

Association of interleukin 2, interleukin 12, and interferon- γ with intervertebral disc degeneration in Iranian population

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Research Article

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Abstract

Background: Intervertebral disc degeneration (IVDD) is an age-related degenerative disease, presenting low back pain or radicular pain in a variety of severity. As the inflammatory changes in discs, the inflammatory and anti-inflammatory cytokines, as well as their respective genes, have been proposed to play role in pathophysiology of disease. This study has been conducted to elucidate the role of IL-2, IL-12, and IFN- γ single nucleotide polymorphisms (SNP) in this disease.

Method: 76 patients diagnosed with IVDD and 140 healthy subjects who have complied with eligibility criteria have been included. A total volume of 5cc peripheral blood of each participant has been used for investigating the IL-2 +166G/T, IL-2 -330G/T, IL-12 -1188A/C, and IFN- γ +847A/T single nucleotide polymorphisms (SNP) through PCR-SSP method.

Results: The 'TG' and 'TT' genotypes of IL-2 -330G/T polymorphism have been significantly more common among patients and healthy controls respectively and therefore were associated with disease. The 'GT' and 'TT' haplotypes of IL-2, comprised of -330G/T, and +166G/T, have been also more common among patients and controls respectively.

Conclusion: This study has indicated significant role of IL-2 genotypes and haplotypes in IVDD, as they have been differently distributed in cases and controls. Therefore, alteration in IL-2 gene structure could play an important role in pathophysiology of IVDD through alteration of its function.

Introduction

Intervertebral disc degeneration (IVDD) is one of the common causes of discogenic low back pain, which is considered a frequent health problem of adults affecting their quality of lives as well as the negative impact on the length of their productive lives [1]. Although the mechanical loads on the discs, as well as aging would be considered as the most important causes of IVDD, genetic and immunologic predispositions have been widely discussed especially in the past 2 decades [1]. Accordingly, the individuals with specific genetic predispositions, such as single nucleotide polymorphisms (SNP) of immunologic modulators and cytokines would be more prone to develop IVDD, or be affected with severe grades, or disease onset in younger ages [1].

Generally, innate immunity and inflammation plays important roles in IVDD occurrence. The inflammation would cause destructive damages to the brain through its mediators including pro-inflammatory cytokines and also other cytokines (interleukins, interferons), enzymes and growth factors. Interleukin 2 (IL-2), located on 4q27, is mainly produced by mature T cells and participates in development of T cell and B cell, as it can function as a growth factor for them [2].

The gamma interferon (IFN- γ), which is located at 12q15, is a cytokine with important roles in immunity and immune related diseases. Its expression would remarkably increase during the inflammatory reactions [2]. In the process of disc degeneration, and disc herniation in specific, the IFN- γ is one of the

inflammatory components which is upregulated in disc nucleus pulposus (NP), and affects tissue-specific macrophages in NP [3]. When produced by T helper 1 lymphocytes in the discs, IFN- γ participates in macrophage activation, which could be considered as an immune response to herniation of NP in discs [4]. Moreover, IFN- γ plays a role in pathogenesis of neuropathic pain as well [5]. As the IFN- γ is upregulated in neuroinflammation, as well as affecting the nociceptive neurons, any structural change in its gene which results in higher expression, could possibly play role in pathogenesis of disease. Therefore, some specific SNPs of this gene have been found to affect its expression levels [3].

Interleukin 12 (IL-12) is a cytokine with main role of connecting innate and acquired immunity [2]. Together with other factors and immune-related molecules, this cytokine plays a role in IVDD as well. Similar to IFN- γ , the IL-12 level was found higher in disc fragments caused by disc herniation [4].

As the important role of genetic and immunologic predisposition to IVDD, we have investigated the association of IVDD with different cytokines so far, including the proinflammatory cytokines, interleukin 4, 10, and TGF- β [6–8]. Although the expression levels of some other cytokines including IL-2 and IFN- γ have been of interest in some other studies, the association of their SNPs have not been investigated yet. Therefore, the aim of current study was to evaluate the association of IL-2, IL-12, and IFN- γ SNPs in Iranian patients with IVDD, as well as their association with post-operative pain reduction in this population.

Method

Patient selection

76 Adult patients of both sexes with history of chronic low back pain who were diagnosed with intervertebral disc degeneration, and indicated for surgical intervention confirmed with both clinical manifestations and lumbosacral MRI, have been included in this study. Patients with other etiologies of low back pain including trauma, malignancies, scoliosis, infections, spondylolysis, and spondylolisthesis, and inflammatory disease affecting spine were detected ineligible to participate in this study. The control group (N = 140) has been selected from adult patients of both sexes and same ethnicity without history of chronic low back pain or intervertebral disc degeneration. Participation in the study has been voluntary for all study subjects. Written informed consent to participate was obtained from all study participants before recruitment and sampling. The study has been approved by Ethics Committee of Tehran University of Medical Sciences.

Blood sampling, DNA extraction, and polymerase chain reactions (PCR)

A total volume of 5 cc peripheral blood was obtained from all patients and controls in falcon tubes containing EDTA and stored in -20°C prior to DNA extraction. Previously described by Loparev V. et al. [9], genomic DNA was extracted with Phenol-Chloroform method. Detecting the quality of extracted DNA, the optical density (OD) and 260/280 ratio has been measured with NanoDrop. Four SNPs including IL-2 +

166G/T, IL-2 -330G/T, IL-12 -1188A/C, and IFN- γ + 847A/T have been genotyped through Sequence Specific Prime PCR (PCR-SSP) method using Cytokine CTS-PCR-SSP TRAY KIT (University Clinic Heidelberg, Heidelberg, Germany) protocol. In brief, the 96-well reaction plate of cytokine kit has contained a total volume of 10 μ l/well solution, comprised of 1.44 μ l Master Mix, 3.43 μ l dH₂O, 0.57 μ l DNA, and 0.04 μ l Taq enzyme. The PCR reactions have been performed accordingly to kit protocol.

Statistical analysis

Distributions of qualitative variables as allele and genotype distributions were reported as frequencies. The association of two qualitative variables was detected calculating odds ratio (OR) and Chi² test. The respective P-value was determined as indication of significance and P < 0.05 was the point of significance in all tests.

Results

Association of cytokine (IL-2, IL-12, and IFN- γ) allele and genotype distributions with intervertebral disc degeneration

Genotype distribution of IL-2 promoter polymorphism (-330 G/T) has been significantly associated with IVDD, as 'TG' genotype has been 2.05 times more common among patients (P = 0.05) and 'TT' has been more frequent among healthy subjects (P = 0.03). Other investigated polymorphisms, however, have failed to show any significant association with IVDD for either allele or genotype distribution. (Table 1)

Table 1
IVDD-Control allele and genotype frequency comparison

Cytokine	Position	Alleles/ Genotypes	IVDD (N =	Control (N =	P- Value	OR	95% CI
			76)	140)			
			N (%)	N (%)			
IL-12	-1188 (rs3212227)	A	103 (72.5)	204(72.9)	1.00	0.98	0.63– 1.55
		C	39 (27.5)	76(27.1)	1.00	1.02	0.65– 1.58
		AA	40 (56.3)	72(51.4)	0.56	1.22	0.69– 2.16
		CA	23 (32.4)	60(42.9)	0.18	0.64	0.35– 1.16
		CC	8 (11.3)	8(5.7)	0.17	2.09	0.75– 5.84
IFN-γ	+ 874 (rs2430561)	A	85 (57.4)	140(50.7)	0.22	1.31	088– 1.96
		T	63 (42.6)	136(49.3)	0.22	0.88	0.51– 1.31
		AA	26 (35.1)	43(31.2)	0.65	1.19	0.66– 2.18
		AT	33 (44.6)	54(39.1)	0.47	1.25	0.71– 2.19
		TT	15 (20.3)	41(29.7)	0.15	0.60	0.31– 1.18
IL-2	-330	G	68 (46.3)	110(39.6)	0.21	1.31	0.88– 1.97
		T	79 (53.7)	168(60.4)	0.21	0.76	0.50– 1.13
		GG	4 (5.4)	8(5.8)	1.00	0.94	0.27– 3.22
		TG	60 (81.1)	94(67.6)	0.05	2.05	1.03– 4.06
		TT	10 (13.5)	37(26.6)	0.03	0.43	0.20– 0.93
IL-2	+ 166 (rs2069763)	G	113 (76.4)	219(78.8)	0.62	0.87	0.54– 1.40
		T	35 (23.6)	59(21.2)	0.62	1.15	0.71– 1.85

Cytokine	Position	Alleles/ Genotypes	IVDD (N =	Control (N =	P- Value	OR	95% CI
			76)	140)			
			N (%)	N (%)			
		GG	40 (54.1)	82(59)	0.56	0.82	0.46–1.44
		GT	33 (44.6)	55(39.6)	0.56	1.23	0.69–2.17
		TT	1 (1.4)	2(1.4)	1.00	0.94	0.08–10.52

Association between cytokine haplotype distribution and intervertebral disc degeneration

Considering two polymorphisms of IL-2 together, -330G/T and +166G/T, two haplotypes have been differently distributed in cases and controls. While 'GT' haplotype has been 42.63 times more frequent among patients ($P < 0.001$), the 'TT' haplotype has been more common among healthy subjects (OR = 0.44, $P = 0.009$). (Table 2)

Table 2
IVDD-Control haplotype frequency comparison

Cytokine	Haplotype	IVDD (N = 76)	Control (N = 140)	P-Value	OR	95% CI
		N (%)	N (%)			
IL-2 (-330, +166)	GG	48 (32.2)	107(38.8)	0.20	0.75	0.49–1.14
	TG	65 (43.6)	112(40.6)	0.60	1.13	0.76–1.69
	TT	15 (10.1)	56(20.3)	0.009	0.44	0.24–0.81
	GT	20 (13.4)	1(0.3)	0.000	42.63	5.66-321.15

Association of IL-2 and IFN- γ polymorphisms with Oswestry disability index and visual analogue scales

The allele and genotypes of IL-2 and IFN- γ were considered for evaluating their association with post-operative pain reduction. The of association between each allele or genotype and pain indices have been shown in detail in Tables 3–6. Among them all, only IL-2 -1188A/C indicated significant association with 6-month post-operative ODI reduction.

Table 3
Association between cytokine genotypes and 2 months or 6 months postop OSW changes in IVDD patients

Cytokine	Position	Genotype	2 months postop		6 months postop	
			MD ± SD (MD)	P-value	MD ± SD (MD)	P-value
IL-12	-1188	AA	-18.75 ± 5.69	0.19	-24.54 ± 5.60	0.07
		CA	-21.36 ± 6.01		-27.59 ± 3.69	
		CC	-21.50 ± 5.08		-26.50 ± 5.16	
IFN-γ	+ 874	AA	-20.08 ± 5.44	0.99	-25.82 ± 4.54	0.98
		AT	-20.11 ± 5.92		-25.92 ± 5.18	
		TT	-20.35 ± 6.12		-25.57 ± 6.33	
IL-2	-330	GG	-27.50 ± 0.70	0.12	-28.00 ± 2.82	0.82
		TG	-19.54 ± 5.90		-25.80 ± 5.27	
		TT	-21.37 ± 4.03		-25.50 ± 4.75	
IL-2	+ 166	GG	-20.66 ± 5.38	0.33	-26.21 ± 4.27	0.27
		GT	-19.12 ± 6.14		-25.19 ± 5.85	
		TT	-26.00		-33.00	

Table 4
Association between cytokine genotypes and 2 months or 6 months postop VAS changes in IVDD patients

Cytokine	Position	Genotype	2 months postop		6 months postop	
			MD ± SD (MD)	P-value	MD ± SD (MD)	P-value
IL-12	-1188	AA	-4.37 ± 1.42	0.52	-5.62 ± 1.29	0.49
		CA	-4.77 ± 1.10		-5.90 ± 1.06	
		CC	-4.50 ± 0.83		-6.16 ± 1.47	
IFN-γ	+ 874	AA	-4.65 ± 1.19	0.49	-5.86 ± 1.09	0.92
		AT	-4.37 ± 1.49		-5.74 ± 1.48	
		TT	-4.85 ± 0.94		-5.85 ± 0.94	
IL-2	-330	GG	-6.00 ± 0	0.22	-6.00 ± 0	0.88
		TG	-4.56 ± 1.31		-5.83 ± 1.21	
		TT	-4.25 ± 0.88		-5.62 ± 1.50	
IL-2	+ 166	GG	-4.57 ± 1.27	0.90	-5.84 ± 1.12	0.18
		GT	-4.58 ± 1.31		-5.70 ± 1.29	
		TT	-4.00		-8.00	

Table 5
Association between cytokine allele frequencies and 2 months or 6 months postop OSW changes in IVDD patients

Cytokine	Position	Allele	2 months postop		6 months postop	
			MD ± SD (MD)	P-Value	MD ± SD (MD)	P-Value
IL-12	-1188	A	-19.35 ± 5.81	0.07	-25.23 ± 5.33	0.05
		C	-21.41 ± 5.55		-27.20 ± 4.13	
IFN-γ	+ 874	A	-20.09 ± 5.54	0.89	-25.86 ± 4.72	0.89
		T	-20.23 ± 5.91		-25.74 ± 5.68	
IL-2	-330	G	-20.08 ± 6.04	0.90	-25.94 ± 5.14	0.81
		T	-19.95 ± 5.54		-25.73 ± 5.09	
IL-2	+ 166	G	-20.17 ± 5.62	0.58	-25.88 ± 4.80	0.83
		T	-19.54 ± 6.17		-25.66 ± 5.97	

Table 6
Association between cytokine allele frequencies and 2 months or 6 months postop VAS changes in IVDD patients

Cytokine	Position	Allele	2 months postop		6 months postop	
			MD ± SD (MD)	P-Value	MD ± SD (MD)	P-Value
IL-12	-1188	A	-4.46 ± 1.35	0.41	-5.68 ± 1.24	0.20
		C	-4.67 ± 1.00		-6.00 ± 1.18	
IFN-γ	+ 874	A	-4.54 ± 1.30	0.75	-5.82 ± 1.23	0.92
		T	-4.61 ± 1.25		-5.80 ± 1.22	
IL-2	-330	G	-4.66 ± 1.32	0.45	-5.84 ± 1.17	0.78
		T	-4.49 ± 1.22		-5.78 ± 1.26	
IL-2	+ 166	G	-4.57 ± 1.27	0.90	-5.80 ± 1.16	0.85
		T	-4.54 ± 1.27		-5.84 ± 1.37	

Discussion

To our knowledge, the current study was one of the firsts to investigate the association of cytokine polymorphisms including IL-2 (-330T/G, + 166G/T), IL-12 (-1188A/C) and IFN-γ (= 874A/T) with intervertebral disc degeneration (IVDD) in Iranian patients. Among all the investigated SNPs, only the IL2 -330T/G was significantly associated with disease was twice more common in patients than controls. Besides, the haplotype of IL-2 (-330, + 166) was also significantly associated with IVDD, as the GT haplotypes was 42 times more frequent in IVDD patients. However, other investigated SNPs failed to show any association with IVDD in this population.

The role of immunity, as well as the cytokines of interest in the current study, were investigated in a number of studies so far. Although the biomarker levels of these cytokines were of interest in a number of studies or animal model investigations, the role of SNPs were not widely investigated in the literature.

The IFN-γ level would significantly change in low back pain. A recent investigation in 2019, indicated higher levels of IFN-γ in acute low back pain compared with chronic low back pain or healthy subjects. Interestingly, the IFN-γ level was not significantly different between chronic low back pain and asymptomatic individuals [10]. A considerable number of patients with IVDD who experience chronic low back pain may require surgical intervention, nevertheless, some of them may not response to this treatment either. The IFN-γ expression level was associated with increased numeric rating scale, and higher levels of IFN-γ was detected in patients who did not respond to surgery. Spinal cord stimulation might be considered as an option for improving the pain and quality of life in these patients. However, in an investigation, IFN-γ level was not significantly altered after the stimulation [5]. IFN-γ levels was associated with acute low back pain, although its association with chronic low back pain was not

significant [10]. While the IFN- γ SNP did not showed significant association with postoperative pain reduction in this study, the A allele of IFN- γ rs2069705 remarkably associated with higher ODI score in a study, as the patients with AA and GA genotypes had significantly higher ODI scores. In addition, the AG and GG genotypes of IFN- γ rs2069718 were associated with higher ODI scores in this Norwegian population [3].

The IL-12 mainly functions together with other cytokines such as IFN- γ . Accordingly, when comparing the cytokine levels in fragments of herniated discs, with degenerative disc tissue, both IL-12 and IFN- γ , as well as other cytokines (IL-4, IL-6) indicated higher levels in the fragments of herniated discs [4]. On the other hands, the expression levels of these cytokines were not remarkably different between nucleus pulposus and anulus fibrosus of healthy discs obtained from autopsies [4].

Although the IL-2 plays an important role in inflammatory processes, its expression level was not significantly different between acute or chronic low back pain, or even the asymptomatic subjects [10]. Moreover, the IL-2 level was not associated with VAS scores either in acute or chronic low back pain [10]. In a study measuring the levels of different cytokines in low back pain, IL-2 levels in the sera of patients with low back pain were remarkably lower than controls, similar to other factors including IL-6, IL-4, and MMP-1. On the other hands, the serum levels of IL-12 and IFN- γ were not significantly different between patients and healthy controls [11]. However, another study indicated higher levels of IL-2 and IL-12 in degenerated disc tissue compared to controls [12]. Another study confirmed this finding, as both the mRNA level and protein level of IL-2 were higher in prolapsed NP cells than controls [13].

In an animal model of disc herniation in rats, presence of NP and IFN- γ in the dorsal nerve root of animals resulted in remarkable higher activity of nociceptive neurons [3]. The intervertebral disc degeneration would affect different components of spinal cords, in addition to disc itself. The inflammatory process and destructive damages would also affect the spinal components such as epidural space, as the inflammation in this component would have compressive effect on nerve roots and cause more severe clinical manifestations. Accordingly, in a canine model of disc degeneration, the cytokine levels were assessed in different stages of disc extrusion. Importantly, the expression level of IL-2 was significantly decreased in disc extrusion, especially in the subacute stage [14]. However, another animal model in rabbits showed contradictory results, as the IL-2 level was significantly increased not only in degenerated disc tissue, but also in the sera of animals. Moreover, in the current model, the effect of plasma vaporization ablation was evaluated for treatment of IVDD. The expression levels of IL-12, as well as some other cytokines such as IL-4 and TNF- α , were decreased after applying this method, which could indicate its effectiveness in treating IVDD [15]. The mRNA expression of IL-2 was also remarkably higher in another model of disc herniation in rats [16]. In an animal model of disc herniation in rats, dorsal root ganglions were exposed to NP, and epidural lavage indicated higher expression of IFN- γ , as well as other cytokines such as IL-4, IL-6, and TNF- α [17].

Taking limitations of current study to account, small sample size could be considered as one of them, and therefore, a much larger sample size could improve random error or increase the study power.

Meanwhile, the genetic factors may have co-effect on each other, and therefore a wider panel measuring the role of different cytokines and other genetic factors could be helpful if better understanding of the role of each factor.

Conclusion

Cytokines variants including IL-2 -330T/G could be considered as a predisposing factor for IVDD, as the TG genotype was twice more common in Iranian adults with IVDD. As the importance of IVDD in daily lives of working population, understanding genetic predispositions could be invaluable to detect high risk individuals, and therefore, consider some preventive conditions for them, such as avoiding them to choose jobs which involve high load on lumbar discs.

Abbreviations

IFN- γ

Interferon gamma

IL-12

Interleukin 12

IL-2

Interleukin 2

IVDD

Intervertebral Disc Degeneration

NP

Nucleous Pulposus

OD

Optical Density

ODI

Owestry Disability Index

OR

Odds Ratio

PCR

Polymerase Chain Reactions

PCR-SSP

Sequence Specific Prime PCR

SNP

Single Nucleotide Polymorphisms

TNF- α

Tumor Necrosis Factor alpha

VAS

Visual Analogue Scale

Declarations

Ethical approval and Consent to Participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All the study participants were adults over 18 years, and written informed consent to participate was obtained from all of study participants prior to recruitment and sampling. The study was approved by Ethics Committee of Tehran University of Medical Sciences.

Consent for publication

Not applicable

Availability of data and materials

The data that support the findings of this study are available from the corresponding author (Nima Rezaei) but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of the corresponding author (Nima Rezaei).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors have read and approved the final version of manuscript. The authors' contribution in the study was as follow:

SH prepared the proposal, performed laboratory tests, interpreted the results, drafted the manuscript, and confirmed the final manuscript.

SA designing the study, provided samples, revised the manuscript, and confirmed the final manuscript.

MS performed the laboratory tests, interpreted the results, and confirmed the final manuscript.

MHM provided samples, cooperated in collecting the clinical data, and confirmed the final manuscript.

EF provided samples, cooperated in collecting the clinical data, and confirmed the final manuscript.

ARK provided samples, supervised the project as the clinical expert, and confirmed the final manuscript.

NR designed the study, critically revised the manuscript, supervised the whole study as the expert, and confirmed the final manuscript.

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