Association between Nanobacteria and Urinary Calcium Stone Disease

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Purpose: Nanobacteria have been reported to induce various pathologic calcifications like atherosclerosis and nephrolithiasis, and they do so by forming an apatite envelope, however, this concept is still controversial. We tried to elucidate whether nanobacteria might be related with urinary calcium stone by performing comparative study.

Materials and Methods: This study included 38 urinary stone patients who were proved to have calcium-containing stones and 37 healthy adults without urinary stone disease as controls. The subjects' age and gender were well matched between both groups. For the detection of nanobacteria, the serum and urine of all subjects were collected and western blotting for the samples was performed.

Results: There was no significant difference in the positive rate of nanobacteria from the serum samples between stone and control groups (52.6% vs 48.6%, respectively, p=0.469). But on the urine samples, the stone group showed a significantly higher positive rate than the control group (71.1% vs 21.6%, respectively, p<0.05).

Conclusions: Nanobacteria might have a relation with urinary calcium stone disease. (Korean J Urol 2007;48:512-516)

Key Words: Bacteria, Urolithiasis, Calcium

PURPOSE

Many diseases such as kidney stone disease, gall bladder stone, atherosclerosis, rheumatoid arthritis, etc are related with biocrystallization of calcium.¹ Especially, calcium is the main component in urinary stone and has much attention in the study of urolithiasis. The known mechanism of stone formation is the subsequent procedures such as urine supersaturation, crystal nucleation and aggregation, bringing about retention of crystals (nidi) and continued growth on the retained crystals. But all others who develop stones due to any unknown cause are referred to as idiopathic stone formers. Several theories have been put forward to explain the etiology of nephrolithiasis but none has been able to answer fully the questions concerning the mechanism of renal calculi formation.²

Recently, it was reported that a novel bacteria, nanobacteria, may be involved in the genesis of renal stone. These bacteria have been isolated from renal stones.³,⁴ These are the smallest described bacteria to date and are phylogenetically close relatives of mineral forming bacteria.⁵ It has been proposed that nanobacteria may serve as biomineralization centers as they produce carbonate apatite on their cell walls for the initiation of kidney stones, atherosclerosis, dental pulps, malignant calcification, etc.⁶-⁸ However, a significant controversy has erupted over the existence and significance of nanobacteria⁹,¹⁰ and there have been few clinical studies for the relationship between nanobacteria and urinary stone disease.

Therefore, the present study was conducted to investigate the detection rates of nanobacteria in serum and urine between calcium containing stone patients and healthy adults and evaluate the relationship between nanobacteria and urinary calcium stone.
MATERIALS AND METHODS

1. Patients population

Institutional Review Board approved of this study. This study included 38 urinary stone patients (mean age, 41.5 years from 19 to 77) who were proved to have calcium containing stones after percutaneous nephrolithotomy or ureteroscopic stone removal in Seoul National University Hospital between Sep., 2005 and Feb., 2006. As control, 37 healthy adults (mean age 47.9 years from 19 to 70) without urinary stone disease who visited Seoul National University Hospital Health Center were selected in the same period. History of urinary tract infection (UTI) had been absent in the healthy volunteers throughout their lifetime. Individuals having the history of cancer, gall bladder stone, and atherosclerosis which are known to be related with nanobacteria were not included in our study population.

2. Methods

1) Serum and urine sample collection: 10cc of each serum and urine samples were collected from the calcium stone patients before the operation and from the healthy volunteers when they visited the Health Center. In the stone patients, the serum and urine sample collection were done before intravenous administration of preoperative prophylactic antibiotics.

2) Detection of nanobacteria in serum and urine samples: For the detection of nanobacteria, serum and urine of all subjects were collected and western blot for the samples was performed. First, protein was extracted from serum and urine samples by the ordinary methods. Secondly, Samples containing equal amounts of serum and urine protein (20μg) were separated on each lane of 12% SDS-polyacrylamide gel and then transferred to 0.45 μm Western membrane (Amersham Pharmacia Biotech., USA) using standard electrophoresis procedures. The membrane was blocked with 5% skim milk in TBS-T (trypsin buffered saline, 1% Tween-20). Blots were immunolabeled with primary antibodies (1:1,000) for nanobacteria (NanobacOy, Kuopio, Finland). Immunoblots were washed and then incubated with secondary antibody (NanobacOy, Kuopio, Finland). Immunoreactivity was detected by chemiluminescence detection system (Intron, Gyeonggi, Korea).

3. Statistical analysis

Differences in nanobacteria detection rate were compared by Student’s t-test (unpaired). Categorical comparisons were performed using the chi square-test. Data are presented as means ± SD unless otherwise stated. The level for significance was set at p < 0.05.

RESULTS

1. Demographic data of patients

Table 1 shows the demographic data between stone group and control. There were no statistically significant differences in distributions of age, sex, and body mass index (BMI) between two groups. In stone group, 21 patients (55.3%) had a single stone episode, 12 (31.6%) had two episodes and 5 (13.1%) had more than two episodes.

2. The detection rate of nanobacteria in serum and urine protein

20 (52.6%) out of 38 calcium stone patients showed the positive results in Western blot assay for nanobacteria in serum and 18 (48.6%) of 37 healthy volunteers were positive in serum Western blot. There was no significant difference of the detection rate for serum Nanobacteria between two groups. In urine samples, the detection rates of nanobacteria in stone group and control were respectively 71.1% (27 out of 38) and 21.6% (8 out of 37). The positive rate of nanobacteria in urine sample in stone group was significantly higher than in control

Table 1. Demographic data and the positive rate of nanobacteria in serum and urine between control and stone group

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Stone</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>37</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Mean age (range)</td>
<td>41.5 (19-70)</td>
<td>47.9 (19-77)</td>
<td>0.110</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>0.270</td>
</tr>
<tr>
<td>Male</td>
<td>10 (27%)</td>
<td>7 (18.4%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>27 (73%)</td>
<td>31 (71.6%)</td>
<td></td>
</tr>
<tr>
<td>Mean BMI</td>
<td>24.6 ± 2.1</td>
<td>24.3 ± 2.3</td>
<td>0.18</td>
</tr>
<tr>
<td>Stone episodes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>—</td>
<td>21 (55.3%)</td>
<td></td>
</tr>
<tr>
<td>Two</td>
<td>—</td>
<td>12 (31.6%)</td>
<td></td>
</tr>
<tr>
<td>More than two</td>
<td>—</td>
<td>5 (13.1%)</td>
<td></td>
</tr>
</tbody>
</table>

BMI: body mass index
**Table 2.** Detection rate of nanobacteria in serum and urine between stone group and control in the Western blot

<table>
<thead>
<tr>
<th>Stone group</th>
<th>Control</th>
<th>Stone</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>18/37 (48.6%)</td>
<td>20/38 (52.6%)</td>
<td>0.465</td>
</tr>
<tr>
<td>Urine</td>
<td>8/37 (21.6%)</td>
<td>27/38 (71.1%)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

**Table 3.** Detection rate of nanobacteria in stone group according to the frequency of stone episodes

<table>
<thead>
<tr>
<th>Stone episodes</th>
<th>Serum (%)</th>
<th>Urine (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>10 (47.6)</td>
<td>11 (52.4)</td>
<td>21</td>
</tr>
<tr>
<td>Two</td>
<td>5 (41.7)</td>
<td>7 (58.3)</td>
<td>12</td>
</tr>
<tr>
<td>More than two</td>
<td>2 (40)</td>
<td>3 (60)</td>
<td>5</td>
</tr>
</tbody>
</table>

**Fig. 1.** Western blot analysis of urine protein. Nanobacteria Western blot of urinary protein from stone patients and normal control showed a single band at 39kDa. The detection rate of nanobacteria in urine from stone group is higher than normal control. C1-3: control group, S1-4: stone group, L: ladder.

(p<0.05) (Table 2) (Fig. 1).

**3. The detection rate of nanobacteria in stone patients according to the frequency of stone episode**

The detection rates of nanobacteria in single, two, and more than two stone episodes were respectively 52.4%, 58.3%, and 60% in serum (p=0.922) and 71.4%, 75%, and 80% in urine (p=0.919) (Table 3).

**DISCUSSION**

Kajander et al. also demonstrated a cytotoxic effect on renal tubules.

Further studies revealed that nanobacteria might cause atherosclerotic plaques, psammoma bodies seen in ovarian tumors, gall bladder stones, malacoplakia, acute periarteritis in which there are short of evident mechanisms in their pathogenesis. A common factor in these diseases is pathologic tissue calcification.

In urologic field, pathologic calcification is related with nephrolithiasis. Especially the theory of Randall’s plaque to explain the calcium stone formation supports the association between nanobacteria and urinary stone. Carr and Randall found small shiny deposits of calcium phosphate in kidney lymphatics and collecting ducts. Calcium phosphate formed above the collecting duct might induce heterogeneous nucleation at lower levels of the renal collection system. According to the hypothesis, the formation of kidney stone starts from these plaques due to a primary lesion in the tissue.

Recent studies on nanobacteria have produced findings suggesting that nanobacteria might be calcium phosphate nidi for kidney stone formation. Nanobacteria are renotropic, as reported from rabbit experiments using radiolabelled nanobacteria. Nanobacteria were found to adhere, invade and damage cells in collecting tubuli and the papillary area in the rat and rabbit models. Cuerpo et al. showed that when these bacteria were injected intravenously, they accumulate in the kidney and produced apatite.

Clinical evidences to show the relationship of nanobacteria to urolithiasis have been reported in a few studies. Ciftioğlu assessed nanobacteria in 72 consecutively collected kidney stones from Finnish patients and identified nanobacteria in 97.2% of the stones. Khullar et al. reported the presence of apatite forming, ultrafilterable gram negative, coccoid micro-
organisms in 62% of renal stones from an Indian population and characterized morphological and immunological feature of the nanobacteria. These results suggest that nanobacteria may cause urolithiasis like that Helicobacter pyroli develops chronic gastritis.

Our study compared the detection rate of nanobacteria in serum and urine of urinary stone patients and normal control. Nanobacteria were shown to be detected at higher rates in the urine of persons with urinary calcium stone, although there was no significant difference in nanobacterial presence in serum. Taken together, these results implicate that urinary nanobacteria may have more important role in the formation of nephrolithiasis than serum nanobacteria.

We analyzed the detection rate of Nanobacteria in stone patients according to the frequency of stone episode. Although there is no statistically significant relationship between the number of stone episodes and the detection rate of nanobacteria in serum and urine, the patients with more than two stone episodes show higher detection rates of nanobacteria in serum and urine than patients with one stone episode. In spite of a limitation of study size, the proportional tendency of nanobacteria detection rate and frequency of stone episodes suggests relationship between nanobacteria and urinary calcium stone.

Unfortunately, we admit the fact that the present study has a few limitations. It would be difficult to conclude that the higher positivity of nanobacteria in urine from stone patients implicates that nanobacteria cause urinary calcium stone. But in addition to the established studies to identify nanobacteria from renal stones, the existence of nanobacteria in more urine samples of calcium stone patients compared to non-stone patients in our study suggests that urinary calcium stone may be related with nanobacteria. Nanobacteria research, in spite of its promising and exciting theme to explain new mechanism of pathologic calcification, have elicited some controversy. In their short existence, there has been controversy over whether they are microorganisms, self-replicating particles, or by-products of known unicellular organisms. But many studies containing the present study to elucidate the association between nanobacteria and urinary stone disease support the existence of nanobacteria and further study for nanobacteria will lead to a breakthrough in the fight to nephrolithiasis.

## CONCLUSIONS

It is likely that nanobacteria might have a relationship with urinary calcium stone disease because urinary calcium stone patients have higher detection rate in urine than healthy volunteer.

## REFERENCES