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Is it possible to contaminate the temporomandibular joint by arthrocentesis?

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Abstract

Introduction: Bacterial contamination of the temporomandibular joint (TMJ) by needle penetration of the skin has previously been discussed as a contributing factor in joint arthritis. The purpose of this study was to investigate the presence of bacterial deoxyribonucleic acid (DNA) in synovial fluid, and to detect any possible iatrogenic contamination during arthrocentesis of the TMJ in patients with juvenile idiopathic arthritis and rheumatoid arthritis.
Method: Synovial fluid (SF) and skin swab samples (before and after disinfection) were collected from 54 TMJs in 30 patients with TMJ arthritis. Bacterial detection and classification was performed using 16S rRNA pyrosequencing.
Results: Bacterial DNA was detected in 31 joints (57%) in 19 patients (63%). In six 12 joints (20%) in six patients specific bacterial species were detected in both skin samples and in the TMJ, indicating a possible contamination. 22 different bacterial species were detected in synovial fluid from these six patients.
Conclusion: Bacterial DNA in TMJ SF with contamination was detected in 20% of the patients. Studies are needed to evaluate the consequences of potential contamination with bacterial DNA in SF with regard to arthrocentesis and treatment of TMJ arthritis.

Clinical relevance

Scientific rationale for the study

Invasive procedures such as injections, blood sampling, arthrocentesis and open surgery may hold the risk of contamination the underlying tissue. To prevent this, different disinfective procedures are used, such as chlorhexidine and ethanol, on instruments and on the skin.

Principal findings

In this study, we demonstrate the possible contamination with bacterial DNA from skin into the TMJ in six patients of a total of 30 patients.

Practical implications

During injections in the TMJ, one might contaminate the joint and disinfection techniques are very important before joint injections.

Introduction

Synovial fluid (SF) contains hyaluronic acid and interstitial fluid filtered from blood plasma and was previously believed to be aseptic. Fourteen different bacterial species were detected in the temporomandibular joint (TMJ) SF in a previously published study in children with juvenile idiopathic arthritis and adults with rheumatoid arthritis[1]. Studies on large joints, have detected *Salmonella* spp., *Shigella*

spp., *Mycobacterium tuberculosis* and *Campylobacter* spp. in patients with juvenile onset spondylo-arthropathologies[2]. Other studies have detected *Borrelia burgdorferi*[3] and *Yersinia* spp.[4] With regard to the TMJ, a small joint, studies have detected bacteria in SF in healthy TMJs, and TMJs with disc displacement and the non-healthy joints have a higher prevalence of bacterial presence and *Staphylococcus aureus,* in particular[5]. Other researchers have detected bacteria only in TMJ SF from temporomandibular disorder (TMD) patients, while the healthy joints have been found to be aseptic[6].

Table 1 Clinical data for patients with bacteria detection in synovial fluid

Joint	JIA	RA	Age (yr)	MIO (mm)	Lateral excursion	Pain VAS	Function VAS	Disease duration (yr)	TMJ duration (yr)
J15H	X		8	34	4	10	8	1	1
J15V	X		8	34	8	10	8	1	1
J16H	X		12	24	0	0	82	11	9
J16V	X		12	24	4	0	82	11	9
J18H	X		12	32	10	80	15	3	3
J18V	X		12	32	6	80	15	3	3
R8V		X	49	35	2	36	73	14	2.5
R8H		X	49	35	2	36	73	14	2.5
R9H		X	29	23	1	93	90	4	4
R9V		X	29	23	7	93	90	4	4
R10H		X	71	34	4	43	46	43	9
R10V		X	71	34	2	43	46	43	9

JIA, juvenile idiopathic arthritis; RA, rheumatoid arthritis; MIO, maximal interincisal opening; TMJ, temporomandibular joint; Lateral excursion is contralateral movement in mm; VAS, visual analogue scale.

Table 2 Bacteria in TMJ synovial fluid in patients with contamination during procedure. Data presented as % of detected bacteria PCR product. Abbreviations represents from left: Propionibacteria, Pseudomonas, Pseudomonas fluorescens, Porphyromonas, Streptococci, Alpha-proteobacteria, Gamma-proteobacteria, Staphylococci, Acidobacteria, Moraxellaceae, Veillonella, Rhiziobiales, Bacteroidales, Corynebacteria, Anaerococci, Actinomyces, Neisseriaceae, Sphingomonas, Treponema, Heamophilus, Methylobacteria, Planococcacaea and Unclassified sp.

	Propioni	Pseudom.	Pseudom F	Porphyrom	Streptoc	Alphapr	Gammapr	Staphyloc	Acidobact	Moraxella	Veillonella
J15R			14		40						
J15L	5		1		11			8			15
J15N			93								
J15D			99								
J16R											
J16L					2			9			
J16N			88								
J16D			99								
J18R			3	3	68						6
J18L			96								
J18N			92								
J18D			97								
R8R	86						8				
R8L	46	2				2	38				
R8N	81				1			1			
R8D	85				1			3			
R9R	56	9			7			5		4	
R9L	83	3		2	5						
R9N	29					12			7		
R9D		94									
R10R	45		27		2						
R10L	31		10		4			3			1
R10N	2	5			1						5
R10D	9	21			1						

R, right TMJ; L, left TMJ; N, skin without disinfection; D, skin disinfected.

The invasion of bacteria into the joint space has been reported to occur via direct spread from a contiguous site of infection[7,8] and by haematogenous spread[7]. The etiology of TMJ arthritis in Juvenile Idiopathic Arthritis (JIA) and Rheumatoid Arthritis (RA) is not fully understood and several studies have been performed to locate a factor that triggers inflammation. Theoretically, bacteria may adhere to the needle and, thus, may contaminate the TMJ during an arthrocentesis.

The purpose of this study was to detect any possible iatrogenic contamination during arthrocentesis of the TMJ as a treatment for arthritis in JIA and RA.

Materials and methods

Subjects

SF was collected from the TMJs of 30 patients (54 joints) with JIA (20 children) and RA (10 adults). None of the patients had any previous TMJ surgery. Six adults had previous TMJ injections, but not within 6 months prior to the SF collection.

The Regional Medical Ethical Committee, East, Norway (S06269a), approved this prospective clinical trial of arthrocentesis of the TMJ in patients with JIA and RA.

Sample collection

Samples were collected from the TMJ using a push and pull technique previously used by Alstergren *et al*[9]. Ultrasound guided sampling of the TMJ SF was performed after prior disinfection of the skin in a 3 cm area surrounding the penetration site, consisting of five washes with chlorhexidine-ethanol 5 mg/mL solution (Klorhexidinsprit 5 mg/mL, Fresenius Kabi, Norway) according to the recommendations of Oslo University Hospital, Norway. Skin swabs were taken from disinfected skin and non-disinfected skin in the puncture area prior to sampling, using a sterile Whatman® Omniswab™ (GE Healthcare, USA). All samples were immediately frozen at –80°C.

DNA isolation, PCR amplification and sequencing

Extraction of genomic DNA was performed using the Masterpure Complete DNA Purification Kit

Rhizobial	Bacteroid	Coryne	Anaerococ	Actinomyc	Neisseria	Sphingom	Treponem	Heamoph	Methylob	Planococc	Unclassifie
						9					37
8	1	1					20	1			29
											7
											1
									75	18	7
	59								11		19
											12
											1
											20
											4
											8
											3
											6
											12
		5	1								11
		3									8
											19
											7
											52
											6
		3									23
		11	1		1						38
		18		31							38
		57			10						2

(Epicentre Biotechnologies, Madison, WI, USA), based on the manufacturer's extraction protocol for fluid samples.

PCR amplification was performed using 16S rRNA gene primers flanking the V3 and V5 region, E334F 5′-CCAGACTCCTACGGGAGGCAGC-3′ and E939R 5′-CTTGTGCGGGCCCCCGTCAATTC-3′[10]. The PCR amplification steps included a denaturation step at 96°C for 2 min, amplification cycles (n = 32): 96°C 30 s, 61°C 40 s, 72°C for 30 s.

Amplicons were gel purified (GenElute, Sigma NA1111) and re-sequenced with fusion primers, Roche 454 FLX adaptor primers, 8-base barcode in front of E334F, reverse adaptor primer (B) fusion with E939, Ampure bead purification (Agencourt's AMPureXP beads, A63880) of fusion PCR products and quality check by Agilent Bioanalyzer high sensitivity chip (Agilent High Sensitivity DNA cat no: 5067-4627).

Amplicons were sequenced at the GS-FLX 454 sequencer at the Norwegian Sequencing Centre (NSC), Centre for Ecological and Evolutionary Synthesis (CEES), University of Oslo, Norway. Sequences (reads) were edited by using RDP pipeline[11] and Mothur[12]. Chimera uchime[13] and reference 16S rRNA (www.arb-silva.de) was used before trimmed fasta sequences were blasted by using the Bioportal at University of Oslo[14]. MEGAN (Center for Bioinformatics ZBIT, Tübingen University, Germany) was used to visualize the clustering and taxonomy of the samples.

Results

Clinical data for the six patients (12 TMJs) with bacterial contamination is presented in Table 1. Data for the twenty-four patients (42 TMJs) without contamination (*16S rRNA* gene fragments were still detected in 19 TMJs in 13 of these patients) have previously been published[1,15,16]. No significant differences were found when comparing clinical data with and without contamination. Patients with contaminated TMJs represented no specific group with regard to age, gender, pain or function.

Each bacterial species detected in the TMJ SF is presented in Table 2 as a proportion (%) of the total of bacteria detected. The most frequent bacterial species in the SF and in skin samples before and after disinfection were *Pseudomonas fluorescens* and Streptococci spp. in children and adolescents with JIA, while the most frequent bacteria in adults were *Propionibacteria* spp., *Pseudomonas* spp., Streptococci spp. and *Corynebateria* spp. In all, 22 different bacterial species were detected in the SF.

Discussion

The invasion of bacteria into the joint space has been reported to occur via direct spread from a contiguous site of infection[6,7] and by haematogenous spread[6]. Haematogenous spread is possible due to the highly vascular synovial membrane of the joint, which lacks a limiting basement membrane. A third possibility is contamination from skin into the joint during open TMJ surgery and during arthrocentesis and injections. This study demonstrates the possibility of contaminating the temporomandibular joint with bacterial DNA by arthrocentesis, despite a thorough disinfection procedure of the overlying skin using Chlorhexidine ethanol.

In all, 22 different bacterial species were detected in the SF. This represents a higher bacterial diversity than previously reported in a comparable group of patients without contamination[1]. Chlorhexidine gluconate kills a range of Gram-positive and Gram-negative bacteria, viruses and fungi, and binds to the top layer of the skin, which results in persistent activity[17,18]. DNA fragments may still be available for PCR detection, and bacterial resistance to Chlorhexidine must also be considered.

Alcohol kills a range of Gram-positive and Gram-negative bacteria and many viruses and fungi. It kills more quickly than chlorhexidine gluconate or povidone iodine, but has little residual effect[17].

In some comparisons of the two antiseptics (chlorhexidine gluconate and iodophors) when used as preoperative hand scrubs and for disinfection of a surgical field, chlorhexidine gluconate achieved greater reductions in skin microflora than did povidone-iodine and also had greater residual activity after a single application[19].

A concern with the push-pull technique[1,9] is that some washing solution persists in the joint and may cause transient symptoms and functional problems. This washing solution may be contaminated, which could lead to the detection of bacterial DNA in the SF. Samples of the actual washing solution were tested with the same PCR-technique and no bacterial DNA was detected.

Previous studies have indicated bacterial DNA and bacterial cell wall structures as a possible initiating or contributing factor to joint disease[8,20], and an increase in TNF-α and IL-6 has previously been associated with the presence of *Chlamydia trachomatis* in tissue samples from the TMJ[21]. The release of pro-inflammatory cytokines can be stimulated in joints by bacterial cell wall peptidoglycan polysaccharide, as has been demonstrated in an animal model[7].

Specific bacterial DNA may also stimulate the production of possibly specific cytokines and bone markers.

Bacteria may stimulate pain, possibly as a protective mechanism. In a study by Chiu *et al.*[22], bacteria were shown to modify the immune response by directly stimulating nociceptors. The role of bacterial DNA in the development of joint arthritis remains uncertain, and bacterial DNA in the joints may induce symptoms and may promote the development of joint pathology. To avoid this, one should always consider the possibility of contamination and take the necessary precautions to assure a disinfected entry needle site.

One might speculate that contamination of the TMJ during arthrocentesis or other types of injection may cause arthritis by itself or worsen an already existing arthritis.

Possibly, the use of a laser may aid in removing bacterial DNA from the penetrating site of the skin but both new disinfection techniques and further studies are needed.

In conclusion, bacteria from the skin may contaminate the joints during arthrocentesis. In our study, bacterial DNA was detected in the SF of the TMJ in 20% of the patients due to contamination. Studies are needed to evaluate the consequences of this bacterial DNA in SF with regard to TMJ arthritis.

Conflict of interest

The authors confirm that there are no conflicts of interest.

Ethical approval

The Regional Medical Ethics Committee East, Norway (S06269a, 1.2006.823) approved this study.

References

1. Olsen-Bergem H, Kristoffersen AK, Bjørnland T, Reseland JE, Aas JA. Juvenile idiopathic arthritis and rheumatoid arthritis: bacterial diversity in temporomandibular joint synovial fluid in comparison with immunological and clinical findings. Int J Oral Maxillofac Surg 2016;45:318–22.
2. Pacheco-Tena C, Alvarado de la Barrera C, Lopez-Vidal Y, Vasquez-Mellado J, Richaud-Patin Y, Amieva RI *et al.* Bacterial DNA in synovial fluid cells of patients with juvenile onset spondyloarthropathies. Rheumatol 2001;40:920–7.
3. Nocton JJ, Dressler F, Rutledge BJ, Rys PN, Persing DH, Steere AC. Detection of *Borrelia burgdorferi* DNA by polymerase chain reaction in synovial fluid from patients with Lyme arthritis. N Eng J Med 1994;330:229–34.
4. Wilkinson NZ, Kingsley GH, Jones HW, Sieper J, Braun J, Ward ME. The detection of DNA from a range of bacterial species in the joints of patients with a variety of arthritides using a nested, broad-range polymerase chain reaction. Rheumatol 1999;38:260–6.
5. Kim SJ, Park YH, Hong SP, Cho BO, Park JW, Kim SG. The presence of bacteria in the synovial fluid of the temporomandibular joint and clinical significance: preliminary study. J Oral Maxillofac Surg 2003;61:1156–61.
6. Sun W, Segami N. Bacterial diversity in synovial fluids of patients with TMD determined by cloning and sequencing analysis of the 16S ribosomal RNA gene. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;105:566–71.
7. Fuseler JW, Conner EM, Davis JM, Wolf RE, Grisham MB. Cytokine and nitric oxide production in the acute phase of bacterial cell wall-induced arthritis. Inflam 1997;21:113–31.
8. Zhang X, Rimpilainen E, Simelyte E, Toivanen P. What determines arthritogenicity of bacterial cell wall? A study on Eubacterium cell wall-induced arthritis. Rheumatol 2000;39:274–82.
9. Alstergren P, Kopp S, Theodorsson E. Synovial fluid sampling from the temporomandibular joint: sample quality criteria and levels of interleukin-1 beta and serotonin. Acta Odontol Scand 1999;57:16–22.
10. Baker GC, Smith JJ, Cowan DA. Review and re-analysis of domain-specific 16S primers. J Microbiol Met 2003;55:541–5.
11. Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ *et al.* The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res 2009;37:141–5.
12. Schloss PD, Gevers D, Westcott SL. Reducing the Effects of PCR Amplification and Sequencing Artifacts on 16S rRNA-Based Studies. PLoSONE 2011;6:e27310.
13. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 2011;27:2194–200.
14. Kumar S, Carlsen T, Mevik BH, Enger P, Blaalid R, Shalchian-Tabrizi K *et al.* CLOTU: an online pipeline for processing and clustering of 454 amplicon reads into OTUs followed by taxonomic annotation. BMC Bioinform 2011;20:182.
15. Olsen-Bergem H, Bjørnland T. A cohort study of patients with juvenile idiopathic arthritis and arthritis of the temporomandibular joint: outcome of arthrocentesis with and without the use of steroids. Int J Oral Maxillofac Surg 2014;43:990–5.

16. Olsen-Bergem H, Bjørnland T, Reseland JE. Temporomandibular joint pain is negatively correlated to TNF alpha and osteoprotegrin content in synovial fluid in patients with juvenile idiopathic arthritis. Endocrinol Metab Synd 2015;3:145.
17. Tanner J. Patient skin preparation. In: Hughes SJ, Mardell A, editors: Oxford Handbook of Peri Operative Practice. Oxford Press: New York, 2009.
18. Mangram AJ, Horan TC, Pearson ML, Silver LC, Jarvis WR. Guideline for prevention of surgical site infection. Infect Control Hosp Epidemiol 1999;20:247–78.
19. Darouiche RO, Wall MJ, Itani KMF, Otterson MF, Webb AL, Carrick MM *et al.* Chlorhexidine-alcohol versus povidone-iodine for surgical-site antisepsis. N Engl J Med 2010;362:18–26.
20. Deng GM, Tarkowski A. The features of arthritides induced by CpG motifs in bacterial DNA. Arthritis Rheum 2000;43:356–64.
21. Henry CH, Whittum-Hudson JA, Tull GT, Wolford LM. Reactive arthritis and internal derangement of the temporomandibular joint. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;104:e22–6.
22. Chiu IM, Heesters BA, Ghasemlou N, von Hehn CA, Zhao F, Tran J *et al.* Bacteria activate sensory neurons that modulate pain and inflammation. Nature 2013;501:52–7.