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Role of Serum 25 Hydroxy Vitamin D Deficiency in Urinary Tract Infection in Children

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ABSTRACT

Background: Vitamin D insufficiency was seen in kids with various infections.

Aim: This study aimed to assess the influence of vit D insufficiency on UTI in kids.

Patients and methods: This case-control investigation involved ninety kids between one and twelve years in the Outpatient and Inpatient of the Pediatric Department, Suez Canal University Hospital.

Results: There was significant variance among vit D level in the two study groups ($p<0.001$). Fever was the most frequent sign in the case group (80%), while dysuria was the most frequent symptoms (60%). The most common organisms identified in the urine culture was E. coli (75.6%). In the urine analysis, the study showed one quarter of the patients had 20-50 pus cells whereas more than 85% of the controls had pus cells below 10 ($p<0.001$). Linear regression analysis revealed that there was a decrease by 14.26 units in the vitamin D level of patients suffering from UTI compared to healthy controls.

Conclusion: We found that concentration of vit D was lesser in kids with UTI than without, also there was correlation among concentration of Vit D and development of UTI manifestations, and moreover there was a decrease by 14.26 units in the concentration of vit D of cases suffering UTI compared to healthy controls.

Keywords: Children, UTI, Vitamin D deficiency, Infection.

INTRODUCTION

Urinary tract infection (UTI) is the most prevalent genitourinary disorder and the 2nd most frequent bacterial infection in kids, following infections of the respiratory tract. Gram-negative bacteria are the predominant etiological agents of urinary tract infections, which can impact either the lower or upper urinary tract [(1)].

Vit D insufficiency and deficiency is a worldwide problem. Vit D is recognized for its role in traditional calcium homeostasis. Recent studies from several sources revealed that vit D exhibits a wide range of effects against autoimmune disorders and infections [(2)]. Vit D has been demonstrated to influence infectious disorders. Nevertheless, its correlation with the acquisition and complex progression of urinary tract infections remains not to be fully studied [(3)].

Vit D obtained from food sources or synthesized in the skin is biologically inactive. It requires enzymatic conversion (hydroxylation) in the liver and kidneys for activation [(4)]. Consequently, it isn't an essential dietary component and isn't formally classified as a vitamin [(5)]. It may be seen as a hormone, wherein the activation of vit D pro-hormone leads to the formation of the active form, calcitriol, which exerts actions through a nuclear receptor in various sites [(5)]. Deficiency of vit D is associated not only with bone-related disorders but also with cardiovascular disease, autoimmune conditions like type 1 various malignancies, diabetes mellitus, multiple sclerosis, and inflammatory bowel disease [(6)]. Vit D is essential for immunological regulation and is believed to have a systemic influence on infections [[6]]. Hypocalcemia resulting from Vit D insufficiency diminishes the activities of both lymphocytes and neutrophils [(7)]. The modulation of cytokine responses and the lowering of toll-like receptor activation are postulated outcomes of vitamin D's influence on local and systemic inflammatory responses [(8)].

The investigation aimed to assess the effect of vit D insufficiency on urinary tract infections in kids.

PATIENTS AND METHODS

This was a case control investigation that has been performed on ninety (n=90) kids aged from one year to twelve years in the Inpatient and Outpatient of the Pediatric Department, Suez Canal University Hospital. Patients have been separated into two groups: Group 1involved forty-five (n=45) kids diagnosed with urinary tract infection, confirmed by a positive urine culture (exceeding 105 colony forming units [CFU]/ml of a single pathogen in a clean catch method or midstream urine sample, or 104 colony-forming unit per milliliter of a single pathogen via urinary catheterization). Group 2 included forty-five (n=45) kids in a healthy control group.

Inclusion criteria: Urinary tract infection identified by a positive urine culture (exceeding 105 [CFU]/mL of a single pathogen in a clean catch method or midstream urine sample, or 104 [CFU]/mL of a single pathogen by catheterization of urinary).

Exclusion criteria: Any children with manifestation of chronic vitamin D deficiency, receiving vitamin D supplementation for any reason and children who have anatomical defects considered as risk factors for developing recurrent urinary tract infection.

Methods: For each child, present history, personal data, nutritional history, history and family history were collected in a case sheet. In addition, routine examination was done. Also, laboratory investigations (urine culture,

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urine analysis and serum 25 (oh vitamin D) level were done. Assessing 1α, 25(OH) D may thus result in inaccurate categorization of an individual's vitamin D status, potentially leading to misdiagnosis. Moreover, 1α,25(OH)D is present in concentrations approximately one thousand times lower than 25(OH)D and possesses a half-life of six hours, in contrast to the fifteen-day half-life of 25(OH)D [(9)].

Procedure: The testing methodology was founded on the competitive enzyme linked immunosorbent assay procedure. This method used anti-Vit D monoclonal antibody (mAb Anti-25-hydroxyvitaminD) that has been immobilized in microtiter wells. A precise volume of case sera and standards has been introduced, followed by a specific quantity of extraction buffer, into the microtiter wells to produce vit D from its binding protein (DBP-complex). Following the initial incubation phase, a fixed amount of Biotinylated 25-hydroxyvitamin D was added to compete with the endogenous serum vit D for a finite many binding sites on the covered anti-Vit D antibodies. Following incubation, the streptavidin-HRP conjugate (Conjugate 2) has been introduced to bind to the biotin-containing first conjugation. Following incubation, the wells were thoroughly rinsed to eliminate unbound 25-hydroxy vit D, following which a chromogen-substrate solution has been added & incubated for fifteen minutes, leading to the formation of a blue hue. The progression of the color stopped by the addition of a stop solution, resulting in a yellow hue, which was then detected spectrophotometrically at 450 nanometers. The color intensity correlated positively with the concentration of biotinylated 25-hydroxy vit D and inversely with the level of endogenous 25-hydroxyvitamin D in the test sample. With reference to a series of vit D standards evaluated in an identical manner **(Abnova, human D Elisa kit, Catalog Number KA1168, 96 assays, version: 03)**.

Ethical considerations: All the procedures of the research were approved by Pediatrics Department and the Investigation Ethics Committee of Faculty of Medicine, Suez Canal University. Administrative consents required were taken. This study was performed in compliance with the Declaration of Helsinki, the code of ethics of the World Medical Association.

Statistical analysis

The data obtained has been recorded in the case sheet. Statistical analysis has been done by Statistical Package for Social Sciences (SPSS) for Windows version 18. Data has been presented through graphs, pies and bars. Significance was calculated through Chi-squared and Fissure exact tests for qualitative variables and t-test for quantitative variables. Correlation was made when or whenever appropriate. P-Value ≤ 0.05 has been deemed statistically significant.

RESULTS

Insignificant distinctions were observed among both groups according to demographic data p>0.05 (Table 1).

Table (1): Demographic data of the studied sample

Variables		Cases (number =forty-five)	Controls (number = forty-five)	test value	p-value
Age (years), mean ± standard deviation		5.05 ± 2.74	5.59 ± 3.21	922.5	0.464 [a]
Gender, n (%)	Male	22 (48.9)	14 (31.1)	2.9	0.08 [b]
	Female	23 (51.1)	31 (68.9)		
Order among siblings, n (%)	1st	15 (33.3)	10 (22.2)	2.4	0.30 [b]
	2nd	16 (35.6)	23 (51.1)		
	≥ 3rd	14 (31.1)	12 (26.7)		
[a] p-values are based on Mann Whitney U test. Statistical significance at P < 0.05 [b] p-values are based on Chi square test. Statistical significance at P < 0.05					

A significant variance was observed among vit D level in the two study groups (p<0.001) (Table 2).

Table (2): Comparative analysis of vitamin D concentration among cases and controls

Variables	Cases (number =forty-five)	Controls (number =forty-five)	test value	p-value
Vitamin D level (ng/ml), mean ± SD	25.44 ± 9.67	39.71 ± 9.30	312	**<0.001** [a]
Vitamin D categories, n (%)				
Deficient (<10 ng/ml)	0 (0)	0 (0)		
Insufficient (10 - 30 ng/ml)	34 (75.6)	6 (13.3)	35.3	**<0.001** [b]
Sufficient (30 - 100 ng/ml)	11 (24.4)	39 (86.7)		

The most frequent organisms identified in the urine culture were E. coli (75.6%) of the culture in UTI patients, followed by klebsiella (11.1%) and proteus (8.9%). All control patients with positive urine culture had E. coli. On performing urinalysis, it was found that about one quarter of the patients had 20-50 pus cells whereas more than 85% of the controls had pus cells below 10 (p<0.001) (Table 3).

Table (3): Urinalysis and urine culture among the studied sample

Variables	Study groups		test value	p-value
	Cases (number =forty-five)	Controls (number =forty-five)		
Urine culture				
Positive	45 (100)	7 (15.6)	65.8	**<0.001** [a]
Negative (no growth)	0	38 (84.4)		
Organism				
None	0 (0)	38 (84.4)	77.6	**<0.001** [b]
E-coli	34 (75.6)	7 (15.6)		
Klebsiella	5 (11.1)	0 (0)		
Proteus	4 (8.9)	0 (0)		
Staph	2 (4.4)	0 (0)		
Urinalysis (pus level)				
< 10	13 (28.9)	39 (86.7)	37.5	**<0.001** [b]
10 -20	18 (40)	5 (11.1)		
20-50	11 (24.4)	1 (2.2)		
>50	3 (6.7)	0		

Fever was the most frequent manifestation in the case group (80%) (Figure 1).

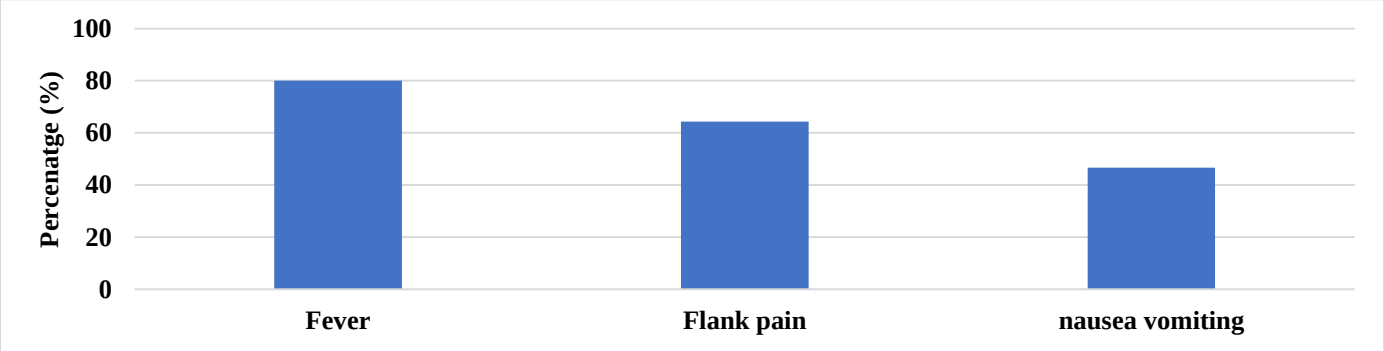

Figure (1): Upper UTI manifestations among cases.

Dysuria was the most frequent symptoms in the case group (60%) (Figure 2).

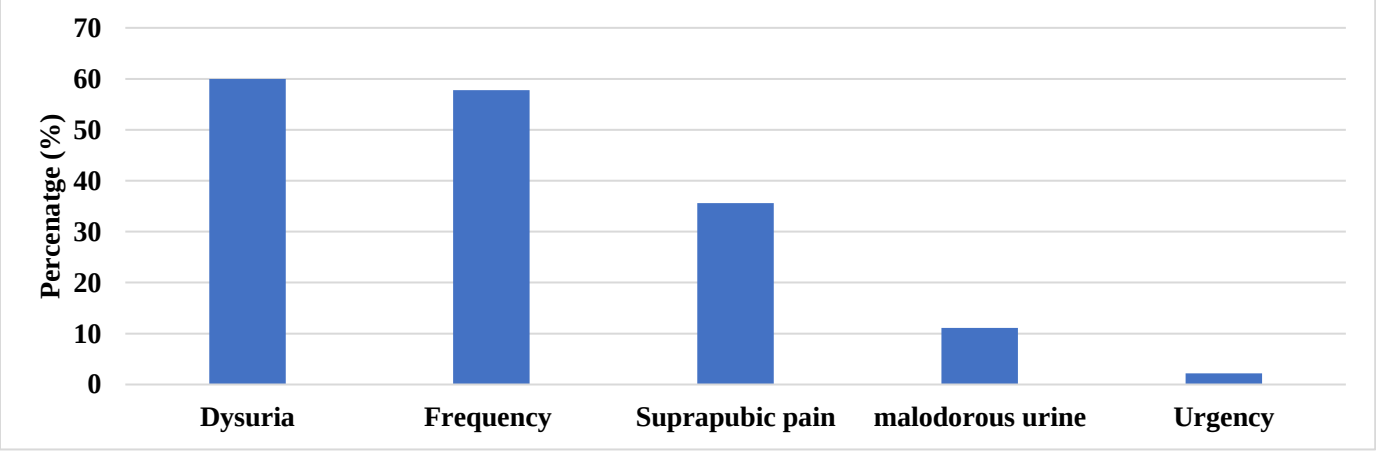

Figure (2): Upper UTI symptoms among cases

The most frequent UTI manifestations among patients were fever (36 patients), flank pain (29 patients) and dysuria (27 patients). Moreover, it was discovered that patients with positive clinical manifestations had significantly lower vit D levels than patients with negative one except for nausea and vomiting. (Table 4).

Table (4): Association among vit D level and development of UTI manifestations among cases

Variables	n=45	Vitamin D level (ng/ml) mean ± SD	test value	p-value
Fever				
Absent	9 (20)	27.14 ± 9.54	59	**0.002** [a]
Present	36 (80)	18.67 ± 7.16		
Flank pain				
Absent	16 (35.6)	28.31 ± 10.13	111.5	**0.004** [a]
Present	29 (64.4)	20.25 ± 6.16		
Dysuria				
Absent	18 (40)	29.26 ± 10.03	94.5	**0.001** [a]
Present	27 (60)	19.72 ± 5.5		
Frequency				
Absent	19 (42.2)	27.88 ± 10.02	150	**0.026** [a]
Present	26 (57.8)	22.11 ± 8.29		
Nausea & vomiting				
Absent	24 (53.3)	27.13 ± 10.04	195	0.19 [a]
Present	21 (46.7)	23.52 ± 9.07		
Suprapubic pain				
Absent	29 (64.4)	32.75 ± 10.84	86.5	**0.001** [a]
Present	16 (35.6)	21.41 ± 6.05		
Malodorous urine				
Absent	40 (88.9)	26.4 ± 9.79	41	**0.031** [a]
Present	5 (11.1)	17.8 ± 3.19		

A statistically insignificant variation was found among different levels of pus in patients' vitamin D levels (p=0.095) (Table 5).

Table (5): Association among Vit D level and severity of infection among patients with UTI

Variables	n=45	Vitamin D level (ng/ml) mean ± SD	test value	p-value
Urinalysis (pus level)				
5 – 10	13 (30)	27.58 ± 12.65	7.9	0.095 [a]
10 -20	18 (40)	25.82 ± 9.20		
20-50	11 (24.4)	21.94 ± 5.81		
>50	3 (6.7)	17.2 ± 1.53		

Patients with delayed teething had lower vitamin D levels than patients who do not have it. Nevertheless, this variance was statistically insignificant (p=0.08). Moreover, a statistically insignificant variation has been observed in vitamin D level among cases with positive past history of UTI and those with negative one (p=0.19).

Table (6): Association among Vit D level and history of patients with UTI

Variables	n=45	Vitamin D level (ng/ml) mean ± SD	test value	p-value
Delayed walking				
Absent	44 (97.8)	25.14 ± 9.55	43	0.393
Present	1 (2.2)	39		
Delayed teething				
Absent	42 (93.3)	26.02 ± 9.71	24	0.081
Present	3 (6.7)	17.33 ± 4.16		
Past UTI				
Absent	42 (93.3)	24.9 ± 9.5	33.5	0.19
Present	3 (6.7)	33 ± 10.58		
[a] p-values are based on Mann Whitney U test. Statistical significance at P < 0.05				

Linear regression analysis has been utilized to assess predictors of vitamin D levels among the studied children. We found that there was a decrease by 14.26 units in vitamin D level of patients suffering UTI compared to healthy controls (Table 7).

Table (7): Linear regression analysis of determinants of Vit D levels among the studied children

Predictors	Unstandardized Coefficients		Standardized Coefficients Beta	95% CI	P value
	B	Std. Error			
(Constant)	53.97	3.162			<0.001*
Study group					
Cases Vs control (R)	-14.26	2	-0.605	(-18.24) – (-10.293)	**<0.001***
ANOVA <0.001	R^2 = 0.359	* Statistical significance at P <0.05			

DISCUSSION

According to baseline characteristics of the examined sample, the mean age of the patients was 5.05 ± 2.74 years. About one half of them were females (51.1%). 1st order children formed one third of the sample (33.3 %) while 2nd order children formed about (35.6%) of the diseased children. Children with UTI were found to have vitamin D level (25.44 ± 9.67) significant lower than that of healthy controls (39.71 ± 9.30) (p < 0.001).

Our investigation corroborate with the findings of **Hacihamdioglu *et al.*** [10], which aimed to clarify the correlation among serum vit D and urinary cathelicidin levels in kids with Escherichia coli-induced urinary tract infections (UTIs). The investigation involved thirty-six kids with UTIs and thirty-eight healthy controls, with the UTI group having a mean age of 6.8 ± 3.6 years, predominantly female (83.3%). Kids with UTIs exhibited significantly fewer levels of vitamin D (16.5 ± 6.3) compared to healthy controls (23.7 ± 11) (p < 0.05). **Tekin *et al.*** [3] aimed to investigate the correlation among blood concentrations of 25(OH) D3 & UTIs in kids. The investigation involved eighty-two cases who experienced their first UTI episode and sixty-four healthy controls, with a mean age of 2.57 ± 2.56 years, of which 68.3% were females. Kids with UTIs exhibited significantly lower vit D levels (11.7 ± 3.3) compared to healthy controls (27.6 ± 4.7) (p<0.001). **Mahmoudzadeh *et al.*** [11] investigated potential correlation among vit D deficiency and urinary tract infection (UTI) in an investigation involving seventy-five kids with urinary tract infections and seventy-five healthy controls. The mean age of the cases was 4.5 ± 1.6 years, with a predominance of females (88%). Serum 25(OH)D levels in the cases ranged from four to forty nomograms per milliliter, significantly lower than the controls, which ranged from ten to sixty-five nomograms per milliliter (p < 0.001).

According to the most common organisms identified in the urine culture. The majority of the results showed E. coli in the culture (75.6% of the culture in UTI patients) followed by klebsiella (11.1%) and proteus (8.9%) organisms. All control patients with positive urine culture had E. coli. On performing urinalysis, it was found that about one quarter of the patients had 20-50 pus cells whereas more than 85% of the controls had pus cells below 10 (p<0.001). Also 36 (80%) cases had fever while 29 about (65%) cases had flank pain. Finally, 21 about

(46%) of them developed nausea and vomiting. Moreover, three most common lower UTI clinical symptoms were dysuria (60%), frequency (57.8%) and suprapubic pain (35.6 %), respectively. Our results agree with **Qadir *et al.*** [12] who aimed to detect status of vit-D in kids with urinary tract infection, the investigation conducted on 172 kids with confirmed urinary tract infection, reported that the cases with moderate vit D deficiency exhibited that the most pathogenesis was due to E.coli (56.36), followed by klebsiella (14.54%), also most children 87.21% had fever, while 18.02% had vomiting, 12.79% had abdominal pain and about 8.72% had dysuria.

Mahmoudzadeh *et al*. [11] observed that E. coli was the predominant bacterium cultivated in kids with urinary tract infections (89.5%), with the most prevalent clinical signs being abdominal pain, dysuria, fever, and malaise. Furthermore **Yunus *et al*.** [13] aimed to evaluate vit D status in kids with urinary tract infections. This investigation was conducted on ninety-one patients and found that approximately eighty-six percent of urinary tract infections were attributable to E. coli.

Our findings indicated no statistically significant variation in vit D levels in varying degrees of pus in cases ($p=0.095$). Furthermore, it was observed that cases with positive clinical manifestations had significantly lower vit D levels compared to those with negative symptoms, except for nausea and vomiting. Our work aligns with **Foti *et al.*** [13] who aimed to examine the correlation among low urinary tract symptoms and vit D status. The findings indicated that cases with lower vit D levels exhibited a greater risk of low urinary tract symptoms compared to those with normal vit D levels ($P < 0.001$). Conversely, **Aydogmus & Demirdal *et al*.** [14] aimed to determine if a distinction exists regarding the frequency of lower urinary tract symptoms among females with a lack of vit D and control group. The investigation concluded that a lack of association has been discovered among deficiency of lower UTI and vit D.

Our results indicated that cases with delayed teething exhibited reduced vit D levels compared to those without the disorder. Nevertheless, the distinction is statistically negligible ($p=0.08$). Furthermore, there was statistically insignificant distinction in vitamin D levels between cases with a positive history of UTI & those with a negative history ($p=0.19$). Also, patients with positive clinical findings had lower vitamin D levels than patients with negative one. However, these differences are statistically insignificant.

Concerning the linear regression analysis employed to evaluate determinants of vit D concentrations in the examined youngsters. $R^2 = 0.359$, indicating that 35.9% of the variability in vit D concentrations among the examined kids was elucidated by this regression model. We observed a reduction of 14.26 units in vit D levels of cases with urinary tract infections compared to healthy controls.

Investigation, conducted by **Shalaby *et al*.** [15] examined the correlation among 25-hydroxyvitamin D (25(OH) D3) concentrations & the incidence of 1st-time febrile urinary tract infections in kids. The findings indicated that multivariate logistic regression analysis revealed a significant correlation among serum concentration of 25(OH) D3 ≤ 25 nmol/l (OR = 1.94, ninety-five percent confidence interval: 1.61–2.82; $p = 0.04$) and UTI occurrence. Additionally, cases with UTIs exhibited a reduction of 17.4 units in concentrations of Vit D compared to healthy controls.

Furthermore, **Sadeghzadeh *et al*.** [16] assessed the correlation between level of vit D and UTI in kids, indicating a significant correlation among vit D status and urinary tract infections [odd ratio = 2.884, ninety-five percent confidence interval (1.075–7.738), P-value = 0.035]. Cases with Vit D deficiency were 2.884 times more likely to contract an infection compared to those without deficiency.

LIMITATIONS

The sample size was relatively small which may lead to the decreased impact of the results. The study was done in only one hospital, which makes it less representative of the Egyptian population. Sampling method was convenient sampling method, which may have led to bias in cases and control selection.

CONCLUSION

We found that vit D level was lower in kids with urinary tract infection than without. Also, there was association among vit D level and development of UTI manifestations, Moreover there was a decrease by 14.26 units in the level of vit D of cases suffering from UTI compared to healthy controls.

RECOMMENDATIONS

To do research in many centers around the country so that the results be more representative of the Egyptian population. Large scale investigations are needed to confirm the role of vit D on our health. Further research for evaluating the therapeutic role of vit D in UTI is recommended. Vit D supplementations in kids must be encouraged in different age groups because of its important role as a modulator of immune system and other organs in the body.

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- **Availability of data and material:** Available
- **Conflicts of interest:** No conflicts of interest.
- **Competing interests:** None.

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