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## Case Report

## Tonsillolith: A polymicrobial biofilm

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## Introduction

Tonsilloliths are rare concretions found in the tonsillar crypt.<sup>1</sup> Tonsillolith occurs rarely in children compared to the adults.<sup>2</sup> The size of tonsillolith ranges from visible size to pea size.<sup>3</sup> Patients with tonsillolith had increased halitosis and also feel foreign body sensation.<sup>1,4</sup> Tonsillolith is not just a stone but a living biofilm. Mechanism of tonsillolith formation is due to the bacteria forms a three-dimensional structure dormant bacteria being in the center to serve as a constant nidus of biofilm.<sup>5</sup> However, the exact cause and the pathogenesis of tonsillolith are not yet completely defined. Hence, the present case with tonsillolith was studied and tonsillolith was analyzed by physical, chemical and microbiological methods.

## Case report

Eleven-year-old female child along with the parents reported to the Department of Pedodontics and Preventive Dentistry, Army College of Dental Sciences, Secunderabad, Andhra Pradesh, India, with chief complaint of tooth eruption in her right side of throat causing continuous irritation, discomfort and halitosis. On general physical examination observed that she is a normal child having normal growth and development. On intra oral examination, erupted teeth were recorded as 16, 55, 14, 53, 12, 11, 21, 22, 24, 65, 26, 36, 75, 34, 32, 31, 41, 42, 43 and 44, 46 with normal intra oral findings. All teeth were intact with good oral hygiene and also aligned in 'U' shaped dental arches with normal occlusion. Protruding tooth colored material was visible on right oropharynx at the tonsillar crypt (Fig. 1). Orthopantomograph of patient depicted no abnormalities. Radiopaque mass was seen on lateral oblique view (Fig. 2). Patient was co-operative. On the basis of clinical and radiological evaluation the tooth colored material i.e., tonsillolith was removed with sterilized swab (High Media Limited, Mumbai) by pressure application after taking consent from the parents (Fig. 3). Patient was relieved of discomfort after removal of tonsillolith from right oropharynx of the tonsillar crypt. Thorough irrigation of the site of tonsillolith was done with normal saline. Patient was recalled once in 2 months on regular basis to maintain oral prophylaxis and to carry out thorough irrigation of the tonsillar crypt with normal saline. Patient has been advised to come for regular check up to the Army College of Dental Sciences.

Tonsillolith was sent to two different laboratories (Recognized by Govt. of India and they follow Sampling & Analysis as

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**Fig. 1 – Intra oral view of tonsillolith.**

per standard methods prescribed by Bureau of Indian Standards) under sterile conditions for physical, chemical and microbiological analysis.

Physical, chemical and microbiological analysis of tonsillolith revealed the following:

**A. Physical measurement of tonsillolith (Fig. 4)**

1. Length: 1.2 cm
2. Width: 1 cm
3. Color: white
4. Shape: irregular
5. Surface: rough
6. Wet weight: 0.13738 g
7. Dry weight: 0.03238 g
8. Consistency: semi hard
9. Odor: foul odor

**B. Microbiological analysis of tonsillolith**

1. Gram stain

Gram stain was carried out on two sites of tonsillolith, one on the surface of the tonsillolith and other one deep inside the tonsillolith. On surface of the tonsillolith gram-positive cocci, gram-positive bacilli and gram-negative bacilli were



**Fig. 2 – Radiopaque mass on lateral view.**



**Fig. 3 – Removal of tonsillolith.**

observed where as deep inside the tonsillolith gram-negative bacilli was predominant.

**2. Carbohydrate fermentation**

Glucose fermentation test was carried out. It was observed that there is production of acid and pH was measured 5.2.

**C. Minerals in the chemical analysis of tonsillolith (Fig. 5)**

1. pH-6.98
2. Electrical conductivity: 22  $\mu$ /cm (microns/cubic millimeter)
3. Total dissolved solids: 14 mg/l
4. Turbidity: 6.80 NTU (Nephelometric Turbidity Unit)
5. Color: white

**Discussion**

Biofilm forms when bacteria adhere to surface in moist environment by secreting a slimy, glue like substance. Biofilm held together by molecules known as extracellular polymeric substance. The establishment of biofilm is a key event in the formation of tonsillolith in tonsillar crypt. Polysaccharide intercellular adhesion protects bacteria against major components of the human innate immune system.<sup>6</sup> Cell to cell signaling (quorum sensing) and communication with different bacteria enhances the biofilm formation.<sup>7</sup> Calcification of matrix may give further protection for polymicrobial bacterial



**Fig. 4 – Specimen (1 × 1.2 cm).**

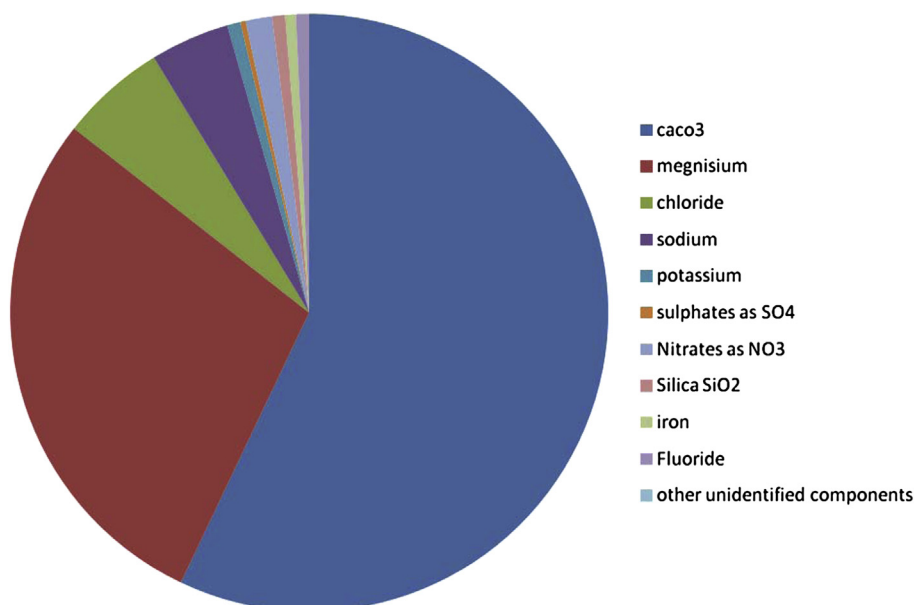


Fig. 5 – Chemical composition of tonsillolith.

flora living in tonsillolith. Present study of tonsillolith by physical, chemical and microbiological analysis confirms that tonsillolith is a polymicrobial biofilm. No recurrence was reported by maintaining good oral hygiene through oral prophylaxis and periodic once in every 2 months thorough irrigation of the tonsillar site with normal saline in the present case. Flattening of the crypts and crevices can be done by using scanned carbon dioxide laser to reduce the retention of tonsillolith were reported.

Tonsilloliths are calcification that forms in the crypts of the palatal tonsils. These calculi are composed of calcium salts either alone or in combination with other mineral salts.<sup>8</sup> Chemical analysis of the present case of tonsillolith indicated that tonsillolith is composed of calcification of calcium salts and presence of other chemical components. Calcium carbonate ( $\text{CaCO}_3$ ) was found to constitute the major portion and also constitutes other chemical components like magnesium, chloride, sodium, potassium, sulphates as  $\text{SO}_4$ , nitrates as  $\text{NO}_3$ , silica as  $\text{SiO}_2$ , iron, fluoride and other unidentified components (Fig. 5). Measurement of turbidity indicated total solids present in tonsillolith whereas measurement of electrical conductivity indicated concentration of salts and other chemical ions present in tonsillolith.

Microbiological analysis of tonsillolith revealed presence of both aerobic and anaerobic microorganisms.<sup>5</sup> Tsuneishi et al (2006) in their study established the presence of wide spectrum of microorganisms such as *Eubacterium* and *Tranterra*.<sup>9</sup> In the present case of tonsillolith gram stain study and carbohydrate fermentation test showed that bacterial flora are present in tonsillolith. On gram staining the surface of tonsillolith showed gram-positive cocci, gram-positive bacilli and gram-negative bacilli whereas deep inside the tonsillolith gram stain showed predominantly gram-negative bacilli. Aerobic bacteria present on surface of tonsillolith whereas deep inside tonsillolith belongs to anaerobic bacteria.<sup>5</sup>

Tonsillolith have the potential to cause oral halitosis. Foul smelling compounds such as volatile sulfur compounds and

sulfur derived gases were produced during bacterial metabolism. A characteristic smell of sulfur gives when production of gases reaches certain concentration.<sup>4</sup> In the present case of tonsillolith, patient expressed irritation, discomfort and halitosis. Irritation and discomfort was due to presence of tonsillolith in right pharynx at the tonsillar crypt and halitosis was due to volatile sulfur compounds produced by bacteria in tonsillolith. This case report will help in increasing awareness amongst pediatric dentist and clinicians about tonsillolith and its effects and modality of treatment.

## Conclusion

Tooth colored material removed from patient was diagnosed as tonsillolith. It has been analyzed by physical, chemical and microbiological methods and indicated that tonsillolith is a polymicrobial biofilm. Discomfort and irritation relieved after removal of tonsillolith from right oropharynx of tonsillar crypt and on subsequent visit during follow up the patient confirmed that there is no halitosis. This case report highlights the importance of thorough oral and perioral examination in the diagnosis of rare conditions such as tonsillolith.

## Conflicts of interest

All authors have none to declare.

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