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Calcifyng Nanoparticles (CNPs) in Human Gallstones

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Abstract: ***Introduction:*** ***CNPs are the only particles discovered in the human body that calcify under normal physiologic conditions, by its discoverer Dr.Olavi Kajander and Dr.Neva Çiftçioğlu.*** ***Materials and Methods:*** ***The present study was analyzed gallstones from operative leached gallbladder of 13 patients. Qualitative and quantitative chemical analysis was used to determine the ratio between Calcium (Ca) and Phosphorus (P).*** ***Results:*** ***This analysis of gall stones showing Ca and P peaks similar to those of hydroxyapatite. The results of this study such its similar with nanobacterial model. In gallstones the calcium-phosphate ratio in five samples (of examined 13), was with specific value to corresponding value of nanobacteria/CNPs.***

Keywords: Nanobacteria, CNPs, gallstones.

1. Introduction

By definition, pathologic calcification refers to the deposition of calcium phosphates (CaP) or other calcific salts at sites, which would not normally have become mineralized. Abnormal accumulation can occur in areas of tissue damage (dystrophic calcification), in hypercalcemic or hyperparathyroid states. Calcifying nanoparticles (CNP) are the first calcium phosphate mineral containing particles isolated from human blood and were detected in numerous pathologic calcification related diseases.

The mineral phase of many kinds of hard tissue in organisms is called biologic apatite (BA). Pure hydroxyapatite (HA) has the formula Ca10(PO4)6(OH)2. BA additionally contains several other ions, mainly carbonate but also trace amounts of other anions such as HPO42−, Cl−, and F−. Other cation elements are also present in minor amounts including Mg2+, Na+, and Fe2+. BA is the primary mineral of normal bone and teeth [1].

Calcium phosphate is deposited in many diseases, but formation mechanisms remain speculative. Nanobacteria are the smallest cell-walled bacteria, only recently discovered in human and cow blood and commercial cell culture serum. Calcium and phosphorous can combine as calcium phosphate, which is an extremely adhesive substance that readily binds organic molecules and nucleic acid and protein fragments. CNPs can assemble these fragments in special combinations, for example in the clotting cascade as published ELISA analysis has shown [2].

Calcifying nanoparticles (CNPs, nanobacteria, nanobacteria-like particles) were discovered as cell culture contaminants by Kajander et al. more than 25 years ago, and the first results of their work were published some years later (Kajander et al., 1997; Kajander and Ciftçioglu, 1998), [3].

Several important human diseases have calcium phosphate deposition as a hallmark, e.g., atherosclerosis and cardiovascular diseases, stone formation in kidneys, gall-bladder, salivary, venous and gingival locations, other urological diseases, e.g., prostatitis, and various forms of autoimmune diseases and arthritis [4]. Gallstones are frequently composed of more than one crystalline compound. Ca and P are the most important elements in them.

2. Materials and Methods

The present study analyzes operative leached gall bladders of 13 patients at the Clinic of Digestive surgery, Medical Faculty in Skopje-University ,,Sts.Cyril and Methdius" with different number of gallstones in them, Figure 1.

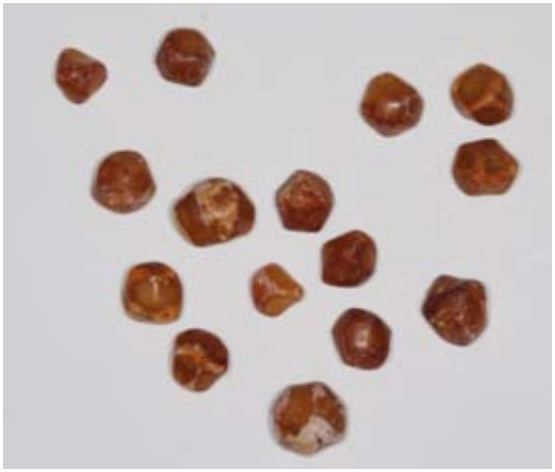

Figure 1

The chemical analysis of the gallstones was conducted at the Faculty of Chemistry, University ,,Sts.Cyril and Methodius" in Skopje.

Qualitative and quantitative chemical analysis was used to determine the ratio between Calcium (Ca) and Phosphorus (P) given in mg/kg and % (percentages).

For the qualitative analysis of the material we used a method of Infrared Spectroscopy, known as FTIR-spectroscopy**.** 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. Control hydroxyapatite was correctly identified in the test. The analytical methods do not exclude the possible presence of minor quantities of other mineral phases.

3. Results and Discussion

This analysis of gall 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.

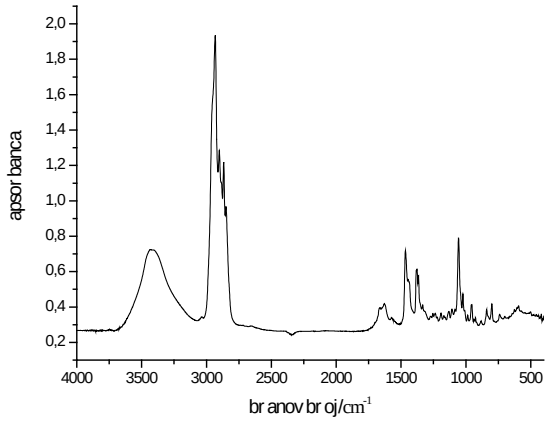

Figure 1

Figure 1. Infrared spectrum in absorbance of from gall stones, made of cholesterol + calcium oxalat.

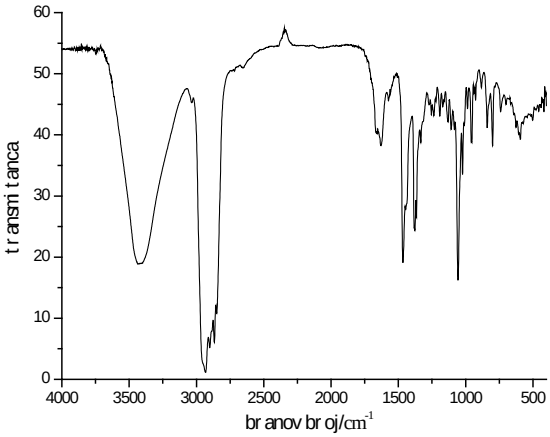

Figure 2

Figure 2: Infrared spectrum in transmittance of from gall stones

In the gallstones the main component is cholesterol. Also there are other minerals like calcium bilirubinate, calcium carbonate, carbon apatite and proteins. The infrared spectra of the gallstones showed that the main components are cholesterol and calcium oxalate.

Table 1: Presentation of the results of the calcium-phosphate ratio in gallstone samples, the blue highlight shows the border values and the yellow the specific values

Gall blader with number of the gall stones	*Ca mg/kg*	*P mg/kg*	*Ca %*	*P %*
2	659	219	0.066	0.022
3	3449	1369	0.345	0.137
15	288	64	0.029	0.006
6	988	640	0.099	0.091
10	54133	6.183	5.413	0.618
14	421	260	0.042	0.036
9	752	480	0.075	0.069
18	43632	6199	4.363	0.620
12	5508	198	0.551	0.020
17	8649	206	0.865	0.021
13	1339	806	0.134	0.129
16	214	134	0.021	0.016
11	136051	979	13.805	0.098

With the method of atom-absorbing spectroscopy, we got the following results: in gallstones the calcium-phosphate ratio in five samples (of examined 13), was with specific value to corresponding value of nanobacteria/CNPs.

In one of examined samples calcium-phosphate ratio was on border on value of nanobacteria/CNPs. Can the method of blood analyzes of Dr. Olavi Kajander, solve the dilemma.

FTIR spectroscopy is a valid method for gallstones composition evaluation [2]. Nanobacteria represent a unique model for evaluating calcification in vitro under physiological conditions [2].

EDX of a nanobacterium (culture of gallbladder stones in rabbits) also showed high concentrations of Calcium and Phosphorus. Calcium-Phosphorus ratio of the nanobacterium was 1.58, which is similar to that of hydroxyapatites. Negative staining of nanobacteria showed 80-250 nm particles and more than 500 nm. The particles appeared in the form of cocobacillar, whether they are independent particles or dominate as clusters. Some of the basic particles were partially or completely covered with a thick or thin layer of hydroxyapatite [5].

4. Conclusion

This study indicates that CNPs/nanobacteria exists in gallbladder bile of gallstones patients and causes the formation of gallstones. Nanobacteria, also known as calcifying nanoparticles (CNP), are controversial infectious agents not matching the current criteria for 'living organism'. Despite the controversy of their classification, they propagate and cause cell death in vitro and are associated or found in many human diseases. Thus, more efforts should be focused on research on pathogenicity of CNP [12].

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Associate Prof. **Aleksova Pavlina** successfully finished Faculty of Dentistry in Skopje, University Sts.,,Cyril and Methodius" and specialization studies in restorative dentistry and endodontics. She got Master degree with the thesis, Dental calcification- reason for special analysis" at same University. She got her PhD degree at same University with the thesis, Correlation of the dental pulp pathological calcification with other calcifications in human organs". She works more than 20 years at the Faculty of Dentistry in Skopje, R.Macedonia.

Ass.Prof. **Daniela Veleska-Stevkovska** is born in 9.12.1976 in Skopje. She successfully finished Faculty of Dentistry in 2001 and specialization studies in oral surgery in the year of 2006. She got Master degree in 2008 and a PhD degree in 2013. She holds a position as an Assistant prof. from the year of 2008 at the Department of oral surgery, Faculty of Dentistry, University Sts.,,Cyril and Methodius". She is honored with a scientific degree this year. Her scientific work is presented throw sixty articles, one monography and takes active participation in the publications of the Faculty of Dentistry and Macedonian Dental Chamber. She is active member of some national and international professional associations.