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Original Article

Elevated IL-6 levels correlate with the development of intervertebral disc degeneration: a case-control study and meta-analysis

Hai-Tao Fan, Wei-Hao Jiang, Bin Zhang, Lei Wang, Shu-Bin Liu

Department of Orthopedics, 307 Hospital of PLA, Beijing 100071, P. R. China

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Abstract: Objectives: We aimed to investigate the correlation between serum IL-6 levels and the development of intervertebral disc degeneration (IDD) by conducting a case-control study and meta-analysis. Methods: Between January 2013 and December 2014, 87 IDD patients hospitalized in 307 Hospital of PLA, were regard as the case group and divided into bulging, protrusion and sequestration IDD types. The other 23 traumatic lumbar fracture patients who have received operations were identified as the control group. Visual analog scale (VAS) and Oswestry disability index (ODI) were used for pain and low back, lower limb dysfunction pre-operatively. The IL-6 levels in nucleus pulposus of intervertebral disc was measured by enzyme-linked immunoassay (ELISA). All relevant case-control studies were retrieved under stringent exclusion and inclusion criteria. R 3.1.0 software was applied for data analysis. Result: Our study showed thatthe IL-6 levels in the case group were evidently higher compared to the control group (all $P < 0.05$), and the highest IL-6 levels were observed in sequestration IDD type. The highest and lowest VAS and ODI score were respectively found on sequestration and bulging IDD patients. Results of meta-analysis also revealed significant correlation between IDD and serum IL-6 levels (SMD = 3.47, 95% CI = 1.73-5.22, $P < 0.001$). Serum IL-6 levels were also found to be correlated with the three IDD types (bulging: SMD = 2.41, 95% CI = 1.71-3.11, $P < 0.001$; protrusion: SMD = 3.27, 95% CI = 2.57-3.96, $P < 0.001$; sequestration: SMD = 7.33, 95% CI = 5.93-8.74, $P < 0.001$). Conclusion: Our findings suggest that elevated IL-6 levels may correlate with the progression of IDD.

Keywords: IL-6, Intervertebral disc degeneration, lumbar intervertebral disc, visual analog scale, oswestry disability index, meta-analysis

Introduction

Intervertebral disc degeneration (IDD), also known as degenerative disc disease (DDD), is a common disease which can result in radiculopathy, axial skeletal pain and myelopathy [1, 2]. IDD incidence is affected mainly by age, with less than 10% prevalence in adolescents and young adults, and about 30~50% in middle adulthood [3]. The production and secretion of extracellular matrix components may be reduced under the circumstances of nucleus pulposus degeneration in discs, which leads to changes in disc structure and the dysfunction of discs [4-6]. In terms of clinical experience, IDD is mainly featured by loss of intervertebral disc (IVD) height and structural failure because of proteolytic degradation of extracellular matrix (ECM), transformed mechanical loading and infiltration of blood vessels along with nerve fibres [7, 8]. IDD, as a multifactorial disease is influenced by both genetic and environmental factors, including mechanical stress to the spine, age, smoking, heavy lifting, and biochemical influences also lead to the risk of IDD [9-11]. And the genetic factors including tumor necrosis factor-α (TNFα), interleukin 1-β, interleukin 6 (IL-6) and so on [12, 13].

IL-6, also called 26-kd protein, hybridomaplasmacytoma growth factor, β2-interferon, or B cell stimulatory factor 2, is located on 7p15-p21 chromosome [14, 15]. It is produced by several types of lymphoid and nonlymphoid cells, such as T and B lymphocytes, fibroblasts, monocytes, endothelial cells and some other tumor

cells [16]. As atypical pro-inflammatory cytokine, IL-6 plays a crucial role in host immune defense responses, inflammatory processes, cellular growth regulation, proliferation of massive types of immune cell [17]. IL-6 is capable of crossing the blood-brain barrier, regulating energy mobilization, increasing the neutrophils production in the bone marrow [18]. Several studies have revealed that elevated IL-6 levels are closely related with type 2 diabetes, cardiovascular diseases, Crohn's disease, rheumatoid arthritis, multiple sclerosis, lymphatic, renal, bladder and colorectal cancer [19, 20]. Interestingly, cumulative evidence has suggested a significant correlation between IL-6 and the pathogenesis of the IDD [21-23]. However, the correlation between IL-6 and IDD remains controversial, and a study showed no correlation between IL-6 and IDD [24]. To further investigate the correlation between serum IL-6 levels and the development of IDD, we conducted a case-control study of IDD to evaluate the expressions of IL-6 levels.

Materials and methods

Study subjects

Between January 2013 and December 2014, 87 IDD patients with typical sciatica symptoms hospitalized in the Department of Orthopedics, 307 Hospital of PLA were regarding as the case group. Combined with the results of the X-ray and clinical manifestations, we divided all enrolled patients into three types according to the degree of disc protrusion: bulging IDD (marginality and limitation bulging; n = 25), protrusion IDD (lateral and central protrusions; n = 31) and sequestration IDD types (subligamentous and transligamentous, sequestration IDD types, Schmorl nodes and transosseous protrusion IDD types; n = 31). Patients were enrolled in case group if they met all the criteria: (1) be satisfied with the diagnosis criteria of prolapse of lumbar intervertebral disc [25] according to the magnetic resonance imaging (MRI) and/or computed tomography (CT) test results; (2) voluntarily joined this study with informed consents; (3) do not take immunopotentiator/immunosuppressant, Antagonistic drug, anti-inflammatory and analgesic drugs within two weeks. The exclusion criteria were as follows: (1) pregnant or lactating women; (2) with serious cardiovascular, liver, kidney disease and mental abnormality; (3) with blood, lymph, endocrine and nervous system diseases; (4) with autoimmune disorders, rheumatic diseases and anaphylactic diseases. All IDD patients were treated by posterior fenestration of vertebral lamina. Twenty-three traumatic lumbar fracture patients who have received operationswere identified as the control group by meeting the following criteria: (1) no typical sciatica symptoms before injury, spinal operation medical history and relevant immune system diseases; (2) no relevant to immune system diseases and acute and chronic infection were confirmed before operation; (3) lumbar disc protrusion was not found in frontal and lateral position of lumbar vertebra through X ray and CT test, and no pathology symptom like prolapse of intervertebral disc in lumbar fracture during operation. All samples were collected by the same clinical team and the study design was reviewed and approved by the ethics committee of 307 Hospital of PLA. All procedures in this study were in compliance with the Declaration of Helsinki [26].

Data collection

All the patients were operated by a veteran doctor. The herniated nucleus pulposus of intervertebral disc was taken different measures according to the degree of hernia of nucleus pulposus: (1) bulging IDD types: contact of vertebral canal and nerve root; (2) protrusion IDD types: contact of annulus fibrous, posterior longitudinal ligament and nerve root; (3) sequestration IDD tissues isolated into vertebral canal and degenerated tissues visible to the naked eye. IDD tissue, removed from the fracture of lumbar vertebra patients during anterior or posterior approach operation which conducted in control group patients under general anesthesia, was collected for use in our study. The specimens were removed after thawing and weighed and then cut into pieces with scissors. Then the pieces were placed into the mortar which was pre-cooled by liquid nitrogen, and pulverized with continual pour of liquid nitrogen. Liquid nitrogen may be added several times because of the volatility until the tissue become powdered during operation. The powdered tissue were placed into the centrifuge tube, added with IP cell lysate (every 20 mg tissue joining 100 μl cell lysate) (main components of IP cell lysate: 20 mmol/L Tris (pH 7.5), 150 mmol/L NaCl, 1% TritonX-100, sodium pyrophosphate, β-glycerophosphate, EDTA,

Na_3VO_4, leupeptin and so on). Further, these powders were lysed at ice or 4°C refrigerator about 30 to 60 min and shook up by agitator every few minutes to make fully lysis. After fully lysis, the powder was removed into a tube and centrifuged at 10000-14000 g/min for 3-5 min, and the supernatant of nucleus pulposus tissue was taken and packed for testing. Ice rewarming was carried out during test, and the quantitative determination of IL-6 in the supernatant of nucleus pulposus tissue was measured using an ELISA kit (Arthur D. Little, Inc., America).

Visual analog scale (VAS) and oswestry disability index (ODI)

Intervertebral disc pain of all the patients was assessed with VAS and ODI before operation. The concrete operations of VAS are as follow: take a 10 cm ruler, the grades 0 to 10 is used to reflect the degree of pain (zero refer to no pain, ten refer to intolerable pain). Patients were asked to rate the pain felt on that ruler, the distance between 0 to the point which patients signed was the degree of pain. ODI consists of nine questions, including the degree of pain, the ability of living independence, the ability of carrying things, walk, sit, stand, disrupt sleep, civil life and travel. Every question has six options, and the highest score of every question is five. If you choose the first option, you will get 0, and so forth, you choose the last one you will get 5 points. The higher score of ODI indicated the more serious symptoms of dysfunction. ODI = the total number of points scored in the scale/45×100%.

Statistical analysis

The statistical analysis was conducted by SPSS 22.0 and R 3.1.0 (Robert Gentleman and Ross Ihaka, Auckland University, New Zealand) software. Continuous data were presented with mean ± standard deviation (SD). Differences between pairwise group means were tested by Student's t test, while variance analysis was applied for mean comparisons among multigroups. The pairwise difference was compared with SNK test. Categorical data were measured by χ^2 test. Pearson's correlation was applied to analyze the relations between IL-6 and VAS and ODI. And $P < 0.05$ was considered as statistically significant. The following databases were electronically and manually searched to identify relevant articles: PubMed, Wanfang database, CNKI (China National Knowledge Infrastructure) and the VIP Database (last updated search in October, 2014). The combination of key words and free words was used in our study, including interleukin-6, IL-6, intervertebral disc degeneration, disk degeneration, lumbar disc protrusion, cervical disc protrusion, lumbar intervertebral disk protrusion. Studies were considered for inclusion if they met the following criteria: (1) the case-control study about the relation between expressions of IL-6 and IDD; (2) all enrolled subjects are IDD patients and the healthy controls; (3) selected studies supplied complete data, including number of case, age, ethnicity, sex, pathological types, the expressions of IL-6 and so on; (4) the latest or complete study was collected when the extracted studies were published by the same authors. Studies were excluded if they were (1) not related to our topic; (2) not case-control study; (3) supplied incomplete data information; (4) not Chinese/English article; (5) published repeatedly. Two investigators used unified data collection form to extract information separately. The following relevant data were extracted from eligible studies prospectively in the final analyses: surname of first author, year of publication, country, language, ethnicity, number of case, age, and pathological types. Differences were resolved by further discussion and reexaminations among all authors during the process of data collection. Standardized mean difference (SMDs) and its 95% confidence interval (95% CI) were calculated to assess the relation between expressions of IL-6 and IDD. The statistical significance of pooled SMDs was estimated by Z-test. Cochran's Q-statistic ($P < 0.05$ was considered significant) and I^2 tests (0%, no heterogeneity; 100%, maximal heterogeneity) was used to quantify heterogeneity among studies. When there was heterogeneity ($P_h < 0.05$ or $I^2 > 50\%$) among studies, a random effect modelwas used; otherwise, a fixed effect model was used.

Results

Baseline characteristics

Of the 87 IDD patients, 50 were men and 37 were women with mean age of 46.41 ± 18.55 years including 16 aged < 30 years old, 22 aged > 60 years and the remaining 49 aged 30~60 years. The disc level of IDD was $L_{3/4}$ in

Table 1. Comparisons of gender, age and different nodes in the control group and bulging, protrusion and sequestration intervertebral disc degeneration types

Parameter	Control group (n = 23)	Case group (n = 87)			χ^2/F	*P*
		Bulging (n = 25)	Protrusion (n = 31)	Sequestration (n = 31)		
Gender (M/F)	12/11	16/9	20/11	18/13	1.065	0.785
Age	45.7 ± 15.6	46.00 ± 21.14	49.61 ± 19.08	43.55 ± 15.68	0.601	0.616
$L_{3/4}$	2	2	5	3		
$L_{4/5}$	12	13	17	17		
L_5/S_1	9	10	9	11	1.747	0.941

M: male; F: female.

Table 2. Comparison on IL-6 levels between bulging, protrusion and sequestration intervertebral disc degeneration types in case group and traumatic lumbar fracture patients in control group

Groups	Number of cases	IL-6 (ng/L)
Control group	23	5.5 ± 2.2
Case group	-	-
Bulging	25	13.4 ± 5.8*
Protrusion	31	20.5 ± 7.9*,#
Sequestered	31	28.9 ± 9.9*,#,&
F	-	49.35
P	-	< 0.001

Note: *: compared to the control group, P < 0.05; #: compared to bulging intervertebral disc degeneration type, P < 0.05; &: compared to sequestration intervertebral disc degeneration type, P < 0.05.

10 cases, $L_{4/5}$ in 47 cases and L_5/S_1 in 30 cases. According to the degree of disc protrusion, we had 25 cases of bulging IDD (mean age: 46.00 ± 21.14 years), 31 protrusion IDD (mean age: 49.61 ± 19.08 years) and 31 sequestration IDD (mean age: 43.55 ± 15.68 years). In 25 bulging IDD patients, there are 8 aged < 30 years, 7 aged > 60 years and the remaining 10 aged 30-60 years. Patients had herniated disc at $L_{3/4}$ (2 cases), $L_{4/5}$ (13 cases) and L_5/S_1 (10 cases). Among 31 protrusion IDD patients, who had single-level lumbar disc disease (five at $L_{3/4}$, seventeen at $L_{4/5}$, and nine at L_5/S_1), 6 aged < 30 years, 10 aged > 60 years and 15 age 30~60 years. Among the 31 sequestration IDD patients (3 aged < 30 years, 4 aged > 60 years and 24 aged 30~60 years), the disc level of IDD was $L_{3/4}$ in 3 cases, $L_{4/5}$ in 17 cases and L_5/S_1 in 11 cases. In the 23 patients in control group, there are 12 man and 11 women with a mean age of 46.22 ± 15.61 years. The disc level of IDD was $L_{3/4}$ in 2 cases, $L_{4/5}$ in 12 cases and L_5/S_1 in 9 cases. As for the ages of patients, 5 aged < 30 years, 3 aged > 60 years and the remaining 15 aged 30~60 years. Proportionality can be seen in the gender and age of patients in the two groups (**Table 1**).

Serum IL-6 levels in intervertebral disc tissues

Comparison between case and control groups showed evidently statistical significance (t = 8.559, P < 0.001) that serum IL-6 levels in intervertebral disc tissues is (21.46 ± 10.22) and (5.5 ± 2.2) ng/L in the case and control groups respectively. The IL-6 level in intervertebral disc tissue in bulging, protrusion and sequestration IDD types were significantly higher compared to the control group (F = 29.920, P < 0.001); and those in bulging and protrusion IDD types were lower than those in sequestration IDD types, all having statistically significant differences (all P < 0.05) (**Table 2**). Patients in case group with age ranged < 30 years, 30~60 years and > 60 years presented higher IL-6 levels than those in control groups (all P < 0.05), and the IL-6 level in case group elevated with the increase of age. Moreover, the highest and lowest IL-6 levels were respectively found in patients aged > 60 years and < 30 years (all P < 0.05) (**Table 3**).

Correlations of serum IL-6 levels with clinicpathological features of IDD patients

Our results showed that IL-6 levels in bulging, protrusion, sequestration types in case group elevated with the increase of age (all P < 0.05). There was no such statistical significance in IL-6 levels among bulging, protrusion, and sequestration patients aged < 30 years (P > 0.05). IL-6 levels in protrusion and sequestration IDD patients aged > 60 and 30~60 years

Table 3. Comparison of age and IL-6 levels in intervertebral disc tissues between case and control groups

Groups	Ages			*F*	*P*
	< 30	30-60	> 60		
Control group	4.98 ± 0.89	5.55 ± 2.43	6.17 ± 3.86	0.237	0.792
Case group	7.90 ± 3.83	22.93 ± 8.09*	28.05 ± 8.80*,#	33.710	< 0.001
t	2.816	13.220	4.190		
P	0.011	< 0.001	< 0.001		

Note: *: compared to patients aged < 30 years in case group, P < 0.05; #: compared to patients aged 30~60 years in case group, P < 0.05.

Table 4. Comparison of age and IL-6 levels among the three pathological types in case group

Types	IL-6			*F*	P
	< 30 years	30-60 years	> 60 years		
Bulging	6.40 ± 2.87	13.16 ± 1.95*	19.84 ± 2.64*,#	55.600	< 0.001
Protrusion	9.02 ± 3.95	19.62 ± 3.35*,&	28.72 ± 4.20*,#,&	52.530	< 0.001
Sequestered	9.13 ± 5.69	29.06 ± 6.35*,&,@	42.78 ± 1.42*,#,&,@	27.260	< 0.001
F	1.045	41.540	58.410		
P	0.378	< 0.001	< 0.001		

Note: *: compared to patients aged < 30 years old in the same type, P < 0.05; #: compared to patients aged 30~60 in the same type, P < 0.05; &: compared to the same age grade in bulging type, P < 0.05; @: compared to the same age grade in protrusion type, P < 0.05.

Table 5. Comparisons of visual analog scale and Oswestry disability index before operation among different pathological types in case group

Groups	Number of cases	VAS test before operation	ODI test before operation
Bulging	25	5.8 ± 0.66	45.58% ± 9.8%
Protrusion	31	7.9 ± 0.54*	68.21% ± 10.6%*
Sequestered	31	8.70 ± 0.71*,#	85.17% ± 9.43%*,#
F		148.4	108.6
P		< 0.001	< 0.001

VAS: visual analog scale; ODI: Oswestry disability index; *: compared to patients aged < 30 years in the same type, P < 0.05; #: compared to patients aged 30~60 in the same type, P < 0.05.

Table 6. Comparisons of visual analog scale and Oswestry disability index before operation among different ages in case group

Groups	Number of cases	VAS test before operation	ODI test before operation
< 30	16	6.23 ± 1.17	48.82% ± 14.37%
30-60	49	7.85 ± 1.18*	70.49% ± 16.79%*
> 60	22	7.98 ± 1.23*	75.56% ± 17.11%*
F		12.78	13.73
P		< 0.001	< 0.001

VAS: visual analog scale; ODI: Oswestry disability index; *: compared to patients aged < 30 years in the same type, P < 0.05.

were higher compared to those in bulging types, and sequestration patients aged > 60 and 30~60 years had the highest IL-6 among those three types, all indicating statistical significance (all P < 0.05) (**Table 4**).

VAS and ODI test in bulging, protrusion, sequestration IDD patients before operation demonstrated statistically significant differences (VAS: $F = 148.4$, $P < 0.001$; Oswestry: $F = 108.6$, $P < 0.001$), and the highest and lowest VAS and ODI score were respectively found on sequestration and bulging IDD patients (all P < 0.05) (**Table 5**). Statistical difference were found in VAS and ODI in different age grades (VAS: $F = 12.78$, $P < 0.001$; Oswestry: $F = 13.73$, $P < 0.001$), and VAS and ODI score in patients aged > 60 and 30~60 years were evidently higher than patients aged < 30 years, indicating statistical significance (all P < 0.05) (**Table 6**). VAS and ODI test of bulging, protrusion, sequestration patients in case group before operation increased with the increase of age, which revealed the existing of statistical difference (all P < 0.001) (**Table 7**). VAS and ODI test before operation in bulging, protrusion, seques-

Table 7. Correlation between pathological types, age and visual analog scale before operation in case groups

Types	VAS test before operation			*F*	P
	< 30	30-60	> 60		
Bulging	5.01 ± 0.32	5.78 ± 0.22*	6.54 ± 0.30$^{*,\#}$	56.930	< 0.001
Protrusion	7.11 ± 0.33$^{\&}$	7.86 ± 0.26$^{*,\&}$	8.45 ± 0.22$^{*,\#,\&}$	32.240	< 0.001
Sequestered	7.33 ± 0.56$^{\&}$	8.71 ± 0.45$^{*,\&,@}$	9.67 ± 0.29$^{*,\#,\&,@}$	23.760	< 0.001
F	48.320	230.800	205.700		
P	< 0.001	< 0.001	< 0.001		

Note: VAS: visual analog scale; *: compared to patients aged < 30 years old in the same type, P < 0.05; $^{\#}$: compared to patients aged 30~60 in the same type, P < 0.05; $^{\&}$: compared to the same age grade in bulging type, P < 0.05; $^{@}$: compared to the same age grade in sequestration type, P < 0.05.

Table 8. Correlation between pathological types, age and Oswestry disability index before operation in case groups

Types	Oswestry test before operation			F	P
	< 30	30-60	> 60		
Bulging	34.54% ± 3.46%	44.48% ± 4.26%*	56.64% ± 5.68%$^{*,\#}$	45.530	< 0.001
Protrusion	55.18% ± 3.50%$^{\&}$	65.08% ± 4.39%$^{*,\&}$	80.74% ± 5.81%$^{*,\#,\&}$	60.330	< 0.001
Sequestered	69.41% ± 3.28%$^{\&,@}$	84.70% ± 6.72%$^{*,\&,@}$	99.83% ± 0.20%$^{*,\#,\&,@}$	21.260	< 0.001
F	131.700	188.300	92.580		
P	< 0.001	< 0.001	< 0.001		

Note: *: compared to patients aged < 30 years old in the same type, P < 0.05; $^{\#}$: compared to patients aged 30~60 in the same type, P < 0.05; $^{\&}$: compared to the same age grade in bulging type, P < 0.05; $^{@}$: compared to the same age grade in sequestration type, P < 0.05.

tration patients who were at the same age grade also reflected statistically significant differences (all P < 0.05) (**Table 8**). Pearson's correlation revealed that there were positive correlations between age, VAS and ODI with IL-6 levels (age: r = 0.689, VAS: r = 0.874, Oswestry: r = 0.912, all P < 0.001).

Meta-analysis findings

Our present study initially retrieved 112 studies through electronic database searching and manual search, followed by excluding the duplicates (n = 14), letters, reviews or meta-analysis (n = 2), non-human studies (n = 11), and the studies not related to research topics (n = 44). The left 41 studies were further reviewed, and 8 papers (English: n = 3, Chinese: n = 5) were enrolled in the final analysis [27-34], including 263 IDD patients and 129 healthy controls (as shown in **Figure 1**). In this study, comparisons of the expressions of IL-6 between bulging, protrusion, sequestration IDD types and the healthy controls were respectively performed. These 8 enrolled studies were published between 2002 and 2014, and the research objects were from Asian populations (5 studies), Caucasian populations (2 studies) and mixed population (1 study). All the research objects were tissue samples, and the detection method was ELISA. Random-effects model should be used because of the existing of heterogeneity of expressions of IL-6 levels in IDD patients (P_h < 0.0001, I^2 = 95.7%). Result of meta-analysis also reflected significant relationship between IDD and the expressions of IL-6 levels (SMD = 3.47, 95% CI = 1.73-5.22, P < 0.001) (**Figure 2**). And fixed-effects model was applied because of no existing of heterogeneity in the expressions of IL-6 level in bulging, protrusion, sequestration IDD types (bulging: P_h = 0.7764, I^2 = 0.0%; protrusion: P_h = 0.3148, I^2 = 1.0%; sequestered: P_h = 0.2612, I^2 = 20.8%). A significant relationship was confirmed between the expressions of IL-6 levels and different types patients (bulging: SMD = 2.41, 95% CI = 1.71-3.11, P < 0.001; protrusion: SMD = 3.27, 95% CI = 2.57-3.96, P < 0.001; sequestered: SMD = 7.33, 95% CI = 5.93-8.74, P < 0.001), and with the degree of protrusion of the intervertebral disc increasing, the expressions of IL-6 levels increased gradually (**Figures 3**-**5**).

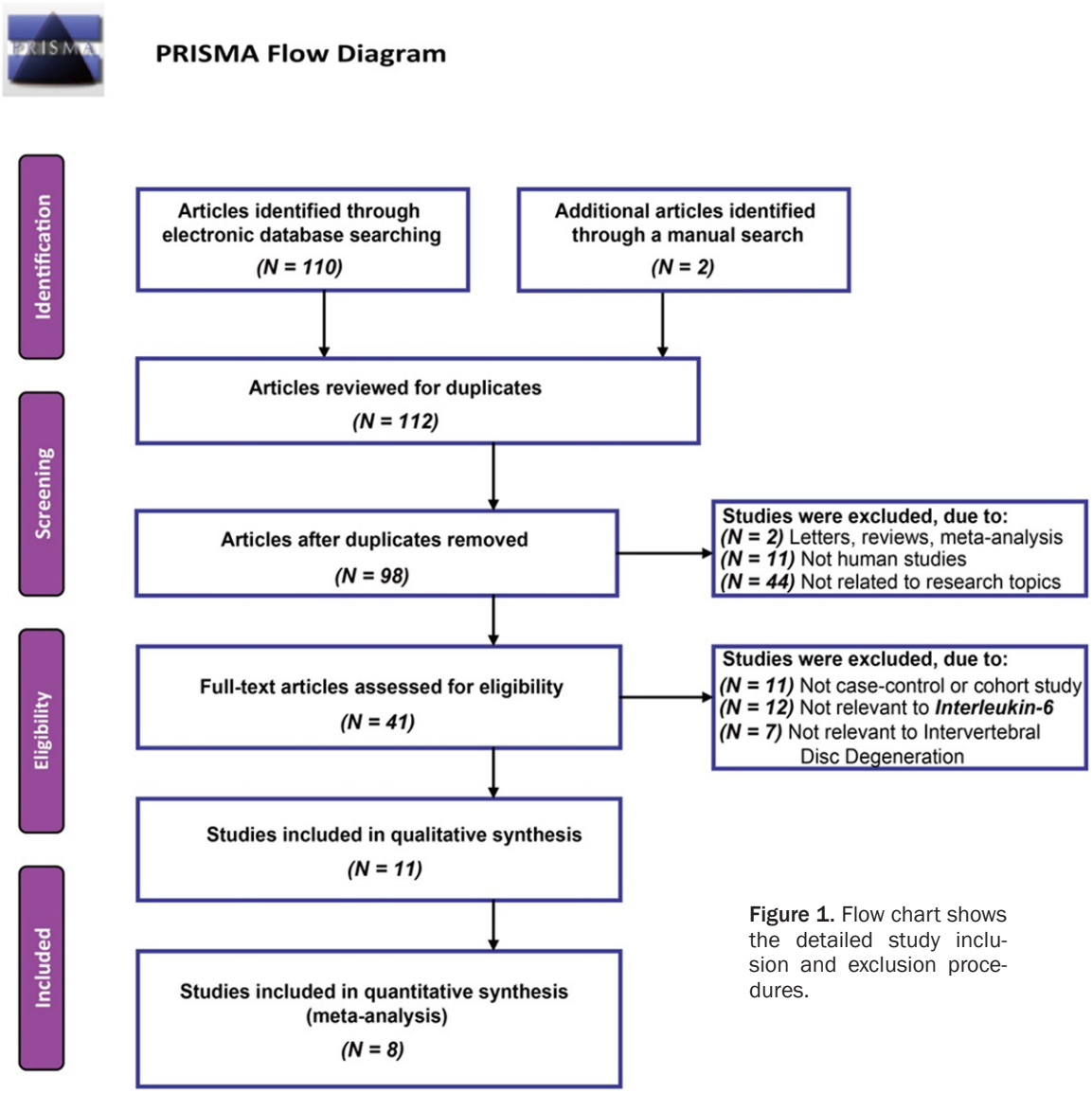

Figure 1. Flow chart shows the detailed study inclusion and exclusion procedures.

The symmetrical funnel plots of IL-6 protein expression suggesting that there was no publication bias in the enrolled studies, and the Egger linear regression analysis confirmed the lack of publication bias (all P > 0.05) (**Figure 6A**), while, specific *P* value was not available because only two includedstudies focused on the IL-6 protein expression of different types (sequestration, protrusion and bulging) of IDD patients (**Figure 6B-D**).

Discussion

The present study was undertaken to verify whether IL-6 was located in the herniated disc tissue and to clarify the possible inflammatory property of the IDD. In this study, we investigated the expression level of IL-6 in IDD in a case-control study and a meta-analysis. Our results revealed that patients with IDD had elevated IL-6 levels in intervertebral disc tissue compared with controls, implying that IL-6 was highly expressed in IDD. In agreement with our major results, our meta-analysis also revealed that patients with IDD presented with increased IL-6 levels compared with healthy controls. Aida *et al.* pointed out that IL-6 and soluble IL-6 receptor may stimulate the production of MMPs and their inhibitor, tissue inhibitors of metalloproteinases (TIMPs) via JAK-STAT and ERK-MAPK signaling in chondrocytes [35]. IDD refers to a complex process with a poorly understood mechanism and recently study document that infiltration with pro-inflammato-

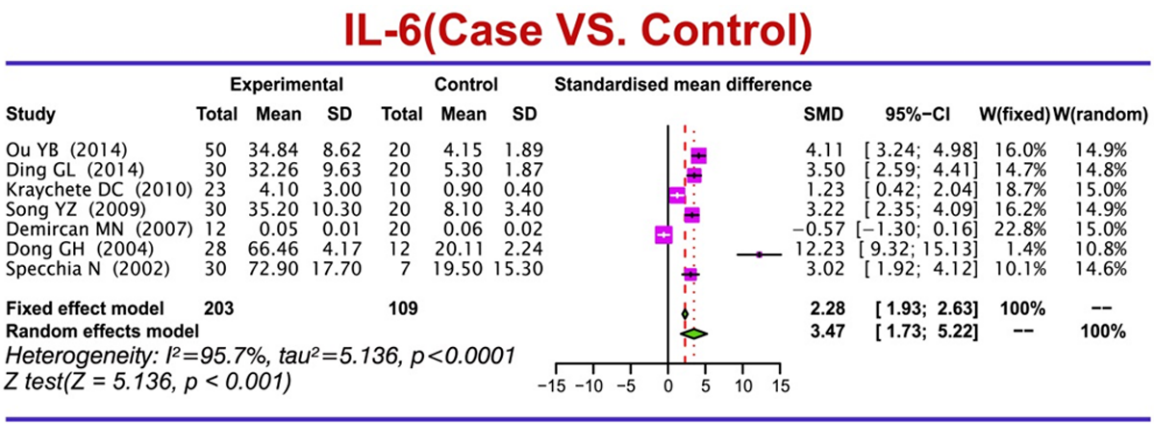

Figure 2. Forest analyses for the relationship between serum IL-6 levels and the development of intervertebral disc degeneration.

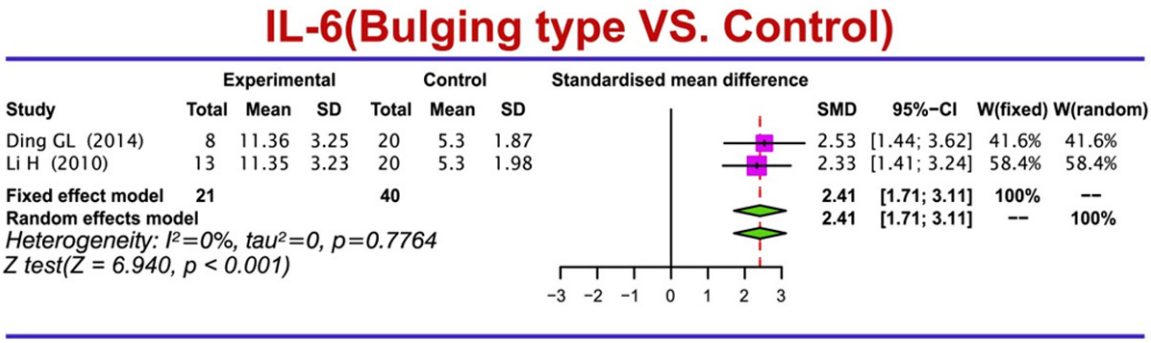

Figure 3. Forest analyses for the relation between serum IL-6 levels and bulging types of intervertebral disc degeneration.

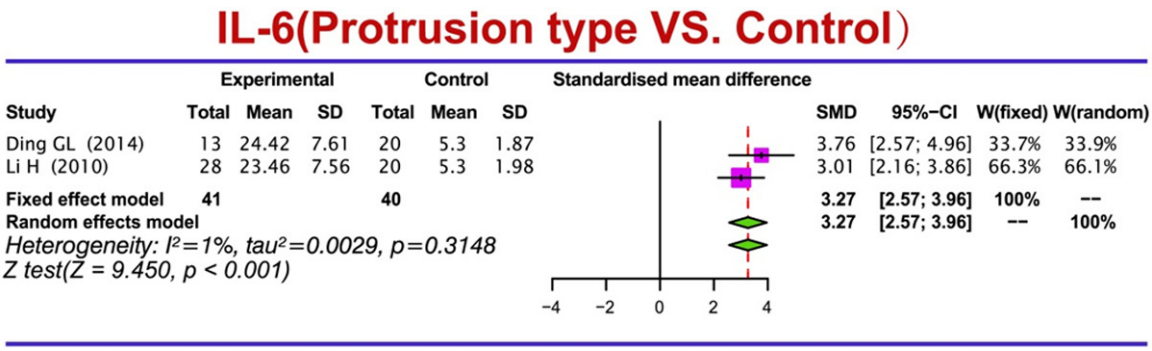

Figure 4. Forest analyses for the relation between serum IL-6 levels and protrusion types of intervertebral disc degeneration.

ry cytokine expression was implicated [36]. Herniated disc tissues express a variety of inflammatory factors such as interleukin-1α (IL-1α), IL-1β, IL-6, IL-8, IL-10, leukotriene B4, and tumor necrosis factor α (TNFα) [37, 38]. Moreover, IL-6 was secreted as a result of TNFα stimulation, contributing to the inflammatory process after disc herniation [39, 40]. IL-6 can upregulate catabolic gene expression and down regulate matrix protein gene expression in chondrocytes, therefore implicates in the synthesis of matrix metalloproteinases (MMPs) [21, 39, 41]. Furthermore, genomic anomalies of MMPs have significant involvement in disc degeneration, thus the progression of disc degeneration strongly correlates with MMP up-

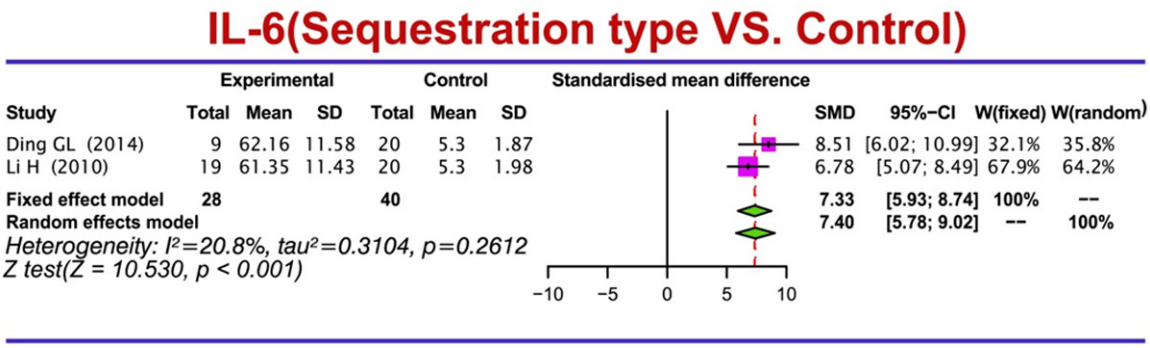

Figure 5. Forest analyses for the relation between serum IL-6 levels and sequestration types of intervertebral disc degeneration.

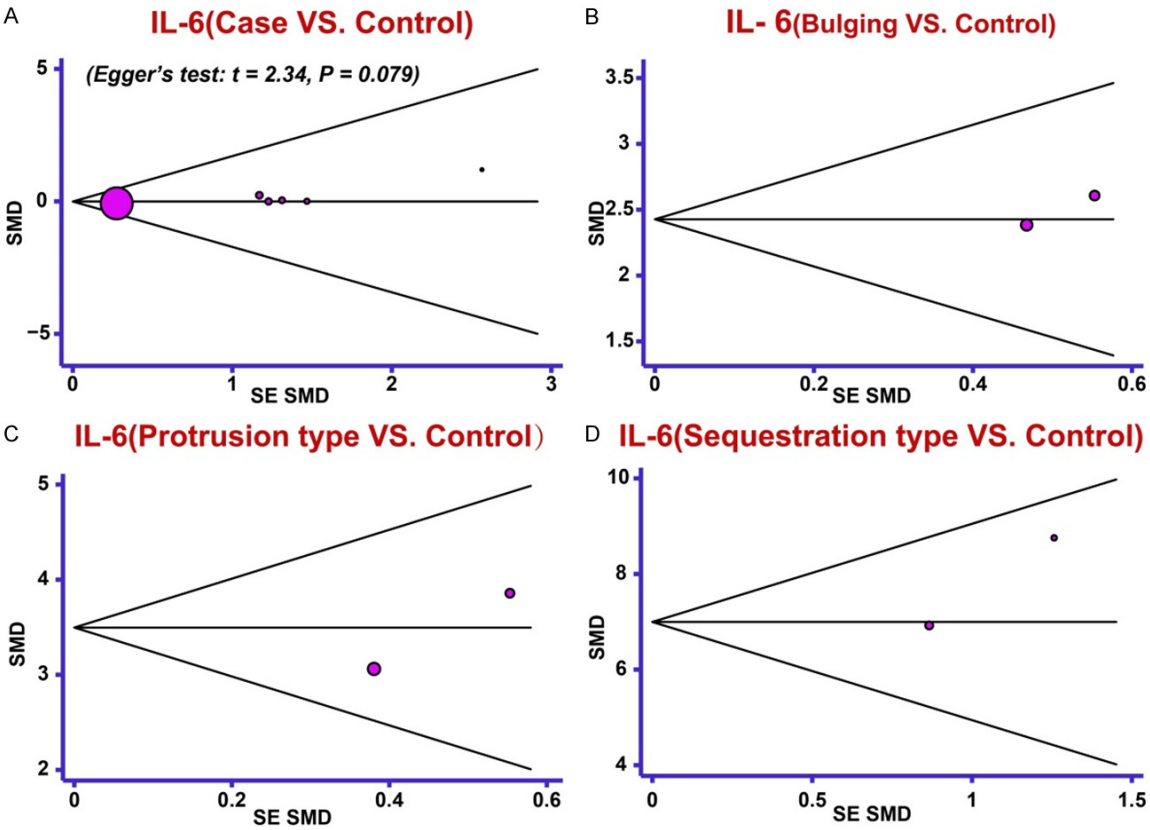

Figure 6. Publication biases ofthe relationship between serum IL-6 levels and the development of intervertebral disc degeneration (A: Case VS. Control; B: Bulging VS. Control; C: Protrusion type VS. Control; D: Sequestration type VS. Control).

regulation [42]. Herein, we hypothesized that elevated IL-6 levels correlates with IDD by regulating the expression of MMPs.

Our results also found a positive association between ages, VAS, ODI and IL-6 expression levels in IDD clinical subtypes, bulging, protrusion and sequestration IDD, which may indicate that IL-6 may serve as a biomarker for disease severity. In consistent with our results, Pedersen et al. found for the first time that chronic lumbar radicular pain may be associated with a continuous increase of pro-inflammatory substances IL-6 and IL-8 in serum after disc herniation [40]. Similarly, Kao *et al.* implied that IL-6 might reflect more active hepatic

necro-inflammation and be associated with the presentation and severity in HBV-infected or HCC studies, suggesting that IL-6 may play an extremely important role to determine the liver progression [43]. A subgroup by our meta-analysis demonstrated that IL-6 expression levels were significantly associated with the IDD clinical subtypes, implying that the positive association between IL-6 expression levels and IDD severity. Mohammed *et al.* revealed that increased levels of mediators of inflammation have been involved in pathologic disc tissue, increasing with grade of degeneration, which was in agreement with our results [36].

In conclusion, our case-control study and meta-analysis both revealed the IDD patients had elevated IL-6 levels compared with controls. Among the three clinical subtypes, sequestration IDD patients with the highest IL-6 levels, suggesting IL-6 levels may be associated with the disease progression in IDD. However, due to the limited sample size of this study, this conclusion needs to be double investigated and validated with a large sample size.

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Disclosure of conflict of interest

None.

Address correspondence to: Drs. Shu-Bin Liu and Hai-Tao Fan, Department of Orthopedics, 307 Hospital of PLA, No. 8 East Street, Fengtai District, Beijing 100071, P. R. China. Tel: +86-10-66947114; Fax: +86-10-66947114; E-mail: liushubin0109@126.com (SBL); fanhaitao0109@126.com (HTF)

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