Nanobacteria can be the Reason for Creating Sialoliths

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Abstract: ***Introduction**: Nanobacteria or calcifying nanoparticles (CNPs), i.e. propagating self-replicating nanoparticles, contribute to pathological calcification in the human body, including diseases such as dental pulp stones, salivary gland stones, kidney stones, gall stones. In this study, we identified calcium phosphate ratio, in the sialoliths or salivary gland stones. **Materials and Methods**: The present study was analyzed operative leached 10 sialoliths. Calcium phosphate ratio, in the sialoliths, were detected in the laboratory at the Faculty of Chemistry, University ‚St.Cyril and Methodius” in Skopje. Qualitative and quantitative chemical analysis was used to determine the ratio between Calcium and Phosphorus given in mg/kg and percentages. For the qualitative analysis of the material we used a method of Infrared Spectroscopy, known as FTIR-spectrometry. For the quantitative analysis of the material, we used a method of Atomic-absorption spectroscopy. Control group was samples hydroxyapatite. Control hydroxyapatite was correctly identified in the test. **Results**: This analysis of sialoliths showing Ca and P peaks similar to those of hydroxyapatite. FTIR-Spectrograms in absorbance were mainly made up of albumin, which is the most common component of sialoliths, as can be seen in the spectra. The intensive line in the spectra in transmittance in the high frequency part, as well as the lines in the area around 1600 to 1300 cm^{-1} show the presence of albumin, and the intensive line in the area around 1000cm^{-1} and the doublet of lines around 600 cm^{-1} are the result of the vibration of phosphate ions present in the stones as carbonate apatite (Calcium Phosphate Carbonate). Atom-absorbing spectroscopy: showing the value are identical of Ca expressed in mg/kg was 584310, and 58.431 in percent. The value of P in mg/kg was 232517, and 23.252 in percent. **Discussion**: Two major chemical elements: Ca and P in the sialoliths concentrations 58.43% and 23.25% respectively giving a Ca/P weight ratio of 2.18 which is very close to the weight ratio of pure stoichiometric hydroxyapatite 2.15. **Conclusion**: Salivary stone formation can be nanobacterial disease initiated by bacterial infection.***

Keywords: nanobacteria, calcifying nanoparticles, pathological calcification, hydroxyapatite, salivary gland stones, sialoliths.

1. Introduction

Pathologic calcification usually is initiated by the biologic membranes of mitochondria or matrix vesicles. Mitochondria frequently initiate intracellular calcification. Matrix vesicles and mitochondria usually initiate calcification through the interaction of phosphatase enzymes with calcium-binding phospholipids, both of which are membrane-bound.

Hydroxyapatite (HA) crystals are formed first within the protective microenvironment of the membrane-enclosed microspace. Once formed and exposed to the extracellular fluid, HA crystals can serve as nuclei or templates, thus supporting progressive, autocatalytic mineral crystal proliferation [1].

Calcification, a phenomenon often regarded by pathologists little more than evidence of cell death, is becoming recognized to be important in the dynamics of a variety of diseases from which millions of beings suffer in all ages. In calcification, all that is needed for crystal formation to start is nidi (nuclei) and an environment of available dissolved components at or near saturation concentrations, along with the absence of inhibitors for crystal formation. Calcifying nanoparticles (CNP) are the first calcium phosphate mineral containing particles isolated from human blood and were detected in numerous pathologic calcification related diseases. Controversy and critical role of CNP as nidi and triggering factor in human pathologic calcification are discussed [2].

Sialolithiasis (also called salivary stones, calculi) refers to the formation of stones in the salivary glands. Stones are most commonly found in the submandibular gland, where stones can obstruct Wharton's duct. It is frequently associated with chronic infection (Staphylococcus aureus, Streptococcus viridans) of the glands, dehydration (phenothiazines), Sjögren's syndrome and/or increased local levels of calcium, but in many cases can arise idiopathically. Pain, when present, usually originates from the floor of the mouth, although in many cases the stones cause only intermittent swelling [3].

2. Materials and Methods

The present study was analyzed operative leached 10 sialoliths, at the Clinic of Maxilofacial surgery, Faculty of Dentistry, University “St. Cyril and Methodius” in Skopje, (Figure 1 and Figure2). The chemical analysis of the salivary gland stones, was conducted at the Faculty of Chemistry, University ‚St.Cyril and Methodius” in Skopje.

Qualitative and quantitative chemical analysis was used to determine the ratio between Calcium and Phosphorus given in mg/kg and % (percentages). For the qualitative analysis of the material we used a method of Infrared Spectroscopy, known as FTIR-spectrometry. This chosen method is very exact and precise for chemical analysis and is widely used in all laboratories in the world for quantitative analysis. The infrared spectrophotometer was used in the study- Perkin Elmer 580 [Figure 3]. For the quantitative analysis of the material, we used a method of Atomic-absorption spectroscopy [Figure 4]. The control group consisted of samples of hydroxyapatite.

Figure 1: Salivary gland stone

Figure 2: Salivary gland stones

Figure 3: FTIR-spectrometer - Perkin Elmer 580.

Figure 4: Atom-absorbing spectroscop

3. Results and Discussion

This analysis of salivary gland stones showing Ca and P peaks similar to those of hydroxyapatite. With the method of infrared spectrometry, we obtained spectrograms where we could identify the ions present in the gallstones from the intensity and the position of lines in the spectra.

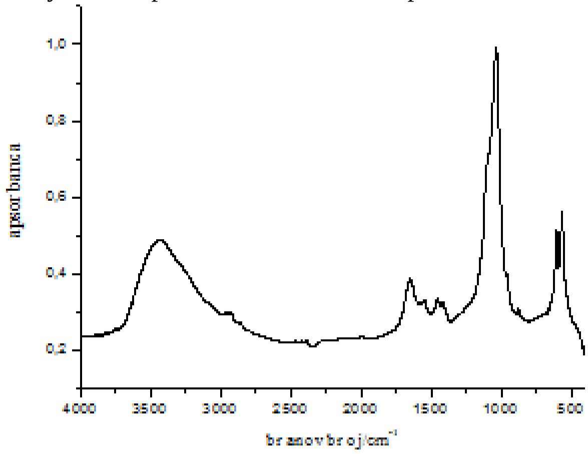

Figure 5: Infrared spectrum in absorbance of from salivary gland stone made of albumin + phosphate (carbonate apatite).

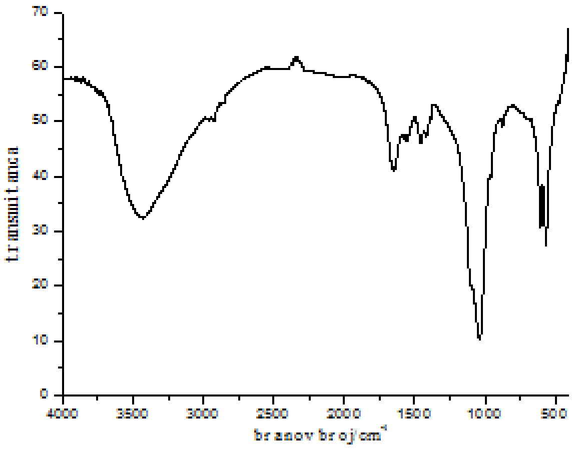

Figure 6: Infrared spectrum in transmittance of from salivary gland stone

FTIR-Spectrograms in absorbance were mainly made up of albumin, which is the most common component of sialoliths, as can be seen in the spectra (Figure 5,6). The intensive line in the spectra in transmittance in the high frequency part, as well as the lines in the area around 1600 to 1300 cm^{-1} show the presence of albumin, and the intensive line in the area around $1000cm^{-1}$ and the doublet of lines around 600 cm^{-1} are the result of the vibration of phosphate ions present in the stones as carbonate apatite - Calcium Phosphate Carbonate (Figure 2). Atom-absorbing spectroscopy: in all 10 samples the value are identical of Ca expressed in mg/kg was 584310, and 58.431in %. The value of P in mg/kg was 232517, and 23.252 in % (Table 1).

Table 1: Ca and P in mg/kg from Salivary Gland stones

Ca	Ca	P	P
mg/kg	%	*mg/kg*	%
584310	58.431	232517	23.252

Two major chemical elements: Ca and P in the sialoliths concentrations 58.43% and 23.25% respectively giving a Ca/P weight ratio of 2.18 which is very close to the weight ratio of pure stoichiometric hydroxyapatite 2.15 [4]. Nanobacterial model has Ca/P ratio similar such hydroxyapatite [5]. Our results indicate that the NB calcium phosphate phase can be formed salivary gland stones. Nanobacteria contribute to pathological calcification in the human and animal body, including diseases such as kidney stones, salivary gland stones, dental pulp stones and atherosclerosis [5].

Chemical analysis revealed that the overall composition of biofilm and solid mineral formation was similar to that of bone, except carbonate apatite was formed, as in most extraskeletal tissue calcification and stones, whereas in bone, hydroxyapatite is the prevalent form [6]. Control hydroxyapatite was correctly identified in the test.

Biofilm-phase of Nanobacterial life appears to be present when the Nanobacteria are chemically attacked, physiologically stressed, environmentally attacked, when they are working together or reproducing. The calcific biofilm that is secreted by the nanobacteria is a potent endotoxin and mediator (cause) of inflammation and swelling. In other words, our bodies react aggressively in response to the presence of this nanobacteria-secreted biofilm with swelling and irritation, the release of cytokines, interleukins, leukocytes, mast cells, collagenase, matrix metalloproteinases and etc. Our bodies react by trying to wall off the area of nanobacterial infection. When Nanobacteria are in an enclosed area, they cause chronic inflammation and swelling. Most of the commonly known medical “markers of inflammation” (C Reactive Protein, MMP's, MPO, Interleukins, etc.) are found to be elevated in response to the endotoxin in the nanobacterial biofilm [7].

In the few years since their discovery, nanobacteria have aroused equal parts of expected success and debate between scientists as possible stimulants of human calcific diseases [8]. They are the first calcium phosphate mineral containing particles isolated from human blood and were detected in numerous pathologic calcification related diseases [2]. As in black pigment gall stones [9,10], dental pulp stones [11], salivary gland stones [12], arterial calcification [13], atherosclerosis [14], heart diseases (local calciphylaxis on the mitral valve) [15], and calcific aortic valve stenosis [16], periodontal diseases (gingivitis and periodonitis) [17].

4. Conclusion

The fundamental importance is that these self-replicating special particles that we call CNP are found in blood and in pathogenic calcification and their properties of promoting ready crystallization and growth of Ca minerals are well established.

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