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SPOTLIGHT ON THE ASSOCIATION BETWEEN NANOBACTERIA AND SOME HUMAN DISEASES

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ABSTRACT

Nanobacteria are the smallest cell-walled bacteria, recently discovered in human and commercial cell culture serum, also known as calcifying nanoparticles (CNPs). Nanobacteria are the name of a proposed class of living organisms particularly cell-walled microorganisms with a size much smaller than the generally accepted lower limit size for life (about 200nm). Recently, histopathological immunohistochemical staining, transmission electron microscopy (TEM), and calcific staining studies revealed the association of nanobacteria in kidney and gall stones. Nanobacteria have been appeared to contribute to different benign and malignant calcifications in the form of calcium phosphate crystals and contribute to malignant calcifications in breast and ovarian cancer. Nanobacteria also cause prostatic stones in patients with chronic pelvic pain syndrome due to chronic prostatitis and are present in dental and pulp calculus. The evidence that nanobacteria exist within the human body and are closely related to numerous sorts of diseases is now overpowering. However, future research is required to reveal their nature and impact on health and disease. This paper aimed to highlight the harmful role of nano-bacteria on human health and to describe the association of nanobacteria and their hypothesized relationship with some human diseases.

Keywords: Nanobacteria, Pathologic calcification, calcifying nanoparticles, human diseases

INTRODUCTION

Nanobacteria (Nb) can be described as a class of living cell-walled microorganisms with a size much smaller than 200 nm, was first described by **Kajander & Ciftcioglu (1998)**, and are classified as Gram-negative organisms. They grow best under aerobic conditions. Nano-bacteria is one of the smallest self-propagating microorganisms that grow well in aerobic conditions and is resistant to heat and various conditions that are not suitable for other bacteria and can be killed by tetracycline or EDTA. They are also known as calcifying nanoparticles (CNPs). In 1997 Folk proposed that nanobacteria are the foremost operators of precipitation of all minerals and crystals on Earth formed in fluid water, that they moreover cause all oxidation of metals, which are abundant in many biological specimens **(Folk, 1993, 2005, Folk & Rasbury, 2007)**. The nature of these obscure cell-culture contaminants and their role in human organ disease has raised a lot of attention in therapeutic and human health research over the final decades **(**Kajander & Ciftcioglu, 1998). Many studies show the significant role of nanobacteria, which may be associated with various organ diseases, especially, disorders with pathological calcifying processes. The first investigated study is its role in kidney stone formation with the risk of nephrolithiasis **(**Kajander & Ciftcioglu, 1998). In the literature, many studies and reports correlate the presence of nanobacteria with human diseases and conditions with pathological calcifications in human organs such as salivary glands, dental pulp, kidneys, cataracts eyes, and arteries **(Alenazy & Mosadomi, 2014)**. Evidence shows that nanobacteria may take part in pathological calcifying forms of the blood vessels, even without their calcified shells **(Schwartz *et al.*, 2008)**. This essentially may point to the presence of particular immunogenic bacterial protein antigens in their structure. It is additionally essential to specify that these proteins may likely have particular toxin-like activity. Nanobacteria (CNPs) are highly susceptible to some chemo-therapeutics agents, such as nitrofurantoin, trimethoprim, potassium cyanide, 5-fluorouracil, trimethoprim-sulfamethoxazole, cytosine arabinoside, 6-aminocaproic acid, and sodium azide. This leads to the suppression of the respiratory enzyme functions as well as the suppression of the biosynthesis of nucleic acids and proteins **(Ciftcioglu *et al.*, 2002)**.

Kidney stone disease

Kidney stone disease is one of the common diseases that affect the urinary tract. However, some complex aspects necessitated the intensive study of the chemical and physical conditions required for stone formation during the past decades. Kidney stone disease is one of the common diseases that affect the urinary tract. However, some complex aspects necessitated the intensive study of the chemical and physical conditions required for stone formation during the past decades **(Lloyd *et al.*, 1996)**. The studies that were conducted showed that the patho-physiology of kidney stone disease was not the only factor responsible for this disease. The crystalline components of kidney stones have been classified as calcium oxalate, calcium phosphate, related bacteria, purines, and cysteine **(Verkoelen *et al.*, 1997)**. Nanobacteria have unique properties and there has been scientific controversy regarding their formation from spherical deposits found in patients suffering from kidney stones **(Bradbury, 1998; Vogel, 1998)**. **Ciftcioglu *et al.*, (1999)** suggest that nanobacteria may act as a common nidus in kidney stone formation, and thus, their eradication could well represent a new approach to therapy for patients with kidney stones. It has been found that the majority of urinary tract stones are a mixture of two or more of these components. The most common were calcium oxalate and apatite **(Kagander *et al.*, 2003; Woodhouse & Robertson, 2004)**. Several studies reported that calcified kidney stones are located on the renal papillary surfaces and consist of an organic matrix and crystals of oxalate or calcium phosphate and approximately 90% of calcium oxalate calculi contained small amounts of phosphates at the calculi core and infection stones or triple-phosphate stones, account for approximately 10 to 15%of all kidney stones **(Grases *et al.*, 1993; Khan, 1997)**. The bacteria commonly present in these stones are the urease producers such as Proteus, Klebsiella, Pseudomonas, Corynebacterium species, as well as *Escherichia coli*. Struvite stone arrangement has been thought to be due to the alkalization of urine by the urease and/or phosphatase action of bacteria **(Du Toit *et al.*,1995; Monk, 1996)**. Nanobacteria are carbonate apatite forming, cytotoxic bacteria recently discovered in human and bovine blood and blood products **(Kajander & Ciftcioglu, 1998)**. Previous reports have proposed that urease-producing bacteria play an important role in the formation of infection-induced urinary stones. The mechanism of stone formation starts by alkalinizing urine causing super-saturation concerning struvite and calcium phosphate, and formation of struvite and apatite crystals. Urinary infections of urea-splitting bacteria in infection stones are thought to be initial factors of stone formation and those of non-urea- splitting bacteria are to be superimposed **(Takeuchi *et al.*, 1989)**.

Salivary stone disease

Salivary gland swelling can happen when one of the ducts that carry spit from the salivary organ to the mouth is blocked. The foremost common cause of blockage is stoning. Salivary gland stones are most common in adults. Blockage makes spit back up interior the conduit, causing the salivary gland to swell. Nanobacteria (NB) contribute to pathological calcification in human and animal bodies. It has been isolated from salivary stones and suggested that it may act as a nucleus for the initiation of these stones **(Harrison, 2009; El Badry *et al.*, 2010)**. Most salivary stones (salivary calculus) are mainly made of calcium, however; there's no variation from the blood calcium level or any other issue with calcium in the body. Mineralogists found that for crystal formation/biomineralization to begin, nidi (nucleus) and an environment of available dissolved components at or close immersion concentrations, along with the absence of inhibitors for crystal formation are needed. Bacteria or other agents producing such nidi, if present in body fluids are exceptionally likely candidates to dispatch and accelerate

pathological calcification in vivo, and these bacteria may play a major role in the recurrence of salivary stone. **(Blair & Fabrizio, 2000; Harrison, 2009)**. El Badry and his co-workers (2010) studied the relationship between nanobacteria in saliva and recurrent salivary gland stones using immune-detection with nanobacteria-specific monoclonal antibodies and scanning electron microscopy (SEM) to provide a method for preventing the recurrence of salivary stones in patients that have suffered from salivary stones, comprising administration of calcium chelator, ethylene diamine tetraacetic acid (EDTA), before or in combination with the suitable antibiotic that is given in an amount effective to inhibit or prevent the growth and development of nanobacteria. They concluded that salivary stone formation is a nanobacterial disease initiated by bacterial infection and subsequently endogenous and dietary factors may influence their progression. These bacteria may play an important role in the recurrence of salivary stones. So the use of calcium chelator such as EDTA before or in combination with the suitable antibiotic may eradicate these stones and prevent their recurrence **(El Badry *et al.*, 2010)**.

Chronic periodontal diseases

The role of nanobacteria in periodontal disease was first proposed based on the association between oral hygiene and the incidence of cardiovascular disease, which was probably mediated via the oral infection inflammation pathways **(Joshipura *et al.*, 2003)**. Investigators reported that probably nanobacteria should have their intrinsic nucleic acids, and their replication and specific proteins biosynthesis system. Inflammation of the supporting tissues of the teeth or chronic periodontal disease mainly includes gingivitis and periodontitis. Dental bacterial plaque is the main cause of chronic periodontal disease, so it must be eliminated by mechanical or chemical methods. It has been found that the mechanism of mineral deposits and dental stones are very similar to the mechanisms of other pathological calculus formation by pathogenic microorganisms. Scientists related the presence of nanobacteria discovered in various pathological calcifications, such as kidney stones and arterial plaques with that of chronic periodontal diseases which are considered as a potential risk factor for chronic periodontal diseases **(Yaghobee *et al.*, 2015)**. Nanobacteria, acting as self-propagating calcifying complexes, found in human blood and blood products, may have an important role in the calculus formation process. The presence of an alkali environment is basic for nanobacteria to cause calcification, as calculus formation is encouraged in such an environment. All these confirmations propose that nanobacteria may be present in the macromolecular structure of the dental calculus **(Ciftcioglu & Kajander, 1998)**. It appears the primary step to the anti-nanobacterial treatment is to weaken the calcified shells by utilizing substances like liquid zeolites and fulvic acid, which loosen the molecular bond. This step might be taken after by including EDTA and/or dimethyl sulfoxide to cause further weakening. Many studies reported that tetracycline is used to inhibit the apatite-binding protein synthesis, chelate calcium, and inhibit metallo-proteinase, however, doxycycline is more highly protein-binding and approximately 10 times more lipophilic than tetracycline. In addition, gentamycin can cause a reduction in the amount of the putative biofilm surrounding nanobacteria **(Demir, 2008; Abo-El-Sooud *et al.*, 2011)**.

Atherosclerotic heart disease

Recent studies have shown that nanobacteria may be a previously unknown and common factor in the development of atherosclerotic heart disease. These studies showed that nanobacteria are present in calcified atherosclerotic coronary arteries and heart valves **(Kumar *et al.*, 2011).** Nanobacteria cannot be identified by standard microbiological methods, and the convention for nanobacterial identification has not been well set up. Light microscopy, immune-histochemistry, electron microscopy, and amplification of 16S rDNA have been utilized by most investigators **(Kajander *et al.*, 1997; Kajander, 2006)**. Since nanobacteria are physically present in the infected atherosclerotic tissues and are measurably connected with heart disease calcification levels, it is additionally sensible to assume that long-term nanobacteria contamination is included within the improvement of the calcification in atherosclerotic heart disease. Atherosclerosis is a lifelong pathological process that can lead to heart disease, coronary artery disease, kidney disease, and many other chronic diseases. There are many available pieces of evidence that atherosclerotic disease is bacterial toxicity that is influenced by genetics and behavior, as small particles of nano-bacteria have been detected in kidney and liver stones that can develop and form a calcium apatite layer in calcified human atherosclerotic plaques. **(Puskas *et al.*, 2005)**. **Ye-Rong *et al.*,(2010)** reported that pathological calcification is present in cardiac valves with rheumatic heart disease. Calcification is one of the main factors that cause valve failure. These authors have detected, isolated, and cultured nanobacteria-like material from calcified cardiac valves with rheumatic heart disease that shared the characteristics of nanobacteria (Se90) recovered from mammalian blood **(Ye-Rong *et al.*, 2010)**.

Prostatic stones disease

The prostate is one of the most commonly diseased organs in Western men, and is responsible for millions of physician visits annually **(Jamal, *et al.*, 2003)**. The etiology of nonbacterial prostatitis and chronic pelvic torment disorder stays tricky and thus treatment is nonspecific, primarily focusing on symptom reduction. If an etiology may be recognized, at that point more particular treatment, focusing on the source might be created. Acute and chronic prostatitis and other incessant prostate conditions are regularly related with prove of inflammation, either acute, chronic, or both, as well as improvement of other histologic discoveries, such as nanobacteria, which contain calcium phosphate, aka apatite **(Stamey, 1981; Khan *et al.*, 2017)**. Investigators reported that nanobacteria are the main cause of prostatic stones, as the apatite core of 98% of prostatic stones was consistent with a nidus formed by nanobacteria. There was indirect evidence of nanobacteria on ELISA in 60% of blood and 40% of urine samples in patients with chronic pelvic pain syndrome due to chronic prostatitis **(Shoskes & Thomas, 2005)**. Prostatic calculi (aka corpora amylacea) are exceptionally common, display in >30% of radiographic studies of the male pelvis and a more prominent rate in men undergoing ultra-sound of the prostate for clinical determination, and transurethral resection of the prostate for the treatment of lower urinary tract side effects **(Geramoutsos *et al.*, 2004; O'Neill *et al.*, 2019)**.

Nanobacteria and Breast Cancer

Nanobacteria (NB) are novel microorganisms interceding microcalcifications in breast cancer. Nanobacteria have been appeared to contribute to distinguishing benign and dangerous calcifications within the shape of calcium phosphate crystals and contribute to threatening calcifications in breast cancer **(Altundag *et al.*, 2006)**. Breast calcifications are deposits of calcium that can be seen on a mammogram of the breast. There are two types: macrocalcifications and microcalcifications. Micro-calcifications are one of the foremost common abnormalities detected on screening mammography for breast cancer. Microcalcifications are specks of calcium within the breast. They may show up alone or in clusters since malignant microcalcifications comprise mostly calcium phosphate within the crystalline shape of hydroxyapatite, and nanobacteria have been shown to contribute to distinguishing benign and malignant calcifications within the frame of calcium phosphate crystals, nanobacteria may also contribute to malignant calcifications in breast cancer. This proposition should be approved by the microbiological investigation of micro-calcified breast cancer tissue **(Kajender & Ciftcioglu, 1998; Azam *et al.*, 2021)**. Calcification of delicate tissues, named ‘‘pathologic calcification,’’ is common in atherosclerotic arteries and engineered biomaterials such as aortic substitutions and heart valves. This kind of calcification moreover has been watched in breast capsules, both macroscopically and microscopically. It results from the accumulation of calcium and phosphate crystals. In any case, the mechanism of the calcification remains speculative **(Benjamin & Gay, 1977)**.

Antinanobacterial therapy

Different suggestions for antinanobacterial therapy are reported but still, it is under the experimental phase. The treatment for systemic diseases incorporates 500 mg tetracycline orally, a proprietary mixture of vitamin C, vitamins B3, B6, B9, selenium, EDTA, coenzyme Q10, bromelain, grapeseed extract, hawthorn berry, quercetin, L-arginine, L-lysine, L-ornithine, trypsin, and papain proteinase, a rectal suppository containing 1,500 mg EDTA for 3 months. Gallium nitrate (120 mg gallium) blended with water making two liters of a gallium mineral water drink to treat chronic, treatment-resistant kidney stones was also hypothesized **(Eby, 2008)**. For oral diseases, the treatment incorporates an antinanobacterial mouthwash or toothpaste containing bisphosphonates specifically etidronate and clodronate (1 mg/ml), gallium nitrate 14%, and EDTA (1%) are mainly recommended **(Kolahi, 2010)**. Chronic pelvic pain syndrome (CPPS) or chronic prostatitis is a common debilitating condition of unclear etiology. Patients often have prostatic calcifications but a link to symptoms is controversial. Nanobacteria are implicated in stone formation in the urinary tract and, therefore, therapy to eliminate nanobacteria and the stones that they produce might have an impact on CPPS symptoms. Wagenlehner and his coworkers performed a study in men volunteers to whom they administered 250 mg levofloxacin and 250 mg

ciprofloxacin orally at the same time and measured the concentrations in plasma, prostatic fluid, ejaculate, seminal fluid, and sperm cells after 3 h. There was a statistically significant difference in favor of levofloxacin for the prostatic fluid concentration, but not for the concentrations in the ejaculate, seminal plasma, and sperm cells **(Naber & Sorgel, 2003; Wagenlehner *et al.*, 2005)**. Fig 1 shows images of the contribution of nanobacteria in different human diseases.

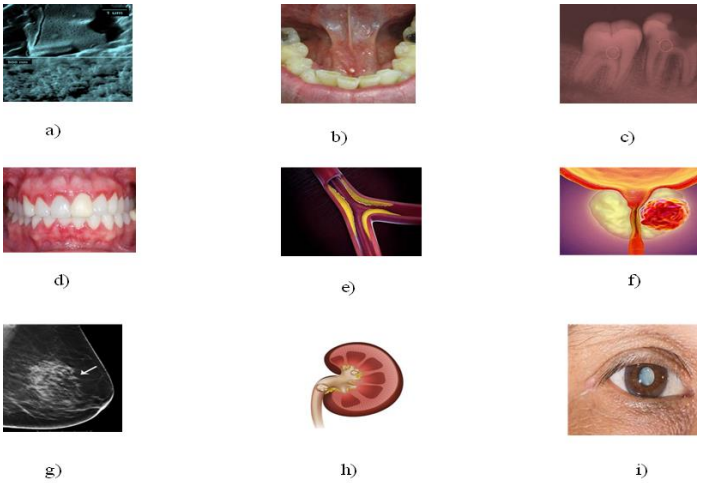

Figure 1 Images of the contribution of nanobacteria in different human diseases: a) Tooth plaque. b) Salivary stone. c) Dental pulp stone. d) Chronic periodontal disease. e) Atherosclerotic heart disease. f) Prostatic stones. g) Breast Cancer. h) Kidney stone i) Cataract eyes.

Mechanism of Calcification by Nanobacteria

Pathologic calcification by nanobacteria may be a complicated process, effectively controlled of mineralization process that is similar to bone formation and remodeling **(Wexler *et al.*, 1996)**. Mineralogists clarify that all that's required for crystal formation to begin is nidi (nucleus) and an environment of accessible dissolved components at or near saturation concentrations, along with the absence of inhibitors for crystal formation **(Jono *et al.*, 2006)**. Nanobacteria creating such nidi, if present in blood and urine, are exceptionally likely to accelerate pathologic calcification, which is clinically critical since blood contains phosphate close to its saturation level **(Driessens *et al.*, 1989; Blair & Fabrizio, 2000)**. Electron microscope studies have demonstrated that biologic calcification happens within the matrix vesicles in two steps, the primary is related to the beginning deposition of hydroxyapatite inside the lumen of the matrix vesicles and the second to the propagation of minerals outside the vesicles **(Poole *et al.*, 1989)**. Most pathologic calcifications all through the body contain blends of carbonate-substituted hydroxyapatite and octa-calcium phosphate. These ultramicroscopic crystals occur as snowball-like clumps which regularly can cause serious inflammation **(Gang *et al.*, 2021)**. Calcifying nanoparticles are the primary calcium-phosphorus mineral containing particles isolated from human blood and had been connected to pathologic calcification related diseases, such as arteriosclerosis, kidney stones, dental pulp stone formation, prostatitis, Alzheimer's disease, amyloidosis, polycystic kidney (PKD), and cancer **(Ciftcioglu, 2002; Ciftcioglu *et al.*, 2002; Hudelist *et al.*, 2004; Wen *et al.*, 2005; Zeng *et al.*, 2006; Zhou *et al.*, 2008)**.

CONCLUSION

Recent studies have proven that the presence of nanobacteria in humans is closely related to many diseases such as, breast cancer, prostatic stones disease, atherosclerotic heart disease, chronic periodontal diseases, salivary stone disease, and kidney stone disease, which need more extensive studies to reveal their nature and their impact on health in general. In addition, further investigations are needed to reveal the mechanism and pathological association between nanobacteria-like material and calcification of cardiac valves with rheumatic heart disease and to further understand the biological characteristics of these unique microorganisms. Nanobacteria may play an important role in the recurrence of salivary stones. So the use of calcium chelator such as EDTA before or in combination with the appropriate antibiotic may eradicate these stones and prevent their recurrence.

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