ORIGINAL ARTICLE

Comparative study of morphology, elemental constitution and crystal structure of two large sialoliths: by way of scanning electron microscope energy dispersive x-ray (EDS) and x-ray diffraction (XRD) analysis

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Abstract

Aim: The purpose of this study was to compare the morphology, elemental constitution and crystal structure of two sialoliths; one from the parotid and the other from the submandibular gland. Also to search for the evidence to suggest the epithelial or bacterial core for the initiation of sialolith.

Materials and Methods: Sialoliths surgically removed from ducts of parotid and submandibular glands respectively were analyzed with Scanning Electron Microscopy (SEM) and X-ray Diffractometry (XRD).

Results and Conclusions: Scanning Electron Microscopy (SEM) and X-ray Diffractometry (XRD) showed that the crystal structure of both sialolith were apatitic in nature. The parotid stone was composed mainly of calcium phosphate, while its submandibular component incorporated calcium carbonate as well. The

الملخص

الهدف: هدفت هذه الدراسة الى مقارنة الشكل الظاهري والبناء العنصري والشكل المجهري لُأثنين من الحصاوي أحدهما من الغدة النكفية والأخرى من الغدة تحت الفك السفلي.

منهج البحث: تم ازالة الحصوات جراحيا من قنوات الغدةالنكفيةوالغدة تحت الفك السفلي وتم تحليلهما باستخدام المجهر الالكتروني والأشعة السينية.

النائج:أوضح كل من المجهر الالكتروني والأشعة السينية أن الشكلالمجهري للحصوتين هما من الفوسفيت في الطبيعة. الحصوة المستخرجة من الغدةالنكفية تتكون بصفة اساسية من فوسفات الصوديوم بينما تحتوي حصوة الغدة تحت الفك

content of carbon also varied between the two large sialolith. The presence or absence of microorganisms could not be determined conclusively from the present study. Thus in accordance with these study results, it may be concluded that sialoliths in the salivary glands may arise secondary to sialadenitis, but not via an organic nidus.
Key words: large sialolith, scanning electron microscopy, x-ray diffractometry

السفلي كربونات الكالسيوم ايضا. يختلف مكون الكربون بين الحصوتين. وجود أحياء دقيقة لا يمكن تحديده من هذّه الدراسة.

الخلاصة: تخلص الدراسة الى أن الحصوات التي تحدث بالغدد اللعابية قد تنتج بطريقة ثانوية من التهاب الغدد اللعابية وليس من مصدر عضوي.

Introduction

Sialolithiasis is a disorder of salivary glands characterised by formation of calculi; resulting from deposition of calcium salts inside the salivary duct or gland proper. It comprises more than 50% of major salivary gland disorders and is a common reason for salivary gland infections [1]. Many theories have been stipulated to explain salivary calculi formation, such as calcification around foreign bodies, desquamated epithelial cells, and microorganisms in the duct[2,3] Sialoliths have an organic matrix of carbohydrates and aminoacids; and are surrounded by calcium phosphate, with traces of magnesium and ammonia. Though the exact mechanism of sialolith formation is still a matter of conjecture; it has been traditionally thought to develop around a central nidus of mucous plugs, desquamated epithelial cells, micro organisms or any other foreign body. This nidus causes salivary stasis and accretion of calcium and phosphate onto it followed by slow crystallization[4].Sialoliths range from 1mm to less than 1cm in size; and rarely grow larger than 1.5cms. Salivary sialoliths are predominantly composed of elements comprising Ca, P, and small amounts of Mg, K, Na, Cl, Al, and Fe.[5,6,7] .In general, sialoliths are composed of an organic and inorganic matrix, the presence of a central core, a laminar peripheral structure, and a major component of calcium phosphate[8,9,10]

This article showcases two large sialoliths, one each from the parotid and the submandibular gland. We have attempted to analyse its chemical composition and micromorphology by X-ray diffraction analysis (XRD), scanning electron microscopy (SEM) energy dispersive X- ray (EDS) respectively, in order to add evidence on the little understood mechanism of sialolith formation.

Materials and Method

Case-1

A 45-year-old South Indian female was examined in the outpatient department, with a swelling in the right cheek of 3 years duration. Her medical and dental histories were otherwise normal. Intraoral examination revealed a hard, ovoid swelling at the opening of the Stenson's duct. Radiographs revealed a radiopaque lesion that was removed

transorally. The sialolith was 1.84 cms long and weighed 5.03 gms (Fig 3A). Postoperative healing was uneventful and the patient was disease free 13 months after surgery.

Case-2

A 42-year-old South Indian male presented to the outpatient department complaining of a painful swelling in the left side of the floor of the mouth of 20 years duration. His medical and dental history were otherwise unexceptional. Intraorally there was a hard, oblong swelling extending along the entire left lingual vestibule. At the posterior most limit, there was a draining sinus. A radiopaque mass was seen in the region of the Wharton's duct on the radiograph (Fig 3B). Under local anesthesia, a 4. 13 cms long sialolith was dissected out transorally (Fig 3C). It weighed 11.04gms and the surface was nodular. Ten months after surgery the patient is symptom free and had a normal salivary flow.

Method

The sialoliths were stored for 12 hours in 10% neutral buffered formalin, rinsed in distilled water and dried in air. They were then halved, one each for scanning electron microscopy and x-ray diffractometry. The samples were analysed for morphology, elemental constitution and crystal structure using Scanning electron microscope coupled with an EDS system (SEM-EDS) and X ray powder diffractometry(XRD) .

Scanning Electron Microscopy (SEM) and Energy Dispersive X ray analysis were done using a FEI- Quanta Environmental scanning electron microscope. The SEM samples were not subjected to any coating. Energy dispersive x-ray microanalysis was done with Large Field Detector (LFD) detector system. The SEM studies were examined at an accelerating voltage of 30 kV and a working distance of 11.7 mm for the inner surface of the parotid sialolith (Fig 1A). The outer surface of the parotid stone was studied at an accelerating voltage of 30 kV and a working distance of 12.1mm (Fig 1B). The inner (Fig 2A) and outer (Fig 2B) surfaces of the submandibular sialolith was examined at an accelerating voltage of 30 kV and working distance was 11.0mm and 10.9mm respectively.

For x-ray diffractometry the samples were powdered in an agate mortar. The procedure was carried out using Model D5005 Siemen, Karlsruhe, Germany using Cu K∞ (Copper K alpha) radiation with scan speed of 5 degree per minute, with a step size of 0.1 degree. The scanning was done for a two theta angle of 10 to 90 degree. As the sample size was minimal no statistical analysis was possible.

Results

SCANNING ELECTRON MICROSCOPE (SEM) analysis of parotid sialolith

The inner part of the sialolith shows fine granular morphology with distributed pores of approximately 5µ in size (Fig 1A). The surface micrograph (Fig 1B) also

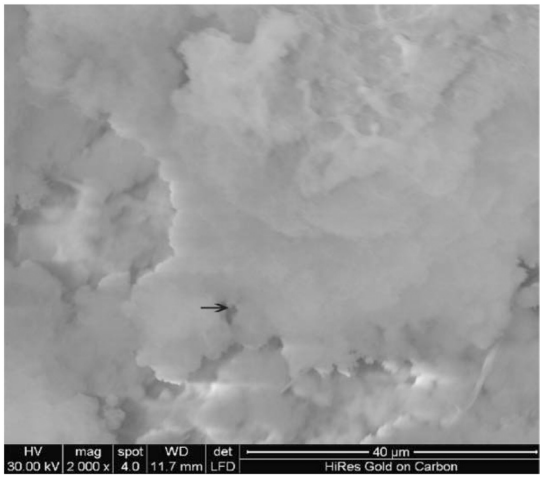


Fig. 1

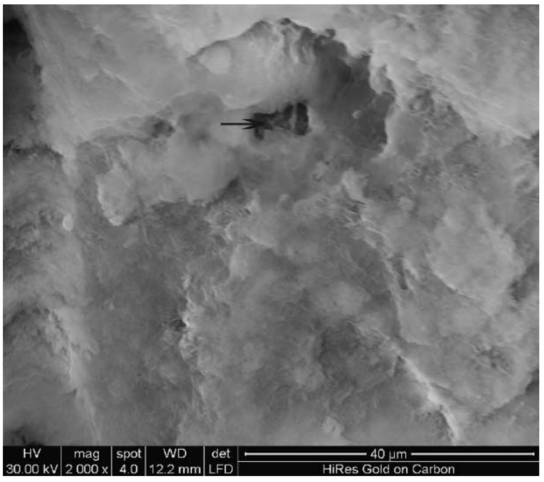


Fig. 2

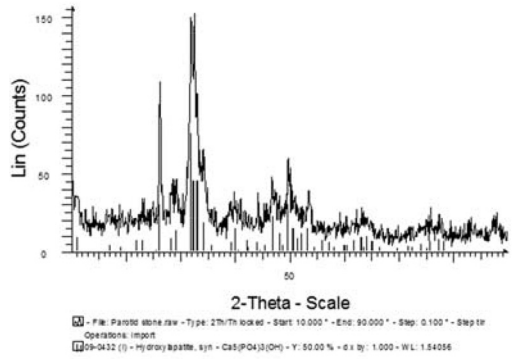


Fig. 3

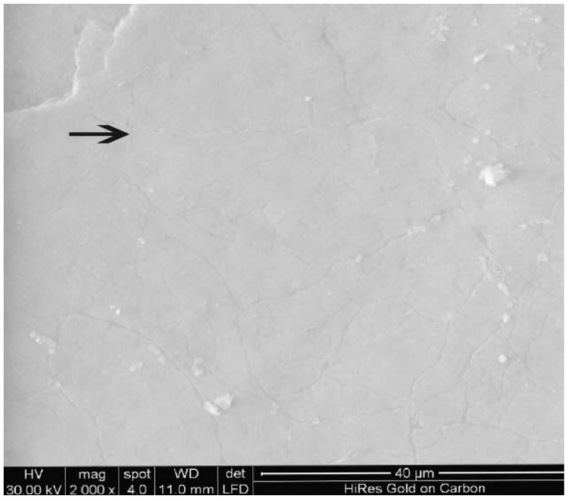


Fig. 4

shows similar microstructure. The inner part clearly shows a brittle fracture morphology emphasizing the high mineral content. The mineral content in the EDS spectra shows calcium and phosphate peaks along with oxygen and small amount of carbon. The ratio Ca/P was 1.65. This means that the mineral phase is apatitic in nature which has been further confirmed by XRD.

SCANNING ELECTRON MICROSCOPE (SEM) - Analysis of submandibular sialolith. The inner and outer surfaces of the sialolith show a smooth architecture (Fig 2 A, 2B) compared to the irregular morphology of the parotid sialolilth. Lot of micro cracks are visible on the micrograph. EDS spectra is similar to the parotid samples in the bulk. A strong presence of carbon in the surface of submandibular samples may be due to the substitution of carbonate in the crystal structure or due to the presence of organic components like micro organisms. Further investigations are required for the confirmation.

X-RAY DIFFRACTION analyses - The XRD scan of parotid stone is Fig 1C and that of the submandibular stone is Fig 2C. Both of them are compared with the

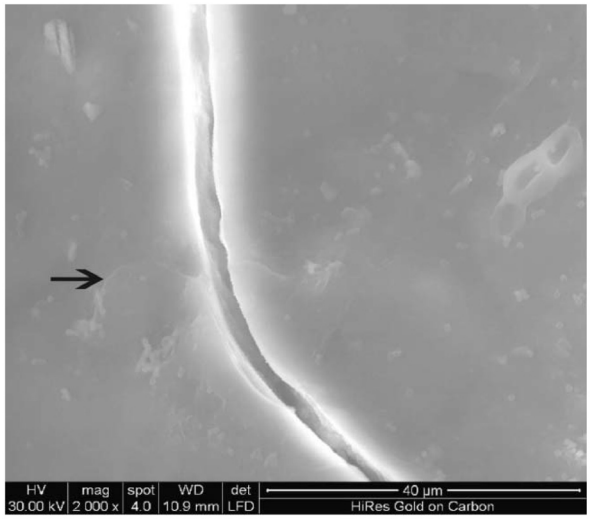


Fig. 5

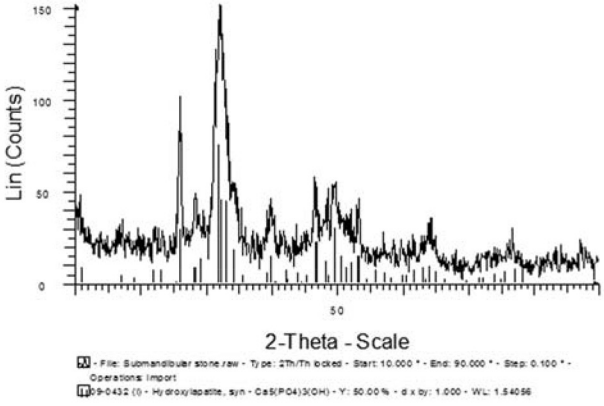


Fig. 6



Fig. 7

standard international database for synthetic hydroxyapatite PDF-9-432[22] All the peaks of the scan are matching and both the sialoliths are calcium hydroxyapatite with chemical formula $Ca_{10}(PO4)_6(OH)_2$. Hence it is evident that both the sialoliths are apatitic in nature. The submandibular stone shows observable amount of organic content on EDS investigation. This can be the reason of its smooth amorphous structure compared to the parotid stones.

Discussion

Approximately 85% of sialoliths occur in the submandibular gland, 10% in the parotid, 5% in the sublingual gland and rare involvement in the minor salivary glands[11]. Males are more prone to develop salivary stones with a peak occurrence between 20 and 50 years. Submandibular stones are commonly located in the ductal system whereas parotid stones are seen in the hilum or gland parenchyma[12]The greater predilection for sialolithiasis in the submandibular system could be due to the flow of saliva against gravity, as the Wharton's duct lies above the gland. Drage et al, postulated that the length of the duct might be of significance in the formation of submandibular stones[13]. The alkaline nature of the saliva, its viscosity, and its relatively high content of calcium salts (3.6mEq/l) compared to 2.0mEq/l of parotid are other reasons. Salivary stasis and viscosity, rather than the calcium content of saliva predispose to the development of sialoliths[14]. The submandibular saliva contains a higher

quantity of mucin protein compared with serous parotid saliva. Partial obstruction plays a greater role in the formation of stones than complete obstruction.

Salivary calculi are generally composed of an organic and inorganic matrix, a core, a laminar peripheral structure and a major component of calcium phosphate[8,9,10]. Kasaboglu et al in an ultrastructural analysis of six submandibular sialoliths, state that Ca and P are predominant on all the surfaces with traces of Na, Mg, Cl, Si, Fe and K on the internal, external and central surface matrices[15]. This may be due to the more unstable, unsaturated structure of the external and internal surfaces compared to the core. No foreign body, organic material or signs of microrganism dependent core formation were detected. Crystalloids of sialoliths are composed of either multiple and polymorphous microcrystals or a large compact crystalline mass and an occasional finding in the parotid gland.[16,17] Crystalloids present in the parotid gland may aggregate to form nuclei of calculi on which successive layers of organic and inorganic material from saliva would be deposited. Hirade and Nomura opine that a foreign body or microrganism that acts as a nucleus of initial stone formation may lose its original contour during calculi development[6]. They indicated that a foreign body or microorganism acts as a nucleus of initial stone formation and the nucleus might be morphologically changed during the calculus development so that the foreign body or microorganism eventually loses its contour[6]. Harrison et al indicate that the stagnation of calcium rich material secondary to inflammation acts as a nucleus which later grows into a sialolith[20] During chronic submandibular sialadenitis, inflammatory swellings would lead to the partial obstruction of a large duct with stagnation of secretory material rich in calcium. This would form a calcified core and later, when this grows, it would become a sialolith.[15] Accordingly, microorganisms have not been found in the core of the sialoliths. Since the mechanisms of calculi formation are largely speculative, all of the above theories may in part contribute to its genesis.

In the present study, the parotid stone surface was more irregular and had a higher mineral content than the submandibular counterpart. Another finding was that the parotid calculi were mainly calcium phosphate whereas the submandibular stone incorporated carbonate too. This should be further evaluated given the fact that submandibular saliva has less bicarbonate concentration than parotid (18mEq/l vs 20 mEq/l). The parotid stone had a lesser carbon content which may suggest lesser microbial content or it may be due carbonate substitution in the submandibular calculi. The higher mineral composition of parotid should also be investigated considering the organic nature of its saliva. Salivary calculi are mainly composed of calcium phosphate and carbonate and small amounts of magnesium, potassium chloride, and ammonium.[15] Likewise, the sialoliths examined in this study contained

predominantly Ca and P on all surfaces.The Ca/P ratio of parotid sialolith was 1.65 which showed the apatitic nature of the mineral phase .

The aim of this study was to analyse sialoliths using SEM and x-ray diffractometer to conduct an elemental chemical analysis on the sialoliths as well as to determine whether or not there was a foreign body or organic core causing the formation of a nucleus in the center of the sialoliths. From the findings we got with our limited methodology, the mechanism for the formation of sialoliths examined in this study was similar to the one suggested by Kasaboglu et al and Harrison et al [15, 20]

Conclusion

Exhaustive research is necessary to elucidate the actual mechanism of evolution of sialoliths. Elemental chemical analysis should be carried out based on ageing of sialoliths to know any changes in the chemical constitution of the sialoliths as it ages.

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