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Research Article

Cathelicidin and Vitamin D Serum Levels in Children with Urinary Tract Infection

Maha Youssif Zein El-Abdin[1], Manal Abd El-Salam[1*], Iman Mouhamed El- Bagoury[2], Nouran Abd Al-Magid[1]

[1]Department of Pediatric, Faculty of Medicine, Al-Azher University, Cairo, Egypt

[2]Department of Clinical Pathology, Faculty of Medicine, Al-Azher University, Cairo, Egypt

[*]**Corresponding author:** Manal Abd El-Salam, Department of Pediatric, Faculty of Medicine, Al-Azher University, Cairo, Egypt. Email: manal24969@gmail.com

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Abstract

Background: Urinary Tract Infections (UTI) are the most frequent bacterial infections in infants and children after respiratory tract infection. The risk of having a UTI before the age of 14 years is approximately 1-3% in boys and 3-10% in girls. *Cathelicidins* are small peptides with amphipathic structures that allow them to disrupt the integrity of the pathogen cell membrane, resulting in its death.

Aim: to assess the association between vitamin D and *cathelicidin* serum levels in children with urinary tract infection.

Subjects and Methods: the study included 25 children with urinary tract infection documented by urine culture and sensitivity, they were selected from children attending the outpatient pediatric clinic and inpatients of the pediatric department, Al-Azhar University hospital. Also, it included 25 healthy children age and sex matched as a control group. Serum vitamin D and *cathalicidin* levels were assessed in both groups.

Results: there was significant increase in *cathelecidin* serum level in patients with UTI compared to the controls, it was (52.8 ± 31.59ng|ml) and (27.06 ± 10.43ng|ml) respectively , ($P < 0.01$), on the other hand there was a significant decrease in vitamin D serum level in patients with UTI compared to the controls ,it was (45.29± 34.17ng|ml) and (48.72 ±17.40ng|ml) respectively($P < 0.05$).A positive correlation between serum *cathelecidin* and vitamin D was detected.

Conclusion: vitamin D stores may influence susceptibility to urinary tract infection in children. There was a strong relation between serum vitamin D and *cathalicidin* levels in those children**.**

Keywords: Cathalicidin; Vitamin D; UTI

Introduction

Urinary Tract Infection (UTI) is one of the most common bacterial infections of humans (most often *E. coli)* and a major cause of morbidity. UTI also accounts for 25 to 40% of all nosocomial infections, making these infections an important medical and financial burden on health care systems. UTI usually starts as a bladder infection but can ascend to the kidneys and may result in renal failure [1]. Antimicrobial Peptides (AMPs) include the gene families of defensins and *cathelicidins*. AMPs have direct lytic properties against a variety of organisms including bacteria, fungi, and viruses. In addition, it is becoming increasingly appreciated that AMPs are also immunomodulatory [2]. *Cathelicidins* are small peptides with amphipathic structures that allow them to disrupt the integrity of the pathogen cell membrane, resulting in its death. *Cathelicidins* are expressed by most immune cells or those epithelial cells that are in contact with the environment. Deficiency in these peptides results in increased susceptibility to infection [3], thus, *cathelicidi*n seems to be a key factor in mucosal immunity of the urinary tract [4]. Vitamin D has important roles in addition to its classic effects on calcium and bone homeostasis, as the vitamin D receptors are expressed on immune cells (B cells, T cells, and antigen-presenting cells), and these immunologic cells are all capable of synthesizing the active vitamin D metabolites. Vitamin D can act in

an autocrine manner in a local immunologic milieu and modulate the innate and adaptive immune responses [5]**.** Vitamin D could influence *cathelicidin* production in the urinary tract and thereby help protection from invading microbes. Urinary bladder cells enhance *cathelicidin* production in response to vitamin D [6]**.**

Subjects and Methods

This is a case control study included 25 children with urinary tract infection, their ages ranged from 3:12 years, with mean age (8.58±3.41 years), these are the patients, numbers who attended at the pediatric department and the out patients, pediatric clinic at the time of the study after the exclusion criteria. They were 19 females (76%) and 6 male (24%).All cases were presented with symptoms of UTI which was confirmed by: Pyuria (pus cell ≥10/ HPF) in mid urine sample collected under sterile condition) and +ve urine culture [7], sixty-eight % of the studied cases were found to be with recurrent attack of UTI while 32%were with the first attack. The frequency of lower UTI and upper UTI in the studied cases were 21 (84%) and 4(16%) respectively. Also 25 healthy children age and sex matched were included in the study as a control group. Patients with: (congenital renal diseases, other acute and chronic infections, chronic illness and children on medications e.g. steroids, cytotoxic drugs) were excluded from the study. Informed consent was obtained from the participating patients or their parents in adherence with the guidelines of the ethical committee of AL-Zahraa hospital, AL-Azhar University, Cairo, Egypt.

Sampling and Procedure

Urine Samples: Urine analysis: significant pyuria is defined as >10 leukocytes per mm^3 in a fresh uncentrifuged sample [8]. Urine culture and sensitivity on a clean catch specimen, more than 10000 colonies in boys suggest likely infection and 100000 colonies in girls makes the diagnosis of an infection likely [9].

Blood Samples

- 2 ml were taken on EDTA vacutainer for C.B.C. and ESR.
- 2 ml were taken on plain vacutainer for, urea and creatinine.
- 3 ml were taken on plain vacutainer for: CRP, serum vit D and *cathelicidin*. samples were separated and stored at -20 ˚C. till the time of the assay.

Assessment of *cathelicidin* serum level using enzyme-linked immune-sorbent assay (ELISA).

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of human (*cathelicidin* LL-37) in samples. Add (*cathelicidin* LL-37) to monoclonal antibody Enzyme well which is pre-coated with human (*cathelicidin*) monoclonal antibody, incubation; then, add (*cathelicidin* LL-37) antibodies labeled with biotin, and combined with Streptavidin-HRP to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme Then add chromogenic solution A, B, the color of the liquid changes into the blue, and at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the human substance (*cathelicidin* LL-37) of sample were positively correlated. The color changes were measured spectrophotometrically at a wave length 450 nm. The concentration of *cathelicidin* (LL-37) in the sample was determined by comparing the optical density of the samples to the standard curve. The results were expressed as ng/ml [10].

Statistical Analysis

Data were collected, revised, coded and entered to the Statistical Package for Social Science (version20). Spearman correlation coefficients were used to assess the relation between two studied parameters in the same group. P value. $P<0.05$ was considered statistically significant.

Results

Age and sex distribution among studied groups, it revealed: female was the predominant sex among patients group, male and female was 19 (76%) and 6 (24%) respectively (Table 1).

Variable	Cases (no=25)	Controls (no=25)	P-value
Age (years)	8.58±3.41 (3-12)	9.18±2.74 (3- 12)	0.537
Gender			
Male	6 (24.0%)	9 (36.0%)	0.496
Female	19 (76.0%)	16 (64.0%)	

Table 1: Age and sex distribution in the study groups.

Comparison between patients and controls regarding some laboratory parameters, it revealed: significant increase in ESR and CRP levels in children with UTI compared to the controls and significant decrease in serum creatinine level in patients with UTI compared to the controls. No significant differences were detected regarding WBCs, and serum urea levels (Table 2).

Parameters	Groups		t/z#	P-value
	Cases group no=25	Control group no=25		
	Mean ± SD	Mean ± SD		
WBCs (x10^3\ul)	8.11± 3.61	8.20 ±2.93	-0.101	0.92

ESR (normal <10)	21.20±19.61	6.76 ±1.81	-5.015#	0.01**
Urea (mg\dl)	24.32 ±7.28	24.00 ± 4.25	0.19	0.85
Create (mg\dl)	0.38 ± 0.13	0.54 ± 0.05	-5.553	0.01
CRP (normal<6)	17.2 ± 14.3	5 ± 2	-12.2#	0.01

Mann-Whitney test

Table 2: Comparison between patients and controls regarding laboratory investigations.

Comparison between patients and control group regarding vitamin D and *cathelicidin* serum levels, it revealed: significant increase in *cathelecidin* and decrease in vitamin D serum levels in children with UTI compared to controls (Table 3).

Parameters	**Groups**		**Z**	**P-value**
	Cases group no=25	**Control group no=25**		
	Mean ±SD	**Mean ±SD**		
Serum vitamin D(ng/ml)	45.29 ±34.17	48.72 ± 17.40	-2.397	0.017*
Serum *cathelecidin* (ng/ml)	52.84 ± 31.59	27.06 ± 10.43	3.774	0.01**

Table 3: comparison between patients group and controls regarding vitamin D and *cathelecidin* serum levels.

Organisms causing UTI in children with UTI, it revealed: *E. coli* is the most common organism causing UTI ,it is detected in 19 cases (76%) followed by Entero-coccus 3 cases (12%), Gm+ve cocci 2 cases (8%) and then Proteus 1 case (4%) (Figure 1).

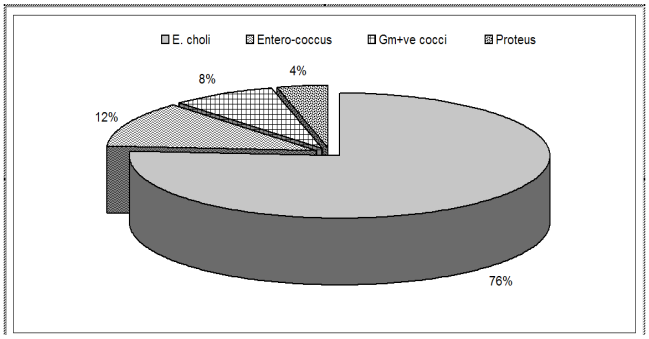

Figure 1: Urine culture results in children with UTI.

(Figure 2) shows significant positive correlation between *cathelecidin* and vitamin D serum level in children with UTI.

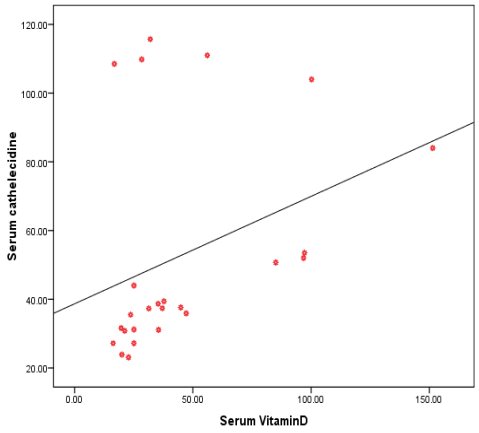

Figure 2: Positive correlation between serum *cathelicidin* and vitamin D levels.

Discussion

E. coli remains the most common organism causing UTI, in the current study, it is detected in 19 cases (76%) followed by Entero-coccus 3 cases (12%), Gm +ve cocci 2 cases (8%) and then Proteus 1 case (4%). This is in agreement with [11-14] they reported that Escherichia coli was the predominant organism causing UTI in their studies. We reported significant increase in ESR and CRP levels in cases with UTI, elevated Erythrocyte Sedimentation Rate (ESR)and C-Reactive Protein (CRP) are indicators of an acute inflammatory process. These tests do not reliably differentiate between children with cystitis and children with pyelonephritis [15]. This study provides evidence that compared to healthy children, serum vitamin D level was reduced in children with UTI, lower levels of serum vitamin D in UTI cases reflect its importance in immunity and protection against infection. This is in agreement with the study by Tekin et al. [17] who reported that a serum 25-hydroxy vitamin D level of <20 ng/mL was associated with UTI in children, in the current study we did not categories' vitamin D to insufficiency or deficiency due to small sample size. Another study done by Hacıhamdioğlu et al. [18] reported that frequency of vitamin D insufficiency was significantly higher in children with a UTI than in those in the control group. Also, Yang et al. [19] reported that vitamin D deficiency were at an increased for UTI, whereas vitamin D supplementation was associated with a lower UTI frequency. There is no enough available data concerned with assessment of vitamin D level in children with UTI but there is a lot of data concerned with assessment of the level in respiratory tract infections [20-21].

Deficiency in vitamin D is associated with an increased susceptibility to UTI, When the bladder cells were infected, a

significant increase in *cathelicidin* expression after vitamin D supplementation was observed [6]**.** Furthermore, vitamin D has a regulatory role on innate and adaptive immune responses. Vitamin D has been shown to promote antimicrobial responses through the production of antibacterial peptides. The epithelial cells of the urinary tract up regulate the production of the human antimicrobial peptide LL-37 upon infection with uropathogenic *E. coli*. Thus, the *cathelicidin* LL-37 plays an important role in the protection against infections of the urinary tract [22]**.** Antimicrobial peptides such as *cathelicidin* constitute an integral part of the innate immune response to a variety of infections especially at barrier sites [23]**.** Our study demonstrated high level of serum *cathalicidin* in children with UTI compared to the controls. *Cathelicidin* expression and secretion were increased during *E. coli* urinary tract colonization in children with cystitis or pyelonephritis [24]. We observed significant positive correlation between serum *cathelecidin* and vitamin D, therapy with vitamin D in animal models of sepsis modulates levels of systemic inflammatory cytokines including TNF-α and IL-6. Furthermore, Vitamin D can enhance the induction of the antimicrobial peptides *cathelicidin* and β-defensin which are found on mucosal and epithelial surfaces and act as the body's first line of defense against viral and bacterial pathogens, so we hypothesized that vitamin D may be involved in the defense against UTI, and can be mediated by *cathelicidin*. In the same line with our results a study done by Hacıhamdioğlu et al. [18] who reported that urine *cathelicidin* level was significantly unregulated in children with UTI and sufficient vitamin D status. In contrast, urine *cathelicidin* levels did not increase significantly during a UTI in children who had vitamin D insufficiency.

Conclusion

We concluded a strong positive relation between serum vitamin D and cathalicidin levels in children with urinary tract infection. Our research suggests that vitamin D enhances immune system and increases antibacterial defense, thus vitamin D can be used as an adjuvant drug in treatment of UTI and may solve the antibiotic microbial resistance.

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