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Propionibacterium acnes Infection in Disc Material and Different Antibiotic Susceptibility in Patients With Lumbar Disc Herniation

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ABSTRACT

Background: Low back pain is an important, worldwide clinical problem from which human populations are suffering. It has been suggested that *Propionibacterium acnes* is associated with low back pain. The objective of this clinical study is to evaluate the *P. acnes* infection in the disc material and antibiotic susceptibility in patients with disc herniation.

Methods: A total of 120 patients with disc herniation surgery were enrolled in the study. The samples were excised during discectomy and then cultured in both anaerobic and aerobic incubations. Minimum inhibitory concentration (MIC) was performed for determination of antibiotic susceptibility.

Results: Of 120 samples, 60 (50%) samples were positive for microorganisms. Disc herniation was at the level of L4-L5 in 63 cases and L5-S1 in 57 cases.

Conclusions: According to the results and presence of *P. acnes* in more than 35% of the cultured samples, the presence of *P. acnes* in lumbar disc herniation is a suspected element leading to this condition. After analysis of the antibiotics, the lowest MIC value was identified for amoxicillin, ciprofloxacin, erythromycin, rifampicin, tetracycline, vancomycin; the moderate MIC value was for fusidic acid; and the highest MIC value was for gentamicin and trimethoprim.

Lumbar Spine

Keywords: *Propionibacterium acnes*, lumbar disc herniation, low back pain, antibiotics

INTRODUCTION

Low back pain (LBP) is the most common social, economic, clinical, and public health problem affecting the human population worldwide.¹ About 70% of adults suffer from LBP with various degrees of severity at some time during their lives.² The cause of most LBPs has not been recognized yet. However, the source of LBP is associated with degenerative joints and disc disease in 5% to 15% of patients.³ In addition, a major cause of discogenic LBP is degenerative changes in the human intervertebral discs especially in the lumbar spine, which occurs during aging.⁴ Discogenic pain in the lumbar spine is related to 3 main factors: mechanical injuries, internal disc disruption, and disc inflammation.⁵ Over the last decade, evidence has emerged that *Propionibacterium acnes* is associated with a number of clinical conditions such as discogenic LBP and sciatica,⁶ discitis,⁷ and infections related to medical devices.⁸ *P. acnes* is a gram-positive, rod-

shaped bacillus that is a human skin commensal and oral microbiota.⁹ It is slow-growing and prefers anaerobic growth conditions and has been documented as a pathogenic factor in the inflammatory skin condition acne vulgaris.^{10,11} Recently, this microorganism has been recognized as a cause of prostatitis leading to prostate cancer,¹² sarcoidosis,^{13,14} synovitis, pustulosis, hyperostosis, and osteitis syndrome, and chronic recurrent multifocal osteomyelitis¹⁵ as well as sciatica.⁶ Also, it has been suggested that *P. acnes* can be observed in various kinds of implant-associated infections, including neurosurgical shunts,^{16,17} ocular implants,¹⁸ breast implants,^{19,20} cardiovascular devices,²¹ spinal hardware,^{22–24} internal fracture fixation devices,^{25,26} and prosthetic joints.^{27,28} Previously, it was reported that bacteria cultures of disc herniations were positive for about 46% of cases, of which 86% were related to *P. acnes*.²⁹ Moreover, using antibiotics could improve LBP in patients with herniated

lumbar discs.³⁰ However, there is no agreement on the origin of this pathogen in the disc tissue. As a closed and avascular structure, an intervertebral disc consists of 2 interlinked but distinct regions, the inner nucleus pulposus and the outer annulus fibrosus. In a normal disc, the posterior annulus is weaker with higher stress load.^{31,32} Therefore, *P. acnes* cannot enter the normal disc. It is reported that stress distributions can be disturbed by aging and disc degeneration. Then, increased stress may lead to posterior annulus injury.³¹ Consequently, new angiogenesis after annulus disruption can directly influence *P. acnes* in the disc tissue.²⁹ Thus, avascular disc tissue can provide a suitable environment for *P. acnes* proliferation, leading to a slow-developing infection.⁶ Because there is no agreement on the sources of *P. acnes* infection in patients with lumbar disc herniation, this study aimed to evaluate the bacterial infection, especially *P. acnes*, in the disc materials from patients with disc herniation.

MATERIALS AND METHODS

Study Participants

From September 2014 to July 2015, a total of 120 patients (referred to Imam Reza Hospital, Tabriz, Iran) aged 18 to 65 years old undergoing discectomy with disc herniation surgery were enrolled into the study. Inclusion criteria were defined as patients with diagnosed lumbar disc herniation at the single level of L4-L5 or L5-S1 confirmed by MRI. In this study, all patients suffering from LBP for 2 to 6 months and not responding to conservative treatment were enrolled. In addition, patients with a history of diabetes, antibiotic treatment (1 month before the surgery date), back surgery, and/or previous epidural steroid injection were excluded.

Biopsy Collection

The operation was performed via posterior approach microdiscectomy by a single senior surgeon with the patient in a prone position. In this study, infection evaluation was performed according to the method of the Infectious Diseases Society of America for investigation of prosthetic joint infection.³³ In this way, 5 separate samples from disc material were surgically removed from each patient during the disc removal. Before surgery, all patients were told to take a bath. To avoid any potential contamination of excised biopsies, stringent antiseptic sterile protocols were

followed and the skin of the operation field was cleaned with Betadine (a surgical scrub containing 7.5% povidone-iodine) preoperatively for 3 minutes, then was allowed to dry. For each individual biopsy, the disc fragment was extracted with a set of sterile instruments. One high dose of cefuroxime (1.5 g) was administered intravenously after the tissue samples were retrieved to prevent growth of any bacteria present in the biopsy samples. Biopsies were placed in separate sterile glass vials and immediately frozen at -80°C . The frozen samples were transported to Tabriz University of Medical Sciences in thermal transport boxes designed for organ transport.

Bacterial Culture

All disc samples were cut into smaller fragments, and the tissue was broken apart and ground up, using an individually packaged sterile scalpel. As a further precaution, all scalpels were dipped in 70% (vol/vol) ethanol and passed through a Bunsen burner flame before use. With a sterilized scalpel, the processed and ground-up tissue sample was first spread across the surface of a Columbia blood agar plate (Oxoid, Basingstoke, UK), and then collectively embedded into the center of the plate.⁶ Two sections were prepared from each individual tissue sample and one section of the tissue was incubated in an aerobic and the other in an anaerobic glove box for 7 days at 37°C (80% dinitrogen, 10% carbon dioxide, and 10% dihydrogen).

Molecular and Phenotypic Identification

Following anaerobic and aerobic culture incubations, resulting colonies were subcultured onto Columbia blood agar plates and then incubated at 37°C for 24 hours in anaerobic conditions. Gram staining was performed for investigation of all colonies. For identification of presumptive *P. acnes*, a rapid ID 32A kit (bioMérieux) was used.

The cultured *P. acnes* was examined by 16S rRNA (ribosomal RNA)-based polymerase chain reaction (PCR). In this way, specific primers were designed for amplification of *P. acnes* 16S rRNA. The forward primer was 5'-GGTTGTAAACCGCTTTCGCCT-3', and the reverse primer was 5'-GGCACACCCATCTCTGAGCAC-3'.²⁹

Genomic DNA was extracted by a rapid boil extraction method.³⁴ The PCR was performed in a 25- μL volume containing 2 μL of template DNA, 19.8 μL of SDW, 2.5 μL of $10\times$ PCR buffer (10 mM

Table 1. Distribution of *Propionibacterium acnes*-infected samples according to age groups.^a

Age Groups, y	<i>P. acnes</i>		Total
	Positive	Negative	
18–29	6	13	19
30–39	16	19	35
40–49	8	19	27
50–59	11	12	23
60–65	5	11	16
Total	46	74	120

^aNumbers show the frequency of positive and negative samples in each group.

TrisHCl pH 8.3, 25 mM potassium chloride, 3.5 mM magnesium chloride), 0.2 µL of dNTPs (10 mM each nucleotide), 0.2 µL of each primer (25 pmol/µL), and 0.1 µL of *Taq*DNA polymerase (1.25 units/µL). A negative control (sterile water as template) and a positive control (with recently amplifiable *P. acnes* DNA) were undertaken. The PCR was performed in the following conditions: initial 94° C for denaturation (4 minutes); 35 cycles at 94° C for denaturation (30 seconds), 54° C for annealing (30 minutes), and 72° C for extension (1 minute); 72°, extension (4 minutes); 4° C, hold. Amplified fragments were separated using a 2% agarose gel containing 1 µg/mL of ethidium bromide. Consequently, electrophoresis was performed in following condition including 0.1% (vol/vol) glacial acetic acid buffer at 100 V and 1× TAE (40 mM Tris, 1 mM EDTA).

Minimum Inhibitory Concentration Determination by Agar Dilution

In this study, the minimum inhibitory concentration (MIC) was determined that each isolate against different antibiotics using the Clinical and Laboratory Standards Institute reference agar dilution procedure.³⁵ The following antibiotics were used: gentamicin, vancomycin, rifampicin, fusidic acid, ciprofloxacin, amoxicillin, erythromycin, tetracycline, and trimethoprim (Mast Diagnostics, UK).

P. acnes type strain NCTC 737 (type IA1) was used as a control. Plates were incubated in an anaerobic condition at 37° C for 72 hours. Then, the MIC was recorded for each antibiotic at the lowest concentration in which there was no visible growth.

Statistical Analysis

Descriptive statistics (mean, standard deviation, frequency, and frequency percentage distribution) were used to describe the basic features of the data. The mean values were compared using a *t* test and

Table 2. MIC values (mg/L) for all *Propionibacterium acnes* isolates recovered from discectomy tissue (N = 221).^a

MIC (mg/L)	≤0.03	0.06	0.12	0.25	0.5	1	2	≥4
Gentamicin								221
Vancomycin		5			81	135		
Rifampicin	221							
Fusidic acid						31	190	
Ciprofloxacin						221		
Amoxicillin		91	130					
Erythromycin	25	196			221			
Tetracycline								
Trimethoprim							35	186

Abbreviation: MIC, minimum inhibitory concentration.

^aTable shows different antibiotic susceptibility. Values correspond to the total number of isolates with that specific.

then exact chi-square test was used for comparing the categorical variables. A *P* value ≤.05 was considered to be statistically significant.

RESULTS

In the present study, 120 participants with a mean age of 43.15 ± 12.62 years (range, 18 to 65 years) were investigated. Of those, 69 (57.5%) were men and 51 (42.5%) were women. The mean age of men was 41.66 ± 12.33 years and of women, 45.15 ± 12.86 years. There was no significant difference in the age of groups (*P* = .135). In this study, disc herniation was at the level of L4-L5 in 63 people (52.5%) and at L5-S1 in 57 people (47.5%). The PCR technique was used for examination of 16S rDNA specific for *P. acnes* in the disc cultures. According to the results, the 16S rDNA gene was identified in 46 (38.3%) disc samples (Table 1). In addition, there was no significant difference in the distribution of *P. acnes*-positive samples according to 5 age groups (*P* = .516; Table 1). The distribution of *P. acnes* infection in the sex groups was evaluated, and 28 (23.3%) men and 18 (15%) women were infected by *P. acnes*. It was shown that there was no significant relationship between sex and *P. acnes* infection (*P* = .556). Antibiotic susceptibility was checked for *P. acnes*-positive samples. The lowest MIC value (≤1 mg/L) was identified for amoxicillin, ciprofloxacin, erythromycin, rifampicin, tetracycline, and vancomycin. A moderate MIC value (1–2 mg/L) was found for fusidic acid, and the highest MIC value (≥2 mg/L) was for gentamicin and trimethoprim (Table 2).

DISCUSSION

In the present study, 60 (50%) samples were infected by different bacteria, of which 76.66% of

infections were related to the anaerobic bacterium *P. acnes*. The findings of the present study confirmed those of other studies stating that the most frequent microorganism in the disc material (more than 70%) is *P. acnes*.^{6,29,36–39} *P. acnes* is an anaerobic-aerotolerant bacteria with a genome that encodes both oxidative phosphorylation components (such as NADH dehydrogenase/complex I, cytochrome C oxidase, cytochrome C reductase, and FOF1-type adenosine triphosphate synthase) and cytochrome D oxidase.^{40–42} Thus, this bacterium can grow in different conditions, can survive under anaerobic conditions *in vitro* for more than 8 months, and is also capable of tolerating exposure to oxygen for several hours.⁴³ In addition, previous studies have shown that *P. acnes* is able to survive in human tissues with low oxygenation for a long period.^{41,43} A spinal disc is a low-oxygen environment due to the lack of vascularization. Therefore, this tissue is an ideal environment for growth of anaerobic bacteria such as *P. acnes*. Stirling et al⁶ showed that there is a relation between severe sciatica and *P. acnes* infection. Nineteen (53%) patients with severe sciatica who had undergone microdiscectomy were positive for *P. acnes* bacterium. The researchers⁶ concluded that these microorganisms could infect the intervertebral disc after a minor trauma. In another study, Stirling et al³⁶ demonstrated that 27 (43%) cases were positive for infection; of these, 22 were associated with *P. acnes*. They showed that epidural injection was not related to a positive culture.³⁶ Corsia et al³⁷ evaluated the presence of bacterial infection in 30 individuals with lumbar and 30 with cervical disc herniation. In contrast, staphylococcus (36%) was more frequent than *P. acnes* (18%) in the lumbar disc herniation. In cervical herniation, they found 37% of the infection involved *P. acnes*.³⁷ Similarly, Agarwal et al³⁸ evaluated the intervertebral disc material from 52 patients who had undergone single-level microdiscectomy for lumbar disc herniation for bacterial infection; 19% of them had microorganism infection, of which 70% was related to *P. acnes*.³⁸ In a similar study, Albert et al²⁹ showed that *P. acnes* was the most frequent microorganism in the disc material from patients with a lumbar disc herniation.²⁹ In this study, all the cases with history of diabetes, back surgery and/or previous epidural steroid injection were excluded to omit the risk factors of disc contamination. Previously, studies have demonstrated that contamination with this

microorganism is associated with the pathogenesis of some disease such as implant-associated infections.⁴⁴ For example, contamination with *P. acnes* was confirmed in the pathology of periprosthetic joint infection, wherein direct contamination of the implant and/or the surgical wound during the operation can lead to this condition.^{45–47} However, all the cases with history of diabetes, back surgery, and/or previous epidural steroid injection were excluded to omit the risk factors of disc contamination from this study. Consequently, the results of the present study showed that the source of infection is not related to previous contamination. Moreover, skin was cleaned preoperatively to prevent possible contamination of biopsy samples. In contrast, other studies were conducted to show that isolated bacteria from disc material in patients with microdiscectomy are associated with tissue contamination, previous back operation, or injection. Ben-Galim et al⁴⁸ suspected that contamination was the reason that 2 of 30 patients had coagulase-negative staphylococcus in the evacuated nucleus material. They used one high-dose cefazolin preoperatively. They explained that low isolation rate of *P. acnes* was related to its sensitivity to this high-dose antibiotic.⁴⁸ Furthermore, Carricajo et al⁴⁹ showed that 2 of 54 of cases were positive for *P. acnes* and that muscle and ligamentum flavum from these 2 cases were also positive for this microorganism. They demonstrated that sample contamination might be the cause of positive *P. acnes* in the lumbar disc tissue cultures.⁴⁹ In another study, Zhou et al⁵⁰ showed that 11 discs and 3 muscle samples were positive for *P. acnes*. As a result, it has been confirmed that *P. acnes* growth in the muscle is related to contamination.⁵⁰ In this study, tissues were cultured. Using culture is a usual technique for identification of bacteria. Our samples were incubated for 7 days. As a slow-growing anaerobic bacterium, *P. acnes* needs a prolonged incubation period in anaerobic condition.^{27,51} The PCR technique has been used for detection of 16S rDNA gene that is specific for *P. acnes*.⁵² This technique is sensitive, effective, and quick for identifying *P. acnes* as compared with culturing methods.^{52,53} In this study, PCR was used for identifying bacterial infection in the cultured samples and then was specifically used for identifying *P. acnes*. Considering its advantages, PCR was also used for identifying the *P. acnes* infection in similar studies.^{50,54} Antimicrobial susceptibility was determined for

cultured *P. acnes* from lumbar herniation, and the findings can provide further information for treatment regimens. In this study, low MIC values were detected for amoxicillin, ciprofloxacin, erythromycin, rifampicin, tetracycline, and vancomycin. Currently, antibiotics such as penicillin/amoxicillin, vancomycin, clindamycin, or rifampicin/linezolid are suggested for the treatment of postoperative or prosthetic infection.^{55–58} Albert et al³⁰ examined the effects of antibiotics (amoxicillin/clavulanic acid) in patients with chronic LBP and type 1 Modic changes. The authors explained that this antibiotic regimen was responsible for the changes in the MRI studies as well as the observed improvement. They suggested that their regimen could act on the low-grade *P. acnes* infection causing the low back pain as well as type 1 Modic changes in the adjacent vertebral endplates.³⁰ In a recent study by Aghazadeh et al,⁵⁹ it was demonstrated that there is a positive relation between *P. acnes* and Modic changes and presence of *P. acnes*.^{59,60} According to the results, it can be concluded that presence of *P. acnes* in the herniated disc tissue has a suspected relationship with clinical manifestations (such as inflammation, Modic changes, and LBP). The lowest MIC value was identified for amoxicillin, ciprofloxacin, erythromycin, rifampicin, tetracycline, and vancomycin. A moderate MIC value was found for fusidic acid; the highest MIC value was for gentamicin and trimethoprim.

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