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ORIGINAL ARTICLE

Bacterial community of chronic rhinosinusitis patients and therapeutic ultrasound efficacy: clinical trial study

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ABSTRACT

Background and Objectives: Bacterial involvement in chronic rhinosinusitis (CRS) condition made it difficult to treat using available antibiotic therapy. Therapeutic ultrasound was investigated here to evaluate bacterial diversity and quantity before and after continuous/pulsed ultrasound strategy compared to control patients.

Materials and Methods: Totally, 34 CRS patients were studied in three groups, including continuous ultrasound, pulsed ultrasound and control. Bacterial culture and identification were done before and after treatment. Computed tomography scan (CT scan) and questionnaire scores were recorded two times before and after intervention.

Results: The most prevalent bacterial isolates were non-hemolytic *Streptococci* (34 patients), coagulase-negative *Staphylococcus* (33 patients), Gram-negative cocci (26 patients), *Staphylococcus aureus* (19 patients), *Streptococcus pneumoniae* (five patients) and *Streptococcus pyogenes* (five patients). Both continuous and pulsed ultrasound could significantly reduce the quantity of bacterial isolates after treatment. CT scan and questionnaire results support the effectiveness of therapeutic ultrasound.

Conclusion: The quantity of clinically important bacteria was significantly reduced using ultrasound treatment and recovery of patients was supported by CT scan and questionnaire scores. Alternative therapeutic ultrasound could be an effective procedure in CRS patients.

Keywords: Rhinosinusitis; Ultrasound therapy; Bacterial infection; Treatment; Computed tomography scan

INTRODUCTION

Chronic rhinosinusitis (CRS) is defined as a complex inflammatory condition of paranasal sinuses and sinonasal mucosa lasting at least 3 months (1) which lead to swelling of the sinonasal mucosa and impairment of normal sinus drainage (2). Development of CRS is multifactorial, including exposure

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to allergens, defects in mucociliary function, immunodeficiency, obstruction of sinus outflow and infections with bacteria, viruses and sometimes fungi (2). Management of CRS is focused on reducing mucosal inflammation, restoring sinus drainage and controlling infections (3).

Infectious rhinosinusitis is the vague face of CRS that is not completely understood and can be result of dysbiosis of bacterial species making up the sinonasal microbiome. Uncultivable microorganisms are likely part of the resident microbiome and may help maintain a healthy sinus mucosa (4). However, some aerobic and anaerobic bacteria can be obtained from those suffering with CRS. The most abundant bacterial pathogens isolated from CRS patients include *Staphylococci aureus*, coagulase-negative staphylococci, *Streptococcus pneumoniae, Haemophilus influenzae* and *Pseudomonas aeruginosa* (5). Inhibition of infectious CRS development is the goal of antibiotic therapy. Nevertheless, infections due to the drug-resistant pathogens including *Pseudomonas* or methicillin-resistant *S. aureus* (MRSA) are notoriously difficult to treat which result in chronic inflammation (6). Surgery may be used to manage such difficult-to-treat CRS patients and newly less invasive therapeutic options are needed to develop for the management of CRS (4).

While ultrasound (US) was as one of the anti-biofilm interventions in industry (7), therapeutic US showed anti-biofilm and anti-inflammatory actions clinically (8). Increasing evidences indicate that therapeutic US presented beneficial effects in CRS management and also in antibiotic effectiveness by increasing cell membrane permeability and hence antibiotic delivery to bacteria (9). The aim of this study was to evaluate the efficacy of therapeutic US as a treatment for CRS patients and changes in the bacterial community of the sinonasal cavities before and after treatment.

MATERIALS AND METHODS

Study design, specimen collection and therapeutic ultrasound. This study was conducted on 34 chronic rhinosinusitis patients during at Faculty of Rehabilitation, Iran University of Medical Sciences, Tehran, Iran from January to September 2018. Patients were included based on the presence of at least two or more of the following main signs and symptoms; facial pain/pressure, hyposmia/anosmia, nasal drainage, and nasal obstruction and inflammation was documented using purulent mucus or edema in the middle meatus or anterior ethmoid region, polyps in nasal cavity or the middle meatus, and/or radiographic imaging showing inflammation of the paranasal sinuses (10). Specimen collection was done using dacron swabs in a sterile tube containing 1 mL saline solution from the nasopharynx, right and left meatus of each patient. Patients were divided into three groups randomly; Continuous ultrasound (12 patients), pulsed ultrasound (12 patients) and control patients (10 patients) without any intervention. Specimen collection, CT scan and filling the SNOT-20 questionnaire were performed before and after US treatment. The SNOT-20 questionnaire includes 20 common symptoms such as need to blow nose, sneezing, runny nose, cough, post nasal discharge, etc., and all patients rate these problems by marking 0 to 5 for 6 already provided answers including, no problem, very mild problem, mild or slight problem, moderate problem, severe problem and problems as bad as it can be. The Lund Mackay CT score (LMCTS) was the method used for radiologic staging of chronic rhinosinusitis (11). US treatment was done 10 times in a three-week period with 4 cm/s of the probe velocity movement, frequency of 1 MHz and intensity of 1 W/cm^2 (maxillary sinuses) and 0.5 W/cm^2 (frontal sinuses). The duty cycle of pulsed ultrasound was 10%. This study was approved by the Ethics Committee of Iran University of Medical sciences (IRCT20140810018754N8) and the informed consent form were signed by all participants.

Bacterial culture and quantification. All samples were cultured immediately after collection. Briefly, 10-fold serial dilutions (10^1 to 10^{-6} concentration) of each sample were prepared and four series of sheep blood, chocolate and MacConkey agar plates were inoculated with 10 µL of prepared concentrations. Inoculated plates from both sample groups collected before and after treatment were incubated in aerobic and anaerobic condition at 37°C for 24-48 hours. Species identification of positive cultures was done according to the biochemical test (12) and colony forming unit (CFU)/mL of samples were accounted using agar plate method (13). The CFU/mL changes were obtained by dividing CFU/mL of after treatment by CFU/mL of before treatment.

Data analysis. To analyze the bacterial CFU/mL results, CT scores and questionnaire values before and after the treatment, Wilcoxon test was applied using SPSS software version 26. A p value of < 0.05 was considered as statistically significant.

RESULTS

Bacterial isolates. Regardless of the specimen site, non-hemolytic *Streptococci* was isolated from all 34 chronic rhinosinusitis patients and the most prevalent pathogenic/non-pathogenic bacterial isolates in patients was as following; coagulase-negative staphylococci (33 patients), Gram-negative cocci (26 patients), *S. aureus* (19 patients), *S. pneumoniae* (five patients), *Streptococcus pyogenes* (five patients), *Corynebacterium* spp. (three patients), *Klebsiella pneumoniae* (one patient), *Escherichia coli* (one patient) and non-pathogenic *Neisseria* (one patient). The highest diversity of isolates was found in the nasopharynx, while bacterial isolates in the right and left meatus were restricted to coagulase-negative staphylococci, *S. aureus*, non-hemolytic streptococci and *S. pneumoniae.*

Continuous ultrasound. Continuous ultrasound significantly reduced the CFU/mL of bacterial isolates after treatment except for two patients (Table 1). *S. aureus* as one of the common pathogens identified in chronic rhinosinusitis patients were detected in five out of 10 patients after treatment those had *S. aureus* before treatment. In addition, 4/7 patients and 0/3 patients were positive for Gram-negative cocci and *S. pyogenes* after treatment respectively. One patient with coagulase-negative staphylococci and *S. aureus* and a patient with non-hemolytic streptococci and *S. aureus* in right meatus were completely negative after treating with continuous ultrasound. In addition, ultrasound treatment result in negative culture results in a patient having coagulase-negative staphylococci in both right and left meatus (Table 1). In contrast to the control patients, the mean value of CFU/mL changes (0.2) at the end of continuous ultrasound treatment procedure was significant (p < 0.05).

Pulsed ultrasound. After pulsed ultrasound treatment, 1/2, 2/4, 0/1 and 0/1 patients were positive for *S. pyogenes*, *S. pneumoniae*, *K. pneumoniae* and *E. coli* respectively. All patients with *S. aureus*, coagulase-negative staphylococci, non-hemolytic streptococci and Gram-negative cocci were positive after pulsed ultrasound with reduced CFU/mL. There were no detectable isolates in right meatus of 3 patients after treatment which already had *S. pneumoniae* (one patient) and coagulase-negative staphylococci (two patients) and a patient with coagulase-negative staphylococci in left meatus was culture negative after pulsed ultrasound intervention. As well, CFU/mL differences before and after the treatment was significantly reduced and this difference was significant when pulsed ultrasound patients compared to the control patients (p < 0.05) (Table 1).

CT scan, questionnaire scores and correlation with bacterial quantity. CT scan and questionnaire scores of patients during 3-weekss follow-up were summarized in Table 1. Based on the Lund Mackay method, CT scan findings scored from 0 to 24. More than 2-fold changes in bacterial quantity were defined as significant increase or reduction. Thus, the values 0.0 to 0.5 means significant reduction, 0.5-1.99 means no significant changes and >2.0 shows a significant increase. The values of the CT scan and questionnaire form were significantly correlated with culture results under continuous/pulsed ultrasound treatment.

DISCUSSION

There are common bacterial phyla existing in chronic rhinosinusitis patients demonstrated previously (14). Furthermore, the total bacterial composition in CRS patients is similar to the controls and sinuses of healthy controls without CRS are also colonized with different bacterial species (15, 16). However, role of the microorganism's interaction in CRS patients remained unclear and involvement of bacteria in recalcitrant CRS disease made challenges in treatment procedures (17, 18). A culture based bacterial diversity of CRS patients and treatment efficacy of therapeutic ultrasound were assessed in this study.

As previously reported, different aerobic and anaerobic bacterial species are involved in the CRS condition (2, 19). The prevalence of Gram-positive bacteria was found to be higher than Gram-negative isolates in this study and more than 50% abundance of Gram-positive bacteria were reported by Roumbax et al. from CRS patients (20). Inconsistent to previous investigations, we found some clinically important bacterial species, including *S. aureus*, *S.*

Table 1. CFU/mL changes, CT scan and questionnaire scores of chronic rhinosinusitis patients under ultrasound treatment and CFU/mL changes of control group.

Patients	Specimen site	Continuous ultrasound treatment					Pulsed ultrasound treatment					Control
		CFU/mL changes	CT scores		Questionnaire scores		CFU/mL changes	CT scores		Questionnaire scores		CFU/mL changes
			Before	After	Before	After		Before	After	Before	After	
1	Nasopharynx	0.05	17	17	40	5	0.3	17	0	38	8	1.3
	Right meatus	0.0					0.01					0.8
	Left meatus	0.01					0.01					1.0
2	Nasopharynx	0.01	6	6	36	25	0.8	15	5	48	25	0.7
	Right meatus	0.0					0.0					0.9
	Left meatus	0.0					0.01					1.2
3	Nasopharynx	0.03	6	6	43	23	0.01	7	0	24	1	1.1
	Right meatus	0.09					0.0					1.0
	Left meatus	0.6					0.04					1.4
4	Nasopharynx	2.5	13	13	25	5	0.02	27	4	36	9	2.0
	Right meatus	2.0					0.01					1.3
	Left meatus	0.01					0.5					0.9
5	Nasopharynx	5.0	20	12	34	17	0.05	6	1	17	2	1.6
	Right meatus	10.0					0.02					1.3
	Left meatus	5.0					0.01					1.0
6	Nasopharynx	0.5	18	0	36	6	0.06	18	4	17	1	0.8
	Right meatus	0.3					0.03					1.2
	Left meatus	0.1					0.0					0.9
7	Nasopharynx	0.01	25	0	49	9	1.7	24	6	40	25	0.9
	Right meatus	0.1					0.8					0.9
	Left meatus	0.1					0.1					1.0
8	Nasopharynx	0.1	8	0	76	18	.06	33	11	85	25	1.7
	Right meatus	0.01					0.02					1.0
	Left meatus	0.05					0.01					1.1
9	Nasopharynx	0.7	36	0	74	48	0.1	11	2	45	15	0.9
	Right meatus	0.2					0.0					2.2
	Left meatus	0.04					0.02					1.4
10	Nasopharynx	0.1	29	0	43	7	0.8	6	0	64	1	0.9
	Right meatus	0.01					0.02					0.8
	Left meatus	1.0					0.01					1.0
11	Nasopharynx	0.01	30	1	22	5	0.2	17	2	48	6	-
	Right meatus	0.08					0.6					-
	Left meatus	0.05					0.07					-
12	Nasopharynx	0.03	25	0	48	5	0.6	24	0	54	0	-
	Right meatus	0.0					0.4					-
	Left meatus	0.2					0.1					-

CT scan; computerized tomography scan, CFU; colony forming unit.

pneumoniae, *S. pyogenes*, *K. pneumoniae* and *E. coli* in our CRS patients. Recent investigations reported that *S. aureus*, Coagulase Negative staphylococci (CNS), *Streptococcus* species, *Propionibacterium* species, *Corynebacterium* species, *Haemophilus* species, *Klebsiella* species, and *P. aeruginosa* were the most common isolated bacteria in CRS patients (21, 22). Although the role of some clinically relevant anaerobic bacterial genera such as genera *Fusobacterium, Peptostreptococcus, Propionibacterium,* and *Prevotella* was demonstrated in CRS patients (19), we did not identify anaerobic isolates using traditional culture technique. According to the notable amount of unculturable microbiota in sinuses and the diversity range of common and uncommon bacterial species in CRS, culture-independent techniques could result in better characterization of bacterial diversity (16).

The bacterial quantity of CRS patients was significantly reduced after both continuous and pulsed ultrasound treatment based on the CFU/mL results. However, the bacterial quantity of some patients in some sampling sites was increased after treatment that may be due to bacterial secondary acquisition during follow-up period or contamination. Meanwhile, there was no significant reduction or increase in the bacterial count before and after the treatment in control patients. CT scan and questionnaire scores before and after the treatment, support this finding and was correlated with the bacterial culture results. As reported in the literature, therapeutic ultrasound showed an effective treatment for both acute rhinosinusitis and CRS (9, 23). The therapeutic effectiveness of ultrasound was demonstrated in clinical studies for both continuous and pulsed strategies and this effect is applied through the following ways; blocking bacterial molecular communication, inhibiting biofilm matrix production and breaking down bacterial biofilms (8). Based on the statistical analysis, the CFU/mL changes among the control patients were not significant ($p > 0.05$). However, the mean values of CFU/mL changes of patients before and after treatment with pulsed ultrasound were lower than with continuous ultrasound group. Significant reduction of bacterial quantity after treatment with pulsed ultrasound was in favor of better efficacy of this procedure in CRS patient's treatment compared to the continuous procedure. Nakhostin-Ansari A et al. (24) was previously reported that therapeutic pulsed ultrasound had a significant effect in relieving the symptoms of CRS by having ability to disintegrate the strong walls of bacterial biofilms (community of bacteria). In addition, we previously demonstrated a significant reduction of *S. aureus* population under continuous/pulsed ultrasound treatment using qPCR technique (25, 26). By the way, analysis of the CRS microbiology using high throughput or molecular techniques are needed to cover our restrictions to better evaluate the bacterial diversity and alterations of clinically relevant bacteria during treatment. As previously demonstrated, despite ultrasound showing positive effects in the treatment of chronic rhinosinusitis, large randomized controlled studies are needed to appoint this method as a potential adjunct resource for treating chronic rhinosinusitis (23).

In conclusion, the prevalence of Gram-positive bacteria like nonhemolytic streptococci, coagulase-negative staphylococci, *S. aureus, S. pneumoniae* was higher than Gram-negative isolates including Gram-negative cocci, *E. coli* and *K. pneumoniae*. The diversity of the bacterial isolates was almost reduced after treatment and CFU/mL changes of bacterial isolates were significantly using continuous and pulsed ultrasound intervention. Pulsed ultrasound showed slightly better efficacy than continuous strategy.

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