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Revealing the Absence of Microbiota in Hypertrophic Mucosa of Inferior Turbinate

Rangga Putra Nugraha[1*], Asti Widuri[2], Luh Putu Lusy Indrawati[3], Budi Yuli Setianto[4], Mahyarudin Mahyarudin[5], Mardhia Mardhia[5], Delima Fajar Liana[5]

[1]Doctoral Program in Faculty of Medicine, Public Health and Nursing Universitas Gadjah Mada (UGM) Yogyakarta, Department Microbiology Faculty of Medicine, Universitas Tanjungpura, Pontianak, Indonesia

[2]Doctoral Program in Faculty of Medicine, Public Health and Nursing Universitas Gadjah Mada (UGM) Yogyakarta, Otorhinolaryngology Department Faculty of Medicine and Health Science Universitas Muhammadiyah Yogyakarta (UMY), Yogyakarta, Indonesia

[3]Department of Ear Nose and Throat, Faculty of Medicine Public Health and Nursing Universitas Gadjah Mada (UGM), Yogyakarta, Indonesia

[4]Department Cardiology and Vascular Medicine, Faculty of Medicine Public Health and Nursing Universitas Gadjah Mada (UGM), Yogyakarta, Indonesia

[5]Department Microbiology Faculty of Medicine, Universitas Tanjungpura, Pontianak, Indonesia

Abstract

Background: Nasal congestion is the most common symptom presented in daily clinical practice, and inferior turbinate hypertrophy (ITH) is the most frequent cause. However, histopathological analysis of turbinate hypertrophy remains unclear. Therefore, this study aimed to assess the presence of microbiota in the mucosa of ITH, to clarify the role in nonspecific immune responses.

Methods and Results: This prospective and cross-sectional study evaluated microbiota in hypertrophic inferior turbinate mucosa. A total of 42 patients and specimens of ITH were enrolled during the endoscopic submucosal rhinoplasty. Intraoperative specimens were collected and inserted into DNA/RNA protectors.The specimens were subjected to DNA extraction, which was followed by PCR electrophoresis. The results showed that metagenomic examination was needed to assess microbiota diversity in tissue, including electrophoresis and sequencing of PCR-amplified DNA such as 16S rRNA in the inferior concha mucosa and the preservation fluid. In this examination, no bands were found at the 1500 bp marker, showing that no bacterial 16S gene fragments were present in either tissue specimen or preservation fluid.

Conclusion: Microbiota were found only in mucus or the nasal mucosa lining and not in the tissue. The results further emphasized the position and role of microbiota as a physiological nonspecific immune response, providing a basis for further studies in learning about microbiota of the respiratory tract, specifically the nasal cavity. (**International Journal of Biomedicine. 2025;15(1):95-100.**)

Keywords: microbiota • inferior turbinate hypertrophy • nonspesific immune response • sinusitis

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Introduction

Nasal congestion is the most common symptom presented in daily clinical practice, and inferior turbinate hypertrophy (ITH) is the most frequent cause, accounting for 72% of the total cases.[1] Inferior turbinate hypertrophy generally occurs independently or in response to septal deformity, known as compensatory hypertrophy. Other causes of ITH are allergic rhinitis, vasomotor rhinitis, and chronic hypertrophic rhinitis,[2] with chronic inflammation being the underlying condition.

The nonspecific immune system plays an important role in the front line of immune response, specifically in the inflammatory process. In this context, the human body is inhabited by a complex microbial community called microbiota, which plays an important role in nonspecific immune response. According to studies by Von Mutius,[3] disturbances or loss of microbiota, called dysbiosis, are correlated with asthma and atopic dermatitis.

Abnormalities in microbiota and subsequent effects on the immune system have attracted significant attention. Other studies have shown that the commensal microbiota regulates susceptibility to allergic diseases. In contrast, the absence of commensal bacteria increases basophil proliferation, elevates the total number of infiltrating lymphocytes and eosinophils, worsens Th2 cell responses and allergic inflammation, as well as reduces the number of RORγt+ regulatory T cells (Tregs) and Th17 cells.[4] Several molecular studies on the nasal microbiota have been published over the past few years. Still, there is no consensus regarding the sampling location, analysis methods, and interpretation, leading to inconsistent results.

Methods

Study population

The subjects were patients with ITH subjected to turbinoplasty surgery from June 2023 to August 2024. During this period, 42 subjects who met the inclusion and exclusion criteria were recruited. Inclusion criteria included nasal obstruction, age between 10 and 60 years, and grades 2 and 3 ITH on nasal endoscopic examination. Exclusion criteria included all patients who had undergone previous nasal surgery and had polyps, nasal infections, septal deviation, and tumors. It was assumed that all cases had a uniform condition of ITH, namely those who had received antihistamine therapy, oral or topical corticosteroids, and decongestants but did not improve.

Specimen Collection and Tissue Preparation

Submucosal resection of turbinoplasty was selected because it is a safe and reliable method having high efficacy and minimal complications with precise excision of hypertrophic stromal tissue in the submucosa that can maintain physiological function in the nose. The specimen was then placed in a tube filled with DNA/RNA protector. This tube was stored in the Microbiology Laboratory of Untan Hospital at -80 C°. The specimens were subjected to a DNA extraction process to be continued in the PCR electrophoresis process.

Protocol PCR 16S

A total of 50 ng in 15uL of DNA template mass was added to 25 uL of LongAmp Hot Start Taq 2X Master Mix and 10uL of 16S Barcode containing 16S primers (27F and 1492R). The 16S barcode was included in the 16S Barcoding Kit 24 V14 (SQK-16S114.24) component. The reaction was then amplified using the following modified PCR protocol:

Cycle Step	Temperature	Time	No. Of cycles
Initial denaturation	95	1 min	1
Denaturation	95	20 sec	35
Annealing	55	30 sec	35
Extension	65	2 min	35
Final extension	65	5 min	1
Hold	4	~10 min	

Variables

Demographic variables included gender, age, and allergic rhinitis (specific IgE and total IgE). In this study, allergic rhinitis was determined based on a history of allergy, positive house dust mite, or total serum IgE levels >87IU/mL.

Statistical Analysis

Baseline characteristics were summarized as frequencies and percentages for categorical variables and mean±standard deviation (SD) for continuous variables.

Results

This study obtained 42 patients and specimens of ITH. Among these patients, 21(50%) were male. The age ranged from 11 to 59 years, with a mean of 27.4±11.25 years (Table 1).

Table 1.

Demographic data.

	ITH
Age, years	
Mmean±SD	27.4±11.25
Minimal	11
Maximal	59
Gender	
Male	21 (50%)
Female	21 (50%)
Allergic Rhinitis	
Specific IgE	8 of 28 cases (28.6%)
Total IgE	11 of 14 cases (78.6%)

PCR and Electrophoresis

Specimens were taken from the results of turbinoplasty surgery on the inferior concha, then placed in a tube filled with DNA/RNA shield. This tube was stored in the Microbiology Laboratory of Untan Hospital at -80 C°. Subsequently, the specimens were subjected to a DNA extraction process, then continued with PCR electrophoresis and 16S sequencing to search for microbiota. The DNA extraction process results are shown in Table 2.

The amount of DNAextracted from the existing specimens was large, showing that the extraction process ran properly. However, to determine whether the results belong to humans or other living creatures, PCR electrophoresis procedures must be carried out before 16S sequencing.

Figure 1 shows the results of PCR electrophoresis on tissue specimens. To assess microbiota diversity in tissue, a metagenomic examination is needed, including electrophoresis and sequencing of PCR-amplified DNA such as 16S rRNA. In this examination, no bands were found at the 1500 bp marker, suggesting that no bacteria 16S rRNA gene fragments were present in the tissue specimen. Tissue and preservative fluid specimen concentrations were compared to find the presence of microbiota. In the DNA extraction process, five preservative fluid specimens obtained results as shown in Table 3.

Figure 2 shows that there is no band at 1500bp, indicating neither the tissue nor the preservation fluid contained bacteria or microbiota. Based on these results, this study did not perform sequencing to map microbiota.

Table 2.

DNA extraction results.

Sample	Total DNA concentration (ng)	Sample	Total DNA concentration (ng)	Sample	Total DNA concentration (ng)
1	3868.8	15	5300	29	5100
2	3630	16	5800	30	4210
3	4020	17	2790	31	5100
4	4670	18	5800	32	3850
5	4990	19	2120	33	5000
6	4060	20	2800	34	5000
7	4230	21	5600	35	4630
8	4570	22	5500	36	4430
9	2650	23	3910	37	2770
10	4310.4	24	3993.6	38	2260
11	5000	25	3657.6	39	4080
12	4860	26	4740	40	2750
13	4630	27	3610	41	4760
14	4406.4	28	3510	42	2450

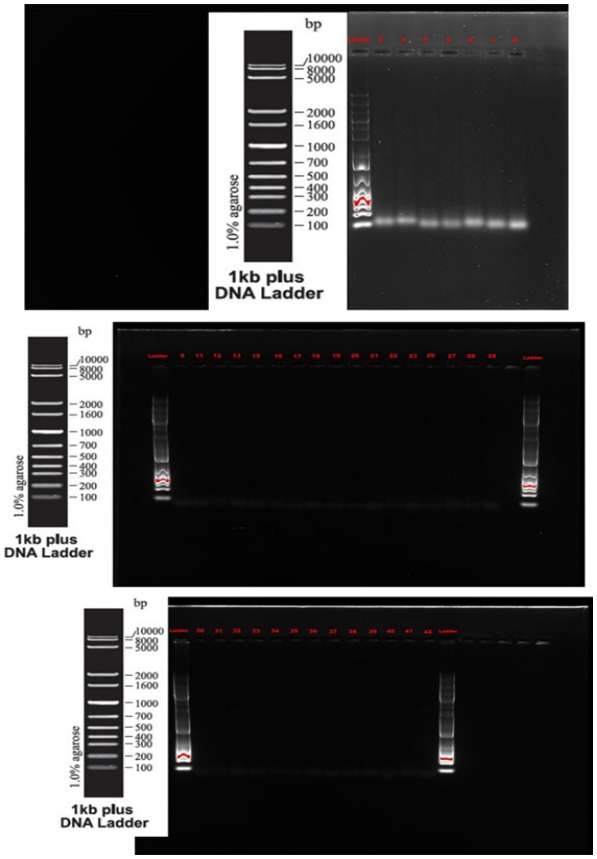

Fig. 1. *PCR electrophoresis of tissue specimens from ITH mucosa.*

The extraction results of the preservative fluid showed a fairly large DNA concentration, but similar results were obtained when the PCR electrophoresis process was carried out. In other words, no band was obtained at 1500 bp, showing no bacteria in the preservative fluid. The specimen obtained in the extraction process, both in tissue and preservative fluids, was suspected to be human DNA.

Table 3.

DNA extraction of preservation fluid specimens

Preservation fluid specimen	Total DNA concentration (ng)
1	1382.4
10	308.16
14	165.12
24	256.32
25	1027.2

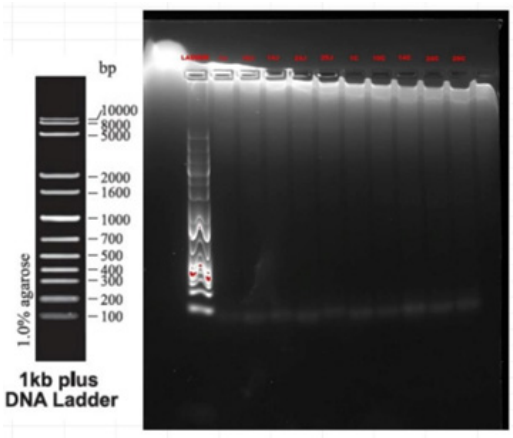

Fig. 2. *PCR electrophoresis on five specimens of ITH mucosal tissue and preservation fluid.*

The results are very different from previous studies on microbiota in cases of chronic rhinosinusitis (CRS), with or without polyps, and allergic rhinitis, and in healthy people. The storage and extraction procedures were in accordance with existing operational standards, and the success of DNA extraction was confirmed. The only difference was in the sampling process, as specimens were taken using the turbinoplasty technique in the operating room, whereas in previous studies, the specimens were taken using a biopsy or swab.

Discussion

The human nasal passage is a major habitat for human pathogens. Other studies have shown that the microbial community plays a key role in pathogen resistance and immunological responses. Viral and bacterial infections and immunological imbalance can change the nasal microbial community.[5]

Variation between studies is largely influenced by the use of different 16S rRNA gene regions to characterize the bacterial microbiota as well as known and unknown biases caused by differences in sampling location, handling, DNA extraction, and sequencing processes.[5] Furthermore, there

were statistically significant differences in the composition of the nasal microbiota, with Proteobacteria (*Moraxella, Haemophilus,* and *Neisseria*) and Firmicutes (*Streptococcus, Dolosigranulum, Gemella, and Granulicatella*) being more abundant in pubertal children, while Actinobacteria (C*orynebacterium, Propionibacterium,* and *Turicella*) were more abundant in adults.[6]

A study by Biswas et al.[7] on microbiota in patients with chronic rhinosinusitis (CRS) showed that more variation in bacteria composition was due to individual differences rather than sampling location or even disease status. Bacteria community diversity was significantly lower in CRS specimens than in those from healthy subjects.

Corynebacterium, *Propionibacterium*, *Staphylococcus*, *Streptococcus*, and an unclassified *Actinobacteria* lineage were the most common bacteria in patients with CRS and healthy individuals, according to the results of the meta-analysis. The bacteria community associated with CRS patients was dysbiotic, with an increased distribution, significantly lower bacterial diversity, and increased abundance of the genus Corynebacterium associated with CRS. Burkholderia and propionibacterium are thought to have potential as gatekeepers, and their presence is important in maintaining the stability of the sinonasal bacterial community.[8]

Patient populations (antibiotic history and ethnicity), environmental factors, genetics methodology or simply natural variation among different nasal passages and sinuses are factors that influence the variation of microbiota and are still poorly understood. Published studies on microbiota are presented in Table 4.

The absence of microbiota in this study was presumably because the specimens were collected from the concha mucosa, not from the mucus lines. Based on the results, microbiota are only found in the mucus that lines the nasal mucosa and not in

Table 4.
Published studies on microbiome

Study	Sampling	Location	Case	Microbiota
Abreu et al.,2012 [9]	Swab during surgery	Sinus maxilla	CRS	Corynebacterium tuberculistearicum
Feazel et al., 2012[10]	ESS-swab operation	Meatus medial	CRS	Coagulase-negative staphylococci, *Staphylococcus aureus, Propionibacterium acnes*
Boase et al., 2013[11]	ESS Operation	Ethmoidal sinus	CRS	*Staphylococcus aureus, Propionibacetrium acnes*
Yan et al., 2013[12]	Swab	AN, MM, SR	CRS	*Corynebacterium accolens, C. pseudodiphtheriticum*
Ramakrishnan et al., 2013[13]	Swab	Meatus medial	CRS	*Staphylococcus aureus*
Biswas et al., 2015[7]	Swab	AN, inferior turbinate, Meatus medial	CRS	*Staphylococcus, Corynebacterium*
Mackenzie et al., 2017[8]	Swab, meta-analysis	Sinus	CRS	*Corynebacterium*
Hoggard et al., 2017[14]	ESS Operation	Sinus	CRS	*Moraxella, Staphylococcus, Fusobacterium, Haemophilus, Streptococcus, Pseudomonas*
Cope et al., 2017[15]	ESS Operation-brushing	Sinus	CRS	*Staphylococcus, Streptococcus, Psedomonadaceae, Corynebactericeae*
Hyun et al., 2018[4]	Biopsy	Inferior turbinate	Allergic rhinitis	*Staphylococcus, Actinobacteria*
Ramakrishnan et al., 2018[16]	Nasal swab	Nasal mucosa	Rinitis kronik	*Staphylococcus, Actinobacteria*
Mahdavinia et al., 2018[17]	Swab	Meatus medial	CRS	*Corynebacterium and Peponiphilus*
Kuhar et al., 2018[18]	ESS-Swab Operation	Sinus	CRS	*Firmicutes* and *Bacteroidetes*
Koelleher et al., 2018[19]	ESS Operation – Swab	Meatus medial	CRS with/without polyps	*Flavobacterium., Pseudomonas, Pedobacter, Porphyromonas, Stenotrophomonas*, and *Brevubdumonas* only in CRS with polyp
Gan et al., 2020[20]	Swab	Meatus medial	Allergic rhinitis, CRS and normal	*Firmicutes, Proteobacteria, Actinobacteria, Bacteriodetes*
Alammar et al., 2023[21]	Swab	Meatus medial	CRS with polyps	*Staphylococcus, Micrococcus*
Fernandes-Rodriguez et al., 2024 [22]	Swab	Nasal mucosa	Normal patients	Corynebacterium spp, Staphylococcus spp, Dolosigranulum spp.

MM, meatus medial; SR, sphenoethmoidal recess; ESS, endoscopicsinus surgery; AN, anterior nasal; CRS, chronic rhinosinusitis

the tissue. This is based on the analysis of previous studies in which specimens were collected with mucosal swabs or mucus on the concha and nasal mucosa, as well as medial meatus and sinuses. In only one study were specimens collected with inferior concha biopsies and obtained microbiota sequencing results. This is possible because mucus fluid was also found during the biopsy.

In this study, specimens were taken from the mucosa/epithelial tissue of the inferior concha during turbinoplasty surgery. These results further confirm the position and role of microbiota, which remain unclear regarding the nonspecific immune system of the nasal cavity. As commonly known, the immune system can be divided into the natural/nonspecific/innate and the acquired/specific/adaptive system.

According to previous studies, microbiota are included in physiological nonspecific immunity, a normal component of the body, always present in healthy individuals and ready to compete with microbes invading the body. The microbiota are located in mucus, not on the surface of the epithelium/mucosa of the concha. Therefore, they play a role in the nonspecific immune system, a physical barrier.

In conclusion, the results can be used as a basis for other studies to learn about the microbiota of the respiratory tract, specifically the nasal cavity. Microbiota are only found in mucus, not in the epithelium, mucosa, or even on the surface of the nasal mucosa. They can be examined by taking specimens with swabs or brushing, not biopsies or mucosal tissue. Furthermore, microbiota are included in physical defenses, including the skin, mucous membranes, cilia, coughing, and sneezing.

Ethics Statement

This study received approval from the Medical and Health Research Ethics Committee (MHREC) FKKMK UGM/RSUP Dr. Sardjito Yogyakarta with No. Ref: KE/FK/0803/EC/2023.

Competing Interests

The authors declare that they have no competing interests.

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***Corresponding author:** Rangga Putra Nugraha. E-mail: ranggaputra.dr@gmail.com*