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Original Article

Do low vitamin D levels facilitate renal parenchymal injury?

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Abstract

Background Decreased vitamin D levels lead to an increase in infectious diseases, including urinary tract infections (UTIs).

Objective To assess serum vitamin D levels in children with renal parenchymal injury secondary to UTIs.

Methods Forty-three upper UTI patients and 24 controls, aged 1-15 years, were included. Vitamin D levels and other laboratory tests were obtained when they first admitted to hospital. 99mTc-labeled dimercaptosuccinic acid (DMSA) scans were performed to evaluate renal parenchymal injury.

Results Mean serum 25-hydroxyvitamin D (25(OH)D) was lower in the upper UTI group compared to the control group [18 (SD 9) *vs*. 23 (SD 10.6) ng/mL, respectively; P=0.045]. The upper UTI group was sub-divided into two groups, those with 22 (51.1%) and without 21 (48.8%) renal parenchymal injury. Mean 25(OH)D was significantly lower in patients with renal parenchymal injury [15.1 (SD 7.1) *vs*. 21 (SD 9.9) ng/mL, respectively; P=0.03]. The renal parenchymal injury cases were further sub-divided into two groups: 8 patients (36.3%) with acute renal parenchymal injury and 14 (63.6%) with renal scarring (RS), but there was no significant difference in 25(OH)D between these two groups [12.5 (SD 8.9) *vs*. 16.6 (SD 5.7) ng/mL, respectively; P=0.14).

Conclusion Decreased vitamin D is associated with renal parenchymal injury in children with upper UTIs. However, vitamin D is not significantly decreased in renal scarring patients compared to acute renal parenchymal injury patients. **[Paediatr Indones. 2020;60:205-11 ; DOI: 10.14238/pi60.4.2020.205-11]**.

Keywords: *child; upper UTI; renal injury; vitamin D*

The prevalence of urinary tract infections (UTIs) is 7.8% in children and adolescents.[1] After infancy, it decreases in boys, while it increases in girls.[2] The complications of UTI have long been known, one of which is renal scarring (RS), which develops in 15-60% of children with upper UTIs.[3] As the number of pyelonephritis events increases, the risk of RS increases concomitantly.[4] The 99mTc-labeled dimercaptosuccinic acid (DMSA) scan is the gold standard method for diagnosing RS.[5] Extensive RS may progress to hypertension, proteinuria, and end-stage renal disease.[6]

Vitamin D is a vital hormone, primarily necessary for calcium homeostasis and bone health. Vitamin D deficiency is defined as less than 20 ng/mL of serum 25-hydroxyvitamin D25[(OH)D].[7] Vitamin D also stimulates cathelicidin synthesis. These molecules have direct antimicrobial effects against many different microorganisms.[8] Observational studies have shown that vitamin D deficiency plays an important

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role in respiratory tract infections, sepsis, UTIs, and hepatic fibrosis.[9-12] The aim of this study was to investigate for a potential relationship between vitamin D and renal parenchymal injury in children with UTIs.

Methods

This prospective study included pediatric patients admitted to our hospital between April 2016 and November 2017 with a diagnosis of upper UTI. Diagnoses were established in patients with complaints like fever, abdominal pain, anorexia, and nausea, as well as positive urine cultures.[5,13,14] Urine samples were collected with a plastic bag or urinary catheter in patients under 2 years of age; midstream urine samples were obtained from older children. Only urine culture-positive patients (≥105 bacterial colony growth in 1 mL of culture in a midstream clean catch or sterile plastic bag, or ≥104 colony count by catheter) were included. Individuals with signs of puberty were accepted as adolescents. Children with ≥2 upper UTIs during follow-up were considered to have recurrent UTI.

The DMSA scanning was ordered to evaluate renal parenchymal injury, and performed no later than 2 weeks after hospital admission.[15] The DMSA was requested only once for each patient. Acute renal parenchymal injury was defined as the presence of focal, multifocal, or diffuse decreased DMSA uptake in the involved kidney without volume loss.[16] Renal scarring was defined as the presence of focal or multifocal decreased DMSA uptake and loss of volume in the involved renal cortex.[16] The DMSA images were investigated by a nuclear medicine specialist. Voiding cystourethrogram (VCUG) was ordered, with the latter performed after completion of antibiotic treatment.

At time of admission white blood cells (WBC), C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were performed. Neutrophil-to-lymphocyte ratio (NLR) was calculated as the ratio of absolute neutrophil count (ANC) to absolute lymphocyte count (ALC). Serum 25(OH)D level was a good indicator of vitamin D status because its half-life is as long as 2-3 weeks.[17] Serum specimens of 25(OH)D collected from patients were frozen at -80°C. Serum 25(OH)D level was measured by enzyme-linked immunosorbent assay (ELISA) kit (*Hangzhou Eastbiopharm Co. Ltd.*, Hangzhou, China).

Children who had recovered from acute nasopharyngitis, acute otitis media, acute gastroenteritis, or seborrheic dermatitis were accepted as the control group. These children were admitted to our hospital during the same time period as the patient group. Subjects' parents or guardians provided written informed consent form. None of the subjects were using supplemental vitamin D. Children with renal disorders, epilepsy, malnutrition, obesity, diabetes mellitus, immune deficiency, or rickets were excluded from the study.

The data were analyzed using *SPSS version 20.0* (*SPSS Inc.*, Chicago, IL, USA). Descriptive analyses were expressed as percentages for categorical variables and mean ± one standard deviation (SD) for continuous variables. In the statistical evaluation, Chi-square test (for categorical variables), student T-test (for continuous variables), Mann-Whitney U test (for non-normally distributed variables), and correlation analyses were used. A P value < 0.05 was considered to be statistically significant. This study received ethical approval from the Ethics Committee of Bolu Abant İzzet Baysal University.

Results

Sixty-seven individuals, comprised of 43 upper UTI patients and 24 controls, were included in the study. Patients'ages ranged from 1-15 years, with a mean age of 6.9 (SD 4.2) years in the upper UTI group. Thirteen (30.2%) upper UTI patients were male and 30 (69.7%) were female. The groups were similar in terms of age, gender, and age group. The mean WBC, ANC, CRP, and ESR values of the upper UTI group were significantly higher than the those of the control group. Additionally, mean serum 25 (OH) D levels in upper UTI group were significantly lower than those in control group [18 (SD 9) *vs.* 23 (SD 10.6) ng/mL, respectively; P=0.045) (**Table 1**).

The upper UTI group was sub-divided into two groups: 22 (51.1%) with and 21 (48.8%) without renal parenchymal injury, according to the DMSA results. Both groups were similar in terms of mean age, age group, and gender. Laboratory findings of ANC, NLR, CRP, ESR, pyuria, and hematuria were

Table 1. Comparison of features of the upper UTI and control groups

Characteristics	Upper UTI (n=43)	Control (n=24)	P value
Mean age (SD), years	6.9 (4.2)	7.9 (3.8)	0.3*
Gender (F/M), n	30/13	13/11	0.2*
Age group (C/A), n	31/12	16/8	0.64*
Mean WBC (SD), /L	10,983 (3,206)	8,198 (2,271)	0.002
Mean ANC (SD), /L	6,229 (1,986)	4,283 (1,765)	0.001
Mean ALC (SD), /L	3,338 (1,873)	2,492 (658)	0.055
Mean NLR	2.4 (1.8)	1.8 (0.7)	0.14*
Mean CRP (SD), mg/dL	51.5 (48.8)	2.6 (2)	<0.001*
Mean ESR (SD), mm/h	39.5 (25)	8 (4.5)	0.01*
Mean 25 (OH) D (SD), ng/mL	18 (9)	23 (10.6)	0.045

*Mann-Whitney U; C: child; A: adolescent

also not significantly different between the two groups. Fourteen (32.5%) cases had been diagnosed with UTI for the first time, and 29 (67.4%) had recurrent UTIs. The majority of both groups had recurrent UTIs. We found that WBC was significantly higher in patients with than without renal parenchymal injury [12,065 (SD 3,359) *vs.* 9,630 (SD 2,518), respectively; P=0.041). In addition, 25(OH)D was significantly lower in patients with than without renal parenchymal injury [15.1 (SD 7.1) *vs.* 21 (SD 9.9) ng/mL, respectively; P=0.03] (**Table 2**).

The 22 patients with renal parenchymal injury were divided into two groups: 8 with acute renal parenchymal injury and 14 with RS, according to the DMSA findings. Mean WBC was significantly higher in patients with acute renal parenchymal injury than those with renal scarring [15,136 (SD 2332) *vs.* 10,530 (SD 2697), respectively; P=0.006]. However, mean 25(OH)D was not significantly lower in patients with acute renal parenchymal injury [12.5 (8.9) *vs.* 16.6 (5.7) ng/ml, respectively; P=0.14]. The other features were also not significantly different between two groups (**Table 3**).

Fifteen of the renal parenchymal injury patients were diagnosed with recurrent UTI. Mean 25(OH)D was lower in patients with recurrent UTI than in those with first UTI, but the difference was not significant [17.4 (10) *vs.* 19.2 (6.7) ng/mL, respectively; P=0.53]. Twelve of the renal parenchymal injury patients had VUR, but there was no significant difference in mean 25(OH)D between those with and without VUR [14.6 (6.2) *vs.* 20.9 (10.7) ng/mL, respectively; P=0.057]. On the other hand mean 25(OH)D was significantly lower in patients with acute renal parenchymal injury

Table 2. Comparison of upper UTI patients with and without renal parenchymal injury

Variables	With renal parenchymal injury (n=22)	Without renal parenchymal injury (n=21)	P value
Mean age (SD), years	6.9 (4.3)	6.9 (4.1)	0.97*
Gender, F/M, n	17/5	13/8	0.27*
Age group, C/A, n	15/7	16/5	0.92*
Mean WBC (SD), /L	12,065 (3,359)	9,630 (2,518)	0.041
Mean ANC (SD), /L	6,854 (1,839)	5,447 (1,953)	0.066
Mean ALC (SD), /L	3,338 (1,442)	3,339 (2,377)	0.32*
Mean NLR (SD)	2.3 (0.9)	2.6 (2.6)	0.79*
Mean CRP (SD), mg/dL	58.8 (63.9)	40.7 (8.9)	0.76*
Mean ESR (SD), mm/h	53 (31.7)	26 (2.6)	0.4*
Pyuria, n	14	10	0.29*
Hematuria, n	10	6	0.25*
VUR, n/total	12/21	3/11	0.18*
Recurrent UTI, %	68.1	66.6	0.91*
Mean 25 (OH) D (SD), ng/mL	15.1 (7.1)	21 (9.9)	0.03

*Mann-Whitney U; VUR: vesicoureteral reflux)

than the those of the control group [12.5 (8.9) *vs.* 23 (10.6) ng/mL, respectively; P=0.018]. In addition, mean 25(OH)D was also significantly lower in patients with renal scarring than the those of the control group [16.6 (5.7) *vs.* 23 (10.6) ng/ml, respectively; P=0.045] (**Table 4**).

Table 3. Comparison of features of renal parenchymal injury types

Variables	Acute renal parenchymal injury (n=8)	Renal scarring (n=14)	P value
Mean age (SD), years	6 (4.1)	7.5 (4.4)	0.48*
Gender, F/M, n	6/2	11/3	0.92*
Age group, C/A, n	7/1	9/5	0.4*
Mean WBC (SD), /L	15,136 (2,332)	10,530 (2,697)	0.006
Mean ANC (SD), /L	7,378 (852)	6,593 (2,171)	0.45
Mean ALC (SD), /L	2,956 (1,189)	3,529 (1,576)	0.48
Mean NLR (SD)	2.8 (1.1)	2 (0.7)	0.17
Mean CRP (SD), mg/dL	65.5 (69)	25 (??)	0.66*
Mean ESR (SD), mm/h	36 (16.9)	87 (??)	0.99*
Pyuria, n	5	9	0.99*
Hematuria, n	4	6	0.81*
VUR, n/total	4/7	8/14	0.98*
Recurrent UTI, %	75	64.2	0.71*
Mean 25 (OH) D (SD), ng/mL	12.5 (8.9)	16.6 (5.7)	0.14*

*Mann-Whitney U

Table 4. Comparison of 25 (OH) D levels of renal parenchymal injury types and control group.

Variables	Acute renal parenchymal injury (n=8)	Renal scarring (n=14)	Control (n=24)	P value
Mean 25(OH)D (SD), ng/mL	12.5 (8.9)[a]	16.6 (5.7)[b]	23 (10.6)[c]	0.018[a,c] 0.045[b,c]

Discussion

Vitamin D deficiency is a major, worldwide, public health problem. It is associated with cancer, autoimmune disease, and cardiovascular disease.[18] Vitamin D deficiency causes dysregulation in the immune system. In particular, changes in cathelicidins are associated with infection. Cathelicidins attracts cell such as dendritic cells, T cells, monocytes, macrophages, and neutrophils to sites of inflammation.[19,20] Therefore, they are considered to be an important component of the innate immune system with a role in inflammation.[21,22] Abundant amounts of cathelicidins in neutrophils have antibacterial (against both Gram-positive and -negative), antifungal, and antiviral effects as they act to destroy cell membrane integrity.[23-25] Since vitamin D receptors are present on dendritic cells, T cells, monocytes, and neutrophils,[20] there is likely a relationship between vitamin D and cathelicidins. Vitamin D deficiency causes an increase in infections as cathelicidins decrease.[18] An UTI is an inflammatory response that occurs as a result of bacterial adhesion to the uroepithelium. Leukocytes, dendritic cells, macrophages, and other cells with cathelicidins move to the affected renal parenchymal area during UTIs.[26] The UTIs can worsen due to impaired function of these cells which have vitamin D receptors. We found a significant relationship between decreased serum vitamin D and upper UTIs, in agreement with another study.[11] Past studies have noted a relationship between vitamin D deficiency and recurrent respiratory infections.[27,28] However, there are few publications on the relationship between recurrent UTIs and decreased vitamin D level.[29] We did not find such a relationship in our study.

Vitamin D deficiency has been associated with upper UTIs.[11] Granulocytes and released bacterial toxins together cause parenchymal injury through

capillary obstruction and hypoxia. Free oxygen radicals released in the hypoperfusion-reperfusion cycle are considered to be the reason for post-infectious renal parenchymal injury.[30] Inflammatory cells, in particular, granulocytes due to the enzymes they contain, are responsible for renal parenchymal injury.[31] Vitamin D was found to be effective on apoptosis, apart from the inflammatory response. Vitamin D has also been shown to protect kidney function by inhibiting apoptosis,[32] and vitamin D deficiency causes increased apoptosis of renal tubular cells.[33] Vitamin D deficiency leads to a decrease in megalin, which is a receptor for 25(OH)D–D-binding protein in renal proximal tubules,[32] thus increasing the effects of the disease even further. Our study showed that renal parenchymal injury may be associated with decreased serum vitamin D levels. Also, serum vitamin D levels were lower in renal parenchymal injury types when compared to the healthy subjects. However we couldn't find a difference in serum vitamin D levels between renal parenchymal injury types. We think the small number of subjects may have caused this result.

Other than bacterial virulence factors, severity of inflammation is the most important factor affecting renal parenchymal injury. The most important indicator of this severity is the increase in acute phase reactants.[34] The NLR is also used as a diagnostic marker of upper UTI.[35] We demonstrated the severity of inflammation with high WBC in patients who had acute renal parenchymal injury. However, the other markers were not significantly different in these patients.

Renal scarring is a result of renal parenchymal inflammation. Bacterial infections activate phagocytotic neutrophils and macrophages. Phagocytosis leads to the release of cytokines, arachidonic acid, oxygen radicals, and lysosomal enzymes. This process, while intending to destroy bacteria, also results in tissue injury and eventually scar formation.[36] Macrophages induce fibrosis through activation and proliferation of fibroblasts.[37] Vitamin D is known to have significant effects on collagen synthesis and degradation; as such, it may have anti-proliferative effects, especially in fibroproliferative diseases. These effects are displayed via transforming growth factor-β1 and hepatocyte growth factor. In vitamin D deficiency, suppression of the transforming growth factor-β1 gene, which is effective for fibrosis, is eliminated, whereas up-regulation of the anti-fibrotic hepatocyte growth factor gene is suppressed.[38] Hence, fibroblasts are effective in scar formation. Although not statistically significant, we found that vitamin D was low in subjects with RS. The small number of patients and short half-life of 25(OH)D may have affected this result. Many factors may change serum vitamin D levels within six-month periods. As such, low vitamin D may facilitate the occurrence of RS. In addition, a study reported that VUR and recurrent UTIs are risk factors for RS following UTIs.[39] However we couldn't find a relationship between the vitamin D levels and recurrent UTI or VUR.

The main limitations of our study were the limited sample size, subjects from only a single center, and not investigating the roles of vitamin D metabolites, vitamin D receptors, or cathelicidin, as well as whether vitamin D replacement prevented RS.

In conclusion, decreased vitamin D is associated with upper UTIs and renal parenchymal injury. Vitamin D is lower in patients with renal scarring compared with acute renal parenchymal injury, but it is not statistically significant. More studies are needed to determine whether decreased vitamin D is a risk factor for renal scarring.

Conflict of interest

None declared.

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