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Original Research

Cytokine and chemokine profile changes in patients with lower segment lumbar degenerative spondylolisthesis

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HIGHLIGHTS

- Significant role of cytokines in DS pathophysiology.
- Cytokines profile corresponding to major pathophysiological processes.
- Involvement of intervertebral disc degeneration in DS development.
- Cytokines as the future potential targets for new biological treatment of pain, or slowed DS progression.

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ABSTRACT

Background: Lumbar degenerative spondylolisthesis (DS) develops as a result of inflammatory and remodeling processes in facet joints (FJs). Several inflammatory cytokines are involved in the osteoarthritic and remodeling changes that occur and in low-back and/or radicular pain, the most prevalent clinical symptom of disease. This study improves knowledge related to the roles that 27 cytokines, chemokines and growth factors play in the pathophysiology of lumbar DS.
Material and Methods: Cytokine levels were examined using capture sandwich immunoassay using the Bio-Plex® 200 System and the Bio-PlexTM Human Cytokine Standard 27-Plex, Group I (Bio-Rad, Hercules, California, USA) separately in intervertebral discs (IVDs) and FJ bone tissue. The samples were obtained during primary spinal surgery from 9 patients suffering from lower segment lumbar DS. The pain intensity was assessed using a visual analog scale. The controls were tissue samples collected from both lower lumbar segment levels of 6 male subjects during a multiorgan procurement procedure.
Results: The Bio-Plex® assay revealed significant differences between the patients and controls in cytokines, chemokines and growth factor profiles:
i, The elevated interleukin-6 (IL-6), IL-7, IL-13, tumor necrosis factor α (TNF-α), interferon γ and platelet-derived growth factor levels in lumbar DS samples of subchondral FJ bone. These indicated ongoing inflammation, bone formation and increased fibroblasts activity in the FJ bone.
ii, The elevated levels of IL-6, IL-8, TNF-α, granulocyte-macrophage colony-stimulating factor and monocyte chemoattractant protein-1 in anulus fibrosus together with increased IL-6, IL-8, TNF-α and eotaxin and decreased IL-1-receptor antagonist in nucleus pulposus confirmed advanced IVD degeneration in the patient samples.
Conclusion: This study identified, for the first time, protective levels of cytokines, chemokines and growth factors in healthy subjects and supported their significant involvement in the pathogenesis of

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lumbar DS. The control samples and analytical methods used avoided any false changes in the cytokine levels due to secondary factors (e.g., death of donor and limited cytokine stability).

List of abbreviations used

AF	annulus fibrosus
ANOVA	analysis of variance
B	bone
CT	computer tomography
CD4	cluster of differentiation 4
DS	degenerative spondylolisthesis
FGF	fibroblast growth factor
FJs	facet joints
FJB	facet joint bone
G-CSF	granulocyte colony stimulating factor
GM-CSF	granulocyte-macrophage colony-stimulating factor
IFN-γ	interferon gamma
IL-1β	interleukin-1 beta
IL-1ra	interleukin-1 receptor antagonist
IL-6	interleukin-6
IL-12 (p70)	heterodimer of interleukin-12
IP-10	interferon gamma-induced protein 10
IVD	intervertebral disc
IVDD	intervertebral disc degeneration
MCP-1	monocyte chemoattractant protein-1
MIP-1α	macrophage inflammatory proteins 1α
MIP-1β	macrophage inflammatory proteins 1β
MRI	magnetic resonance images
NP	nucleus pulposus
PDGF	bb Platelet-derived growth factor
PGE_2	prostaglandin E_2
RANTES	abbreviation of **r**egulated on **a**ctivation
RPM	rotations per minute
TNF-α	tumor necrosis factor alpha
VAS	visual analog scale
VEGF	vascular endothelial growth factor

1. Introduction

Spondylolisthesis is defined as an anterior or posterior slippage of cranial vertebra in relation to the adjacent vertebra. According to the Wiltse classification [1], there are 5 types of spondylolisthesis: dysplastic, isthmic, degenerative, traumatic and pathologic. Although spondylolisthesis can be caused by many pathologic entities, degenerative spondylolisthesis is by far the most frequent. Degenerative spondylolisthesis (DS) is a disease that occurs prevalently in adults older than 50 years of age and is more common in female and African American patients [2]. According to Kalichman and Hunter [3], the following factors are the major local causes of instability of the functional vertebral unit and to the development of degenerative vertebral slippage:

a. arthritis of the facet joints (FJs), with loss of their normal structural support and remodeling;
b. malfunction of the ligamentous stabilizing component, likely due to hyperlaxity; and
c. ineffectual muscular stabilization.

There is contradictory evidence regarding the involvement of intervertebral disc degeneration (IVDD) in the etiology of DS. The general current belief is that IVDD leads to segmental instability in the sagittal plane and may result in DS [4].

Changes in the structure and position of FJs, segmental instability and spinal canal stenosis represent the most probable diagnostic signs/symptoms of DS [5]. Spinal canal stenosis results in the clinical presentation of spinal claudication and/or radicular pain, and *lumbar DS* appears as low back pain and/or radicular pain [6]. Surgical treatment with decompression of the neural structures and fusion is recommended for patients who do not respond to an initial regimen consisting of physical and pharmacological approaches [7].

The various interconnected changes of the functional vertebral unit are involved in the pathophysiology of lumbar DS. Generally, lumbar DS is an anatomic finding that develops as a result of inflammatory and remodeling FJ processes. Except for prostaglandins (e.g., prostaglandin E_2 [PGE_2]), inflammatory cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α), play a major role in the mediation of *osteoarthritic* changes in animals and humans [8–10]. These cytokines and matrix-degrading enzymes disturb chondrocyte metabolism, leading to cartilage degradation. Cartilaginous changes elicit intense pathologic *remodeling* in the subchondral bone [11] and further stimulate the inflammatory process. In addition, IL-1β, IL-6, IL-8 and TNF-α also play a key role in the development of *IVDD*. Their levels in degenerated intervertebral disc (IVD) tissue were shown to increase with the level of degeneration [12,13]. Furthermore, prostaglandins and the inflammatory cytokines IL-1, IL-6 and TNF-α are postulated to play an important role in mediating low-back and/or radicular pain [14], the most prevalent clinical features of lumbar DS.

The current study was designed to increase knowledge of the roles that cytokines, chemokines and growth factors play in lumbar DS pathophysiology. The control group of specimens was carefully selected to strictly avoid any false changes due to donor death and the limited stability of certain cytokines. Similarly, the sample collection, processing and storage methods were selected to achieve accurate and reproducible results.

2. Material and methods

2.1. The source of human IVD and FJ bone

All processes were approved by the Institutional Ethic Committee and registered with the Institutional Review Board in accordance with legislation. Patient recruitment was performed by providing information sheets describing the study, and the patients provided written informed consent.

Information related to the 9 patients (2 males and 7 females; average age 50.7 ± 10.2 years) who were included in study is shown