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Nanobacteria – CNPs the Possibility of Bacterial Association of the Dental Calcifications

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Abstract: ***Introduction:*** ***The discovery of nanobacteria, also referred to as calcifying nanoparticles (CNPs), led to novel theories for pathological calcification. Materials and Methods: The present study was analyzed seven dental pulp stones of the molars from a patient, with kidney stones, were detected and leached at University Dental Clinical Center "St. Panteleimon"- Department of Restorative dentistry and Endodontics, Faculty of Dentistry, University "Ss. Cyril and Methodius" in Skopje.*** *Control group was samples hydroxyapatite.* ***We used a method of Infrared Spectroscopy, known as FTIR-spectrometry and a method of Atomic-absorption spectroscopy in laboratory at the Faculty of Chemistry, University "Ss. Cyril and Methodius" in Skopje. Results: Spectrograms in absorbance were mainly made up of albumin. 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). With the method of atom-absorbing spectroscopy, we got the following results: in denticles, in all 7 samples, the value of Ca expressed in mg/kg was 18.501, and 18.502 in %. The value of P in mg/kg was 11.001, and 17.602 in %.*** *Control hydroxyapatite was correctly identified in the test.* ***Discussion:*** *Generally, the apatite formation required elevated P_i and/or Ca^{2+} levels and AP activity.* ***In the nanobacterial model, apatite was formed at [Ca] 1.8 mM and [P_i] 0.9 mM or less. Values Ca/P ratio in dental pulp stones of this study, is such with values*** *in the nanobacterial model. Infrared spectroscopy showing the distinct phosphate absorption signature at 1, 000 to 1, 200 cm21 seen in all four spectra, with inorganic apatite, bone, kidney stones, and nanobacteria (NB).* ***Conclusions: With this study we confirm that Nanobacteria or CNPs can be the possibility of bacterial association of the dental calcifications.***

Keywords: bacterial association, nanobacteria, calcifying nanoparticles, dental calcifications, pulp stones.

1. Introduction

Since the 1980s, scientists have observed and cultured nanoparticles that share a special trait: they form hard calcified coatings similar to the calcified deposits found in disease.

The type of calcifying nanoparticle found in mammals, including in humans, was informally classified as a "nanobacterium" by its discoverer Dr. Olavi Kajander. Due to newer discoveries by him and Dr. Neva Çiftçioğlu who developed monoclonal antibodies to identify them, these particles were renamed by Dr. Kajander as Calcifying Nanoparticles (CNPs).

They are an active factor for crystallization of Calcium, Phosphorus, minerals and carbonate apatite.

Nanobacteria can be found in part of the dental calcifications or dental pulp stones [1, 2, 3, 4, 5, 6, 7], sialoliths [8, 9, 10], dental plaque [11], kidney stones [12, 13, 14, 15, 16], and in very other organs. They cause the formation of the pathological calcifications.

The etiology of dental pulp stones, one type of extraskeletal calcification disease, remains elusive to date. Calcifying nanoparticles (CNPs), formerly referred to as nanobacteria, were reported to be one etiological factor in a number of extraskeletal calcification diseases. We hypothesized that CNPs are involved in the calcification of the dental pulp tissue, and therefore investigated the link between CNPs and dental pulp stones [2].

2. Materials and Methods

At the clinic for Restorative Dentistry and Endodontics, Faculty of Dentistry, "Ss Cyril and Methodius" University in Skopje, were presence was diagnosed with X-rays, the diagnosis pulpits chronic was given. All dental pulp stones were coronary localized. All dental pulp stones were coronary localized (Figure 1, 2).

After their were extirpated seven dental pulp stones, from molars of a patient over a period of one month (Figure 3, 4), and sent to a laboratory for chemical analysis. The analysis was performed in the Institute of Chemistry at the Faculty of Natural Sciences and Mathematics, "Ss Cyril and Methodius" University in Skopje.

Figure 1: Pulp stone in the lower first molar

Figure 2: Pulp stone in the lower frst molar

Figure 4: Coronal pulp stone

Figure 3: Coronal pulp stone

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.

For the quantitative analysis of the material, we used a method of Atomic-absorption spectroscopy. The control group consisted of samples of hydroxyapatite.

3. Results and Discussion

This analysis of dental pulp 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 pulp stones from the intensity and the position of lines in the spectra (Figure 5, 6).

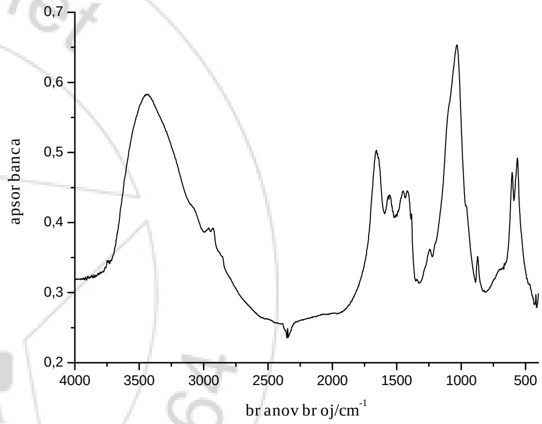

Figure 5: Infrared spectrum in absorbance of from dental pulp stones, made ap of albumin + phosphate (carbonate apatite)

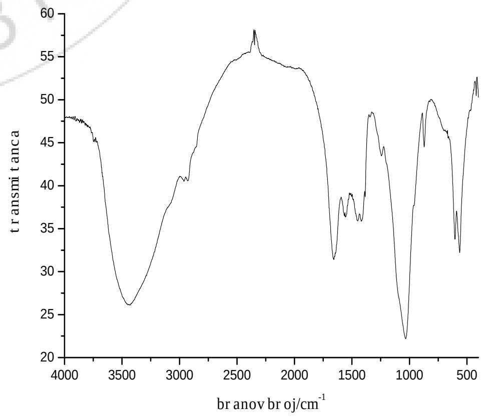

Figure 6: Infrared spectrum in transmittance of from dental pulp stones

Spectrograms in absorbance were mainly made up of albumin, which is the most common component of denticles, 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).

With the method of atom-absorbing spectroscopy, we got the following results: in denticles, in all 7 samples, the value of Ca expressed in mg/kg was 18.501, and 18.502 in percent. The value of P in mg/kg was 11.001, and 17.602 in percent (Table 1).

Table 1: Ca and P in mg/kg from Dental Pulp stones

Ca	**P**	**Ca**	**P**
mg/kg	***mg/kg***	**%**	**%**
185019	110011	18.502	17.602

The values of Ca and P are identical in all samples, probably because they are from the same patient. These values show the presence of calcifying nanoparticles CNPs in the dental pulp stones.

The aim of this study was to determine the ratio of Ca and P in dental pulp stones characteristic for nanobacteria/calcifying nanoparticles regardless of the age of the stone, which will prove their possible bacterial etiology.

Our data indicates that dental pulp stones are associated with apatite forming nanobacteria.

CNPs play an important role in etiopathogenesis of many diseases, and this association is independent from their nature. Consequently, emergence of CNPs in an organism is a pathological, not a physiological, process [16].

4. Conclusions

With the method of atom-absorbing spectroscopy, we got the following results: in the pulp stones, the calcium-phosphate ratio in all seven samples was with specific value to corresponding value of nanobacteria/CNPs.

With this study we confirm that Nanobacteria or CNPs can be the possibility of bacterial association of the dental calcifications.

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