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Oxalate-Degrading Capacities of Gastrointestinal Lactic Acid Bacteria and Urinary Tract Stone Formation

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Abstract

***Background*:** Calcium oxalate is one the most significant causes of human kidney stones. Increasing oxalate uptake results in increased urinary oxalate. Elevated urinary oxalate is one the most important causes of kidney stone formation. This study aims to evaluate oxalate-degrading capacity of lactic acid bacteria and its impact on incidence of kidney stone.

***Materials and Methods*:** This case-control study was conducted on serum, urinary, and fecal samples. The research population included a total of 200 subjects divided in two equal groups. They were selected from the patients with urinary tract stones, visiting urologist, and also normal people. The level of calcium, oxalate, and citrate in the urinary samples, parathyroid and calcium in the serum samples, and degrading activity of fecal lactobacillus strains of all the subjects were evaluated. Then, data analysis was carried out using SPSS-11.5, χ^2 test, Fisher's exact test, and analysis of variance.

***Results*:** The results revealed that the patients had higher urinary level of oxalate and calcium, as well as higher serum level of parathyroid hormone than normal people. In contrast, urinary level of citrate was higher in normal people. In addition, there was a significant difference between the oxalate-degrading capacities of lactobacillus isolated from the patients and their normal peers.

***Conclusion*:** Reduction of digestive lactobacillus-related oxalate-degrading capacity and increased serum level of parathyroid hormone can cause elevated urinary level of oxalate and calcium in people with kidney stone.

Introduction

Hypercalciuria (urinary excretion of more than 800 mg of calcium per day) and hyperoxaluria (urinary excretion of more than 400 mg of oxalate per day) are among the most important pathophysiologic causes of kidney stone formation. They are directly related to calcium-oxalate rich diet. In addition, the mentioned complications result in 50% increase in calcium and oxalate concentration in urinary tracts, as well as increased level of insoluble precipitates of calcium oxalate or calcium phosphate in kidney [1-4]. Park et al. showed that, due to absorption of calcium from the bones, elevated parathyroid hormone (PTH) can intensify hypercalciuria and the formation of calcium oxalate in kidney, up to 80% [5]. Oxalate as a toxic compound is harmful to human. Consumption of oxalate rich plant foods and increased digestive absorption of free oxalate can cause kidney stone formation and other pathologic complications, due to elevated urinary level of oxalate. Almost 60-80% of renal stones are calcium oxalate [6, 7]. Gastrointestinal microbiota causes 40% decrease in urinary level of oxalate and significant reduction of oxalate stone formation in kidneys, when they consume oxalate as a source of carbon and energy [8-10].

Allison et al., for the first time, reported that obligate anaerobic bacteria in the human digestive system and other vertebrates are capable of using oxalate as a source of carbon and energy [11]. In addition, some studies showed that probiotic bacteria, especially bifidobacterium spp. and lactobacillus spp. are capable of degrading oxalate into carbon dioxide and formate [8-12]. Other studies attributed the increased urinary level of oxalate in people with kidney calcium oxalate stone to inadequate and reduced number of digestive oxalate degrading bacteria. These suggested that the existence of oxalate-degrading bacteria is critical in reduction of kidney stones (calcium oxalate) formation [13, 14]. Altermann et al. evaluated the lactobacillus in food, and showed that these digestive Gram-positive bacteria are critical in prevention of kidney stone (calcium oxalate) formation [15]. The underlying reason is that these bacteria are highly potential in degrading oxalate, existing in variety of available foods, to formate and carbon dioxide. In this regard, they reduce the toxicity of oxalate and so prevent formation of kidney stones (calcium oxalate). Kidney stone formation can cause several kidney diseases. Consuming oxalate/ calcium rich foods increases the intestinal absorption of oxalate, formation of calcium

oxalate crystals and deposits, and renal hypertension, which eventually results in acute and chronic renal failure. In recent studies, formation of stone in urinary tracts has been reported [15-18]. The purpose of this study was to compare digestive lactobacillus degradation-capacity of normal people and patients with kidney stone. In addition, its role in decreasing the risk of kidney stones formation was investigated.

Materials and Methods

This case-control study was carried out in 2008-2009 on urinary, fecal, and serum samples of 100 healthy people (without history of kidney stone or urinary problems) and 100 patients (with history of kidney stone), referring to urology division of Shahid Motahhari hospital, Jahrom. The healthy subjects and patients were screened after a kidney ultrasound procedure and based on clinical symptoms under supervision of urologic physician. In addition, the approval of the Ethics Committee of University was beforehand obtained by clarifying clinical and ethical obligations and completing the questionnaire. Regarding one out of seven Iranians are with kidney stone, according the studies, 105 subjects were determined for each of groups (210 subjects in total), i.e. experimental and control, using statistical sample size formula with 95% reliability. In order to measure the concentration of urinary oxalate, calcium, and citrate, 24-hour urine samples of the subjects were collected. To evaluate urinary oxalate, specific enzymatic kit (Kaveh Treatment Co.) was utilized. The fundamental function of the kit is to degrade oxalate to carbon dioxide and hydrogen peroxide by oxidize oxalate enzyme. This enzyme converts hydrogen peroxide to a colored complex in the presence of a suitable chromogen. Then, the concentration of oxalate was measured by taking the readings of reaction product at wavelength of 578 nm and comparing it with the standard curve. In addition, the level of urinary calcium was measured according to the instruction of the enzymatic kit. To measure urinary citrate, citrate measurement enzymatic kit (Kaveh Treatment Co.) was used. It works by converting citrate to alpha-keto acid, using citrate lyase enzyme, and taking readings at wavelength of 330 nm.

After preparation of 5 mm peripheral blood samples, the concentration of calcium ion was measured using the kit's instruction by the means of automatic electrolyte analyzed (AVL, Austria). For measuring serum level of parathyroid hormone, enzyme immunoassay (EIA) technique was used, according to the manufacturer's instruction (Mono Bind, USA). At first, the stool samples were enriched in MRS environment (Merck Co., Germany) at 37°C in anaerobic conditions using Gas-Pak (BBL Co., U.S.A.) for 48 hours. Then, they were transferred to Tomato Juice Agar Special (Merck, Germany) cultivation environment and stored at 37°C for 72 hours in anaerobic condition. Next, gastrointestinal lactobacillus species were determined at the surface based on: morphologic properties, Gram stain, catalase, oxidase, carbohydrates fermentation, and specific biochemical tests introduced in Bergey's Manual of Systematic Bacteriology [19]. First, qualitative capacity of using oxalate of gastrointestinal lactobacillus by the means of B and MRS specific cultures containing 4 mM ammonium oxalate was studied at 37°C and in anaerobic condition for 48 hours [20, 21]. Then, all the strains were inseminated in MRS culture containing 0.05, 0.1, 0.15, and 0.2% (4, 8, 12, and 16 mM, respectively) ammonium oxalate and were stored at 37ºC, for 24 hours. The oxalate enzymatic kit (Kaveh Treatment Co.) was used for quantitative estimation of oxalate degradation. The standard curve was plotted using 0.2, 0.4, and 0.6 mM oxalate. Then, the readings of optical absorption of above mentioned environments were taken at wavelength of 578 nM using spectrophotometer (Apple Co., Japan). Next, the level of oxalate degradation was recorded by comparing the readings with the standard curve. *Lactobacillus acidophilus* (PTCC1643) and *Lactobacillus casei* (PTCC1608) bacteria were used as positive-control. The obtained results were analyzed using SPSS-11.5), χ^2 test, Fisher's exact test, and ANOVA. The significant threshold of $p<0.05$ was set.

Results

By age, it was observed that kidney stone formation is more common in 31-50 (58.06%) and 51-70 (42.11%) years old men and women, respectively. The results from ANOVA revealed a significant correlation between age and gender of the patients under investigation ($p=0.001$). In addition, the incidence of hyperoxaluria and hypercalciuria complications was higher in the patients group. That is, elevated levels of urinary oxalate and calcium were, in turn, observed in 56% and 72% of the patients with kidney stone. ANOVA and Fishetr's exact test revealed a significant correlation between increased urinary oxalate and calcium, and consumption of oxalate rich food and dairy in patients with kidney stone ($p=0.001$ in all cases) (Table 1). Elevated levels of urinary calcium and serum parathyroid hormone were observed in the patient, at the same time. ANOVA test demonstrated a significant correlation between elevated level of parathyroid hormone and increased degree of kidney stone formation ($p=0.013$) (Fig. 1). In addition, the level of serum calcium was higher in the patients. Moreover, ANOVA test depicted a significant correlation between kidney stone formation and increased level of serum calcium in the patients. The results showed that the level of urinary citrate in the patients was lower. ANOVA test revealed a significant correlation between kidney stone formation and decreased level of urinary citrate in the patients to healthy subjects ($p=0.001$) (Fig. 2). In general, lactobacillus spp. bacteria were identified in 128 (64%) subjects. Out of all isolated bacteria, 26 (20.31%) and 64 (50%) cases were related to healthy women and men, and 14 (10.94%) and 24 (18.75%) were related to women and men in the patients group, respectively. Fisher's exact test revealed a significant correlation between kidney stone formation and lack of gastrointestinal lactobacillus

settlement in the patients (p=0.001). In addition, ANOVA test demonstrated a significant correlation between kidney stone formation, reduction of oxalate-degrading lactobacillus, and increased level of urinary oxalate in the patients (p=0.002) (Fig. 3). Estimation of oxalate-degrading capacity in isolated lactobacillus showed that all of them had ammonium oxalate (4 mM) degrading capacity. Moreover, the degradation levels of ammonium oxalate of 8 and 12 mM in the mentioned bacteria were, respectively, 90 and 57.81%. However, none of the studied lactobacillus could degrade ammonium oxalate of 16 mM (Fig. 4). The history of taking antibiotic was lower in the healthy subjects. Fisher's exact test revealed a significant correlation between taking antibiotic and existence of lactobacillus (p=0.001) (Fig. 5).

Table 1. Number and ratio (in percent) of increased urinary and serum parameters in the healthy subject and patients with kidney stone

Urinary and serum parameters	Increasing per day	Healthy subjects	Patients
Urinary oxalate	Over 6 mM	0	56
Urinary calcium	Over 250 mg	22	46
Urinary citrate	Over 50 ng	70	30
Serum parathyroid hormone	Over 3 mM	12	72

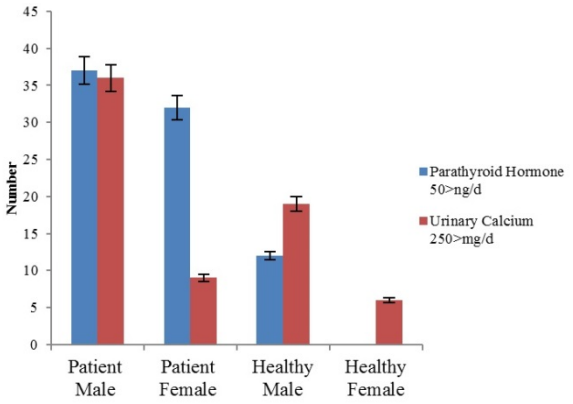

Figure 1. Absolute frequency and the ratio of the studied subjects based on increased level of urinary calcium and parathyroid hormone

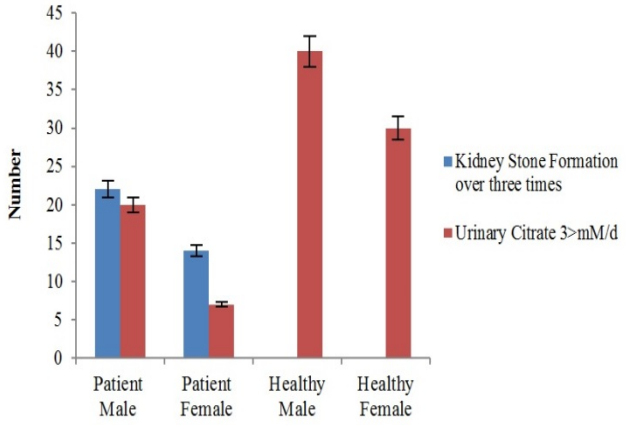

Figure 2. Absolute frequency and the ratio of the studied subjects based on decrease level of urinary citrate and over three times frequency of kidney stone formation

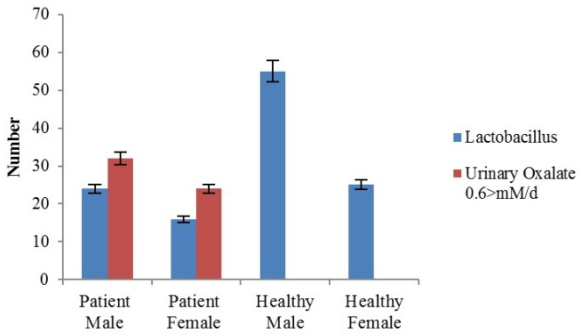

Figure 3. Absolute frequency and the ratio of the studied subjects based on the existence of lactobacillus and increase concentration of urinary oxalate

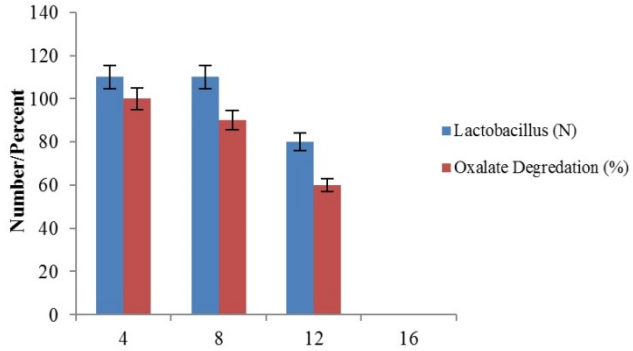

Figure 4. Absolute frequency and the ratio of oxalate-degrading lactobacillus in different concentration of ammonium oxalate

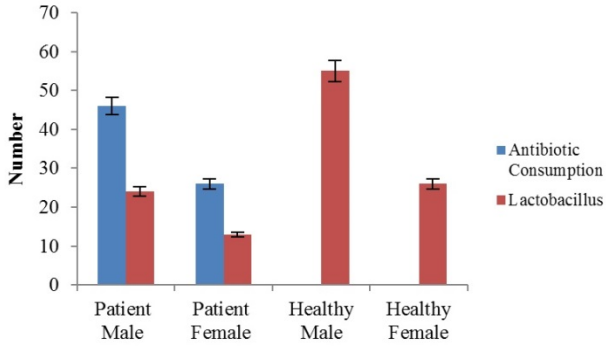

Figure 5. Absolute frequency and the ratio of the studied subjects based on history of taking antibiotic and lactobacillus isolation

Discussion

Excretion of urinary oxalate is one the most important causes of kidney stone formation. Unfortunately, suitable methods for reducing the level of oxalate for preventing urinary tract stone formation have not been identified. Vegetable oilseeds and ascorbic acid metabolic alternate pathways are the main sources of oxalic acid. In addition, in most cases, oxalic acid and calcium ion complex can cause formation of insoluble calcium oxalate salts, leading the renal failure [13, 22]. Studies show that some diets and lack of oxalate-degrading gastrointestinal enzymes can cause uptake and increased level of urinary oxalate, leading to hyperoxaluria and renal failure [20-24].

Moreover, some studies demonstrate that increased consumption of calcium rich foods or excitement of parathyroid hormone secretion can also lead to 35 to 65% increased excretion of urinary calcium and formation of renal calcium oxalate stones [5-7]. Our results from this study show that consumption of oxalate and calcium rich food and increased level of parathyroid hormone secretion are of the major causes of increased excretion of urinary oxalate and calcium and formation of calcium-oxalate stones.

Allison et al., for the first time suggested the role of oxalate-degrading bacteria in ruminant digestive system [11]. Studies by Hoppe et al. showed that digestive system had the greatest role in oxalate-degradation. In addition, they demonstrate that the role of gastrointestinal oxalate-degrading bacteria in reducing the risk of kidney stone was greater than that of diet [21]. Studies conducted by Campiri et al., Lieske et al., and Azcarate-Peril et al., on the role of gastrointestinal bacteria and their impact on urinary oxalate-degradation reveal that probiotic bacteria, especially lactobacillus ones, had high oxalate-degrading potential in the environment containing 5-10 mM ammonium oxalate [8, 9, 13]. In addition, Azcarate-Peril and Altermann proved the critical role of oxalyl-CoA decarboxylase and formyl-CoA transferase coding genes, i.e. oxc and frc, in oxalate-degrading process, by investigating the genomes of lactobacillus bacteria [13, 15]. Additionally, Turroni et al., and Siva et al., in their studies investigated the gastrointestinal lactic acid bacteria and categorized them on a 0-100 percent scale based on oxalate degradation. The underlying reason was the existence of oxalyl-CoA decarboxylase and formyl-CoA transferase as the main causes of oxalate degradation in the mentioned bacteria [17, 25].

Furthermore, Abratt et al., proved the role of lactobacillus strains, containing the above enzymes, in gastrointestinal oxalate degradation [18]. David et al., as well, showed that consumption of lactobacillus by patients led to significant decrease in hyperoxaluria [26].

We, for the first time in Iran, evaluated the role of oxalate-degrading lactobacillus in reduction of kidney stone disease.

The study surveyed the subject by researching one group of healthy people and one group of people with kidney stone. Similar to the mentioned studies, our findings indicate the effective role of lactobacillus bacteria in reducing the incidence of kidney stone problem. In addition, our findings demonstrate that most of the lactobacillus strains could degrade 4-8 mM ammonium oxalate. This is in consistent with Azcarte-Peril study [13]. Lefaucheur et al. in study of the patients, undergoing heart and liver transplant, observed that most of them would encounter with acute renal failures accompanied by acute oxalonephropathy, after a while [27].

They showed that long-term use of antibiotics such as cephalosporins, cotrimoxazole, and quinolones by the mentioned patients would cause decreased settlement of intestinal lactobacillus, leading to acute renal failures. Our results also demonstrated a direct correlation between the history of consuming antibiotics, lack of oxalate-degrading lactobacillus settlement, increased level of urinary oxalate, and frequency of stone formation in the patients under investigation. Therefore, it was determined that lack of gastrointestinal lactobacillus was one of the main causes of increased level of urinary oxalate and formation of renal stones in the population under investigation. Since gastrointestinal lactobacillus bacteria are of the most important gastrointestinal probiotic bacteria, further investigation on them can positively influence people health [28].

Regarding the significant correlation between oxalate-degrading bacteria and decreased incidence of kidney stone in the people under investigation, conducting broader studies on different ethnic groups in other parts of the country is recommended. In addition, informing people who are at more risk can have an important role in correcting their diet and kidney stone prevention. However, the main question is that “whether other gastrointestinal bacteria can degrade oxalate and decrease renal complications caused by it?” For a detailed answer to this question, researching broader populations and investigating the role of other bacteria, especially oxalobacter and other probiotic bacteria, are needed.

The results showed that diet, serum parameters especially parathyroid hormone, urinary factors, gastrointestinal oxalate-degrading bacteria, and antibiotic consumption history are the most important causes of kidney stone formation in the population under investigation. Regarding the probiotic role of lactobacillus, molecular monitoring of the strains that produce genes affecting oxalate degradation and evaluation of their probiotic adequacy in future studies are recommended.

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Authors’ Contributions

The project design and manuscripts revision were performed by Dr. Mohammed Kargar. Practical works and statistical analyses were carried out by Rouhi Afkari and Sadegh Ghorbani-Dalini.

Conflict of Interest

The authors declare no conflict of interest.

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