Brazilian J. Med. Biol. Res. = Rev. Bras Pesqui Medicas e Biol. 49 (7) (2016), https://doi.org/10.1590/1414-431X20165231.
[43] A.S. Wahba, M.E. Ibrahim, D.M. Abo-Elmatty, E.T. Mehanna, Association of the adipokines chemerin, apelin, vaspin and omentin and their functional genetic variants with rheumatoid arthritis, J. Personalized Med. 11 (10) (2021), https://doi.org/10.3390/JPM11100976.
[44] X. Zhu, Y. Jiang, P.F. Shan, et al., Vaspin attenuates the apoptosis of human osteoblasts through ERK signaling pathway, Amino Acids 44 (3) (2013) 961–968, https://doi.org/10.1007/S00726-012-1425-5.
[45] Y. Liu, F. Xu, H.-X. Pei, et al., Vaspin regulates the osteogenic differentiation of MC3T3-E1 through the PI3K-Akt/miR-34c loop, Sci. Rep. 6 (1) (2016), 25578 doi: 10.1038/srep. 25578.
[46] J. Bao, L. Xu, J. Ran, Y. Xiong, L. Wu, Vaspin prevents leptin-induced inflammation and catabolism by inhibiting the activation of nuclear factor-κB in rat chondrocytes, Mol. Med. Rep. 16 (3) (2017), https://doi.org/10.3892/MMR.2017.6911.
[47] M. Carrión, K.W. Frommer, S. Pérez-García, U. Müller-Ladner, R.P. Gomariz, E. Neumann, The adipokine network in rheumatic joint diseases, Int. J. Mol. Sci. 20 (17) (2019), https://doi.org/10.3390/ijms20174091.
[48] X. Li, X. Ke, Z. Li, B. Li, Vaspin prevents myocardial injury in rats model of diabetic cardiomyopathy by enhancing autophagy and inhibiting inflammation, Biochem. Biophys. Res. Commun. 514 (1) (2019) 1–8, https://doi.org/10.1016/j.bbrc.2019.04.110.
[49] P.-P.A. Vergroesen, I. Kingma, K.S. Emanuel, et al., Mechanics and biology in intervertebral disc degeneration: a vicious circle, Osteoarthritis Cartilage 23 (7) (2015) 1057–1070, https://doi.org/10.1016/j.joca.2015.03.028.
[50] D. Yamabe, H. Murakami, K. Chokan, et al., Evaluation of water content in lumbar intervertebral discs and facet joints before and after physiological loading using T2 mapping MRI, Spine (Phila Pa 42 (24) (1976) E1423–E1428, https://doi.org/10.1097/BRS.0000000000002204, 2017.
[51] D.D. Qi, Z.H. Liu, D.S. Wu, Y.F. Huang, A Study on COMP and CTX-II as Molecular markers for the diagnosis of intervertebral disc degeneration, BioMed Res. Int. (2021) 2021, https://doi.org/10.1155/2021/3371091.