Websites:
http://www.sciencepub.net/nature
http://www.sciencepub.net

Emails:
naturesciencej@gmail.com
editor@sciencepub.net

Nature and Science

MARSLAND PRESS
Multidisciplinary Academic Journal Publisher

Association between Serum 25-Hydroxyvitamin D Level and Urinary Tract Infections (UTIs) in Children

Mohamed A. Fareid

Botany and Microbiology Dept., Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt.
* Present address: Basic Science Dept., Prep Year Deanship, University of Ha'il, Saudi Kingdom.
mohamedfareid73@yahoo.com

Abstract: The present study aimed to assessment the relationship between serum 25-hydroxyvitamin D level and Urinary tract infections (UTIs) in both male and female children. Most of the study participants (46.35%) had light brown skin color, approximately one-third had dark brown skin color (27.03%) and few (9.87%) had black skin color. More than 50% of participants were of high economic level (54.93%), 32.61% were of intermediate and 12.44% were of low economic level. Statistically, there is a highly significant difference between skin colors and economic levels in all enrolled participants (p-value < 0.05). Our results showed a high frequency of *Escherichia coli* followed by *Klebsiella pneumonia* and *Proteus mirabilis* in female than in male. They recorded 93 (31.00), 81 (40.91); 76 (25.33), 58 (29.29); 58 (19.33), 40 (20.20), respectively. The association between 25-hydroxyvitamin D level and the frequency of isolated pathogen in both male and female children showed higher frequency of *Escherichia coli* between all enrolled children which have deficient 25-hydroxyvitamin D level in compared to sufficient 25-hydroxyvitamin D level. It recorded 42 (51.85%), 11 (13.58%); 47 (50.54%), 14 (15.05%), respectively. Pearson's correlation coefficient recorded a higher positive significant value (p-value < 0.05). *Klebsiella pneumonia* and *Proteus mirabilis* recorded also a high frequency between deficient 25-hydroxyvitamin levels in both enrolled male and female children in compared to sufficient level. They recorded 31 (53.45), 7 (12.07), 41 (53.95%), 9 (11.84%) and 23 (57.50), 3 (7.50), 33 (56.90%), 5 (8.62%), respectively. Pearson's correlation coefficient between 25-hydroxyvitamin D levels and the frequency of isolated pathogens recorded a higher positive significant value (p-value < 0.05).

[Mohamed A. Fareid. **Association between Serum 25-Hydroxyvitamin D Level and Urinary Tract Infections (UTIs) in Children.** *Nat Sci* 2020;18(8):19-33]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 3. doi:10.7537/marsnsj180920.03.

Key words: Association, 25-Hydroxyvitamin D, Urinary tract infections (UTIs), Children.

1. Introduction

Urinary tract infection (UTI) is an infection of the urethra, bladder and kidney that represent the urinary system of the body. These infections can be defined as the presence of significant quantity or number of bacteria and other parasites in the urine along with signs and symptoms of infection (Abdulhadi *et al*., 2008). In children, one of the most common sites of infection is the urinary tract (Shaikh *et al*., 2010). Bacteria are the most common cause of urinary tract infection and the most common clinical problems in childhood (Allamin *et al*., 2015). *Escherichia coli* (*E. coli*) are the predominant pathogen in childhood UTI, found in 90% of girls and in 80% of boys. An important factor for the predominance of *E. coli* is their ability to attach to the urinary tract endothelium (Brandström and Hansson, 2015). These infections have been reported to be one of the most infections that cause serious health problems affecting millions of people both in the community and hospital setting and may cause permanent kidney damage (Abdulhadi *et al*., 2008; Becknell *et al*., 2015; Hay *et al*., 2016).

Vitamin D deficiency and insufficiency is a global issue (Holick and Chen, 2008). Vitamin D is a steroid hormone which is mainly produced in the skin after exposure to ultraviolet radiation (Guillot *et al*., 2010). It is well known that vitamin D is involved in calcium homeostasis. More recent data from a variety of sources indicate that vitamin D has a broad spectrum of actions against autoimmune diseases and infections (Arnson *et al*., 2007; Borella *et al*., 2014). It was shown that recurrent UTIs in premenopausal women are associated with vitamin D deficiency (Nseir *et al*., 2013). Recently, it was reported that serum 25-hydroxyvitamin D level of <20 ng/mL was associated with UTI in children (Tekin *et al*., 2015). Furthermore, the vitamin D receptor gene polymorphism was an important factor for UTI susceptibility in children diagnosed with UTIs.

Studies show that vitamin D, in addition to calcium-phosphate homeostasis and bone metabolism, has multiple extra skeletal proprieties (Holick, 2010). Immunomodulatory and antibacterial properties are 2 extra skeletal effects of vitamin D (Lagishetty *et al*., 2011; Hewison, 2011) Several studies have been conducted that vitamin D in some infectious diseases including tuberculosis, lower respiratory tract infections and upper respiratory tract infections (Wilkinson *et al*., 2000; Cannell *et al*., 2006; Holick, 2007; Laaksi *et al*., 2007; Nnoaham and Clarke, 2008; Ginde *et al*., 2009; Adams and Hewison, 2010; Sabetta *et al*., 2010; Aydin *et al*., 2011; Berry *et al*., 2011; Nseir *et al*., 2012; Yildiz *et al*., 2012; Science *et al*., 2013; Collak *et al*., 2014; Alladi and Gopal, 2017; Jian *et al*., 2018). The present study aimed to assessment the association between serum 25-hydroxyvitamin D level and Urinary tract infections (UTIs) in both male and female children.

2. Materials And Methods

A school-based cross-sectional study was conducted in Ha'il province, Saudi Arabia. As the study aimed to assess the vitamin D deficiency as well as the association between vitamin D deficiency and urinary tract infection (UTI) in both male and female children, a sample of the population was required, thus school-based survey was found to be a relevant approach to reach all age groups.

2.1. Study population

Urine samples were collected from a total of 233 children between the ages of 6 to 19 years, the present study was conducted for a period from September 2018 to December 2019. Of all 233 urine samples, one hundred thirteen collected from male and one hundred twenty collected from female.

2.2. Urine collection

Clean catch urine samples were collected in sterile universal containers as described by Karlowsky *et al.* (2006) and Solberg *et al*. (2006). Two hundred thirty-three "clean catch" mid-stream urine (MSU) samples were collected inside sterile disposable universal bottles from the children participated in the study. They were instructed on how to collect samples and the need for prompt delivery to the laboratory. The samples were labeled and transported to the medical microbiology laboratory, Ha'il university in iced pack and were analyzed within one hour of collection.

2.3. Sterilization of media and materials

The media used were Nutrient Agar (NA) from Biotec Limited, Nutrient Broth (NB), MacConkey agar (MCA) and Blood Agar (BA) media. All media were supplied by Oxoid Limited. All glass wares were washed with detergent and rinsed with water, then allowed to dry. The glass wares were later wrapped in aluminum foil and sterilized in a hot air oven at 180 ^oC for 2 hr. Media were prepared according to the manufacturer's specifications and sterilized by autoclaving at 121 ^oC and 1.5 atm for 20 min.

2.4. Microscopy

The urine samples were mixed, and aliquots centrifuged at 5000 rpm for 5 min. The deposits were examined using both 10X and 40X objectives lenses. Samples with 10 white blood cells /mm^3 were regarded as pyuric (Smith *et al*., 2003). A volume of the urine samples was applied to a glass microscope slide, allowed to air dry, stained with gram stain, and examined microscopically (Kolawole *et al*., 2009).

2.5. Culturing of bacteria from urine samples

This was carried out as described by Cheesbrough (2006) and Prescott *et al*. (2008). Ten-fold serial dilutions were made by transferring 1 ml of the sample in 9 ml of sterile physiological saline. 1 ml was then poured into molten nutrient agar in Petri dishes and rotated gently for proper homogenization. The contents could set, and the plates were then incubated at 37 ^oC for 24 hr. Bacterial colonies growing on the agar after the incubation period were enumerated to determine urine samples with significant bacteriuria. A loopful of each urine sample was also streaked on MacConkey agar and Blood agar plate for the isolation of bacteria present in the urine sample. After incubation, plates with growth were selected, the colonies were isolated using an inoculating loop and subsequently sub-cultured on agar slants for use in further tests.

2.6. Identification of isolates

The methods used in the identification and characterization of isolated bacteria included Gram stain followed by microscopic examination, motility test and biochemical tests according to Cheesbrough (2006). The isolates were identified by Bergey's Manual for Determinative Bacteriology (Buchanan and Gibbons, 1974). Identification was confirmed with the API 20E system (BioMereiux).

2.7. Vitamin D assessment

To assess vitamin D serum level, 5 ml of blood was collected from everyone participants as clotted sample and was kept on ice until centrifugation on the same day. Serum was kept in aliquots at -20°C until analysis. Serum levels of 25-hydroxyvitamin D were measured using chemiluminescent immunoassay (Liaison 25 OH Vitamin D Total Assay; DiaSorin, Stillwater, MN, USA). This analysis method measures the total vitamin D in the range of 10-375 nmol/L. The sensitivity of the assay is <10 nmol/l and the intra- and inter-assay coefficients of variation were 5% for the intra-assay and 10.4% for the inter-assay. We adopted the Institute of Medicine cutoff points for vitamin D levels (Ross *et al*., 2011; Rosen *et al.*, 2012), classifying serum levels into sufficient (50-125 nmol/L), insufficient (25-50 nmol/L), deficient (<25 nmol/L), and toxic (>250 nmol/l).

2.8. Statistical analysis

To describe the study population and vitamin D serum level, we used frequencies and proportions for the quantitative variables. Significant differences in proportions of vitamin D deficiency in various population subgroups were assessed using Chi-square test. Pearson's correlation was done in order to assess correlation between 25-OH vitamin D deficiency and the frequency of urinary tract infection (UTI) in both male and female children. A *p*-value of <0.05 was defined as the level of significance. Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA), version 15 was used for data analysis.

2.9. Ethical considerations

This study was approved by the ethical committee of the College of Medicine (University of Ha'il, KSA) Research Board. All collected data were kept confidential. Participants were informed on the risks and benefits and their right not to provide information, or to withdraw from the study at any time without any sort of penalty.

3. Results

The present study was conducted for a period from September 2018 to December 2019. A total of 233 participants with an average age 10.33 ± 3.68, consisting of 113 (48.50%) males and 120 (51.50%) females were included in this study (Fig 1). Out of 113 (48.50%) males, there were 19 (16.81%) have sufficient 25-hydroxyvitamin D level, 39 (34.51%) have insufficient 25-hydroxyvitamin D level while 55 (48.67%) have deficient 25-hydroxyvitamin D level. On the other hand, out of 120 (51.50%) female there were 21 (17.50%) have sufficient 25-hydroxyvitamin D level, 41 (34.16%) have insufficient 25-hydroxyvitamin D level and 58 (48.33%) have deficient 25-hydroxyvitamin D level (Fig 2).

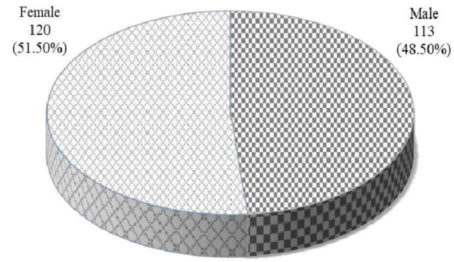

Fig 1. Study population

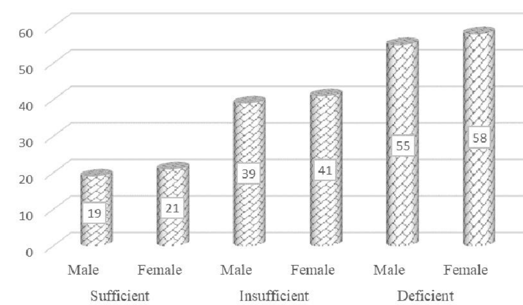

Fig 2. Vitamin D level in the study population

3.1. Characteristics of study participants

Table (1): Characteristics of study participants

Characters		**No. (%)**	**P- value**
Gender	Male	113 (48.5)	0.646
	Female	120 (51.5)	
Age	6-9	33 (14.16)	0.000
	10- 14	78 (33.47)	
	15-19	122 (52.36)	
Skin color	White	39 (16.73)	0.000
	Light Brown	108 (46.35)	
	Dark Brown	63 (27.03)	
	Black	23 (9.87)	
School level	Primary	42 (18.02)	0.000
	Intermediate	111 (47.63)	
	Secondary	80 (34.33)	
Economic level	Low	29 (12.44)	0.000
	Intermediate	76 (32.61)	
	High	128 (54.93)	
Total		233 (100%)	--

As shown in table 1, 233 school students were enrolled in this study with almost equal proportion of males (48.5%) and females (51.5%), statistically there is no significant difference between the percentages of male and female, p-value recorded 0.646. Their ages ranged from 6 to 19 years. Mostly (46.35%) had light brown skin color, approximately one-third had dark brown skin color (27.03%) and few (9.87%) had black skin color. Most of the study participants were from the intermediate school level (47.63%), and the rest were distributed between the primary school level (18.02%) and secondary school level (34.33%). Furthermore, more than 50% of participants were of high economic level (54.93%), 32.61% were of intermediate economic level, and 12.44% were of low economic level. Statistically, there is a highly significant difference between the different age, skin colors and different economic levels between all enrolled participants (p-value < 0.05).

3.2. Frequency of isolated pathogens and its relation to urine analysis in male children

One hundred thirteen urine samples were collected from male children. Urine microscopy recorded highest pus cells (WBCs) in all infected urine samples, while red blood cells (RBCs) and crystals were only found in 24 of samples. Culture plates with bacterial counts greater than or equal to $1x10^5$ CFU /ml were taken as positive, thus indicative of urinary tract infection (UTI). Table (2), showed higher incidence of *Escherichia coli* 81 (40.91%) followed by *Klebsiella pneumonia* 58 (29.29%) and *Proteus mirabilis* 40 (20.20%), while the incidence of *Staphylococcus aureus* was 19 (9.60%). Statistically our result showed higher significant bacteriuria in all infected urine samples (P-value < 0.05).

Table (2): Frequency of isolated pathogens and its relation to urine analysis in male children

Isolated pathogens	Frequency		Microscopic urine analysis (No.)			
	No.	%	WBCs	RBCs	Epi. cells	Crystal
Escherichia coli	81	40.91	78	7	3	5
Klebsiella pneumonia	58	29.29	63	5	1	3
Proteus mirabilis	40	20.20	45	3	2	1
Staphylococcus aureus	19	9.60	27	0	0	0
Total	198	100	213	15	6	9
P-value	0.000	--	0.000	0.067	0.034	0.087

3.3. Frequency of isolated pathogens and its relation to urine analysis in female children

One hundred twenty urine samples were collected from female children. Urine microscopy recorded highest pus cells (WBCs) in all infected urine samples. While red blood cells (RBCs) and crystals were only found in 34 of samples. Table (3), showed higher incidence of *Escherichia coli* 93 (31.00%) followed by *Klebsiella pneumonia* 76 (25.33%) and *Proteus mirabilis* 58 (19.33%), while the incidence of *Pseudomonas aeruginosa* recorded the lowest value. It recorded 29 (9.67%). Statistically our result showed higher significant bacteriuria in all infected urine samples (P-value < 0.05).

Table (3): Frequency of isolated pathogens and its relation to urine analysis in female children

Isolated pathogens	Frequency		Microscopic urine analysis (No.)			
	No.	%	WBCs	RBCs	Epi. cells	Crystal
Escherichia coli	93	31.00	97	13	36	11
Klebsiella pneumonia	76	25.33	79	7	29	7
Proteus mirabilis	58	19.33	58	5	15	4
Streptococcus faecalis	44	14.67	36	1	8	1
Pseudomonas aeruginosa	29	9.67	22	3	4	2
Total	300	100	292	29	92	25
P-value	0.000	--	0.000	0.005	0.000	0.010

3.4. The association between 25-hydroxyvitamin D level and the frequency of isolated pathogen in male children

Table (4), represents the association between 25-hydroxyvitamin D level and the frequency of isolated pathogen in male children. The frequency of *Escherichia coli* isolate recorded the higher percentage between enrolled male children which have deficient 25-hydroxyvitamin D level in compared to sufficient 25-hydroxyvitamin D level. It recorded 42 (51.85%) and 11 (13.58%), respectively, followed by *Klebsiella pneumonia* and *Proteus mirabilis*. They recorded 31 (53.45%), 7 (12.07%) and 23 (57.50%), 3 (7.50%), respectively. While the frequency of *Staphylococcus* *aureus* recorded lowest values in compared to the frequency of *Escherichia coli*, *Klebsiella pneumonia* and *Proteus mirabilis*. It recorded 13 (65.00%) and zero in deficient and sufficient 25-hydroxyvitamin D level groups, respectively. Statistically, there is a higher significant different between 25-hydroxyvitamin D deficiency and the frequency of *Escherichia coli*, *Klebsiella pneumonia* and *Proteus mirabilis* (p-value < 0.05). On the other hand, there is no significant different between the frequency of *Staphylococcus aureus* and 25-hydroxyvitamin D deficiency (p-value = 0.069).

Table (4): The association between 25-hydroxyvitamin D level and the frequency of isolated pathogen in male children

Isolated pathogens	25-hydroxyvitamin D level (No.) %		Frequency of isolated pathogens (No.) %	P-value
Escherichia coli	Sufficient	19 (16.81)	11 (13.58)	0.005
	Insufficient	39 (34.51)	28 (34.57)	
	Deficient	55 (48.67)	42 (51.85)	
Klebsiella pneumonia	Sufficient	19 (16.81)	7 (12.07)	0.010
	Insufficient	39 (34.51)	20 (34.48)	
	Deficient	55 (48.67)	31 (53.45)	
Proteus mirabilis	Sufficient	19 (16.81)	3 (7.50)	0.004
	Insufficient	39 (34.51)	14 (35.00)	
	Deficient	55 (48.67)	23 (57.50)	
Staphylococcus aureus	Sufficient	19 (16.81)	0	0.069
	Insufficient	39 (34.51)	6 (30.00)	
	Deficient	55 (48.67)	13 (65.00)	

3.5. Pearson's correlation between 25-hydroxyvitamin D level and the frequency of isolated pathogens in male children

Table (5) & Fig (3, 4, 5, 6), showed a higher positive significant correlation between 25-hydroxyvitamin D levels and the frequency of *Escherichia coli*, *Klebsiella pneumonia* and *Proteus mirabilis*, (p-value < 0.05). Pearson's correlation coefficient between 25-hydroxyvitamin D and the frequency of isolated pathogens recorded a higher significant value. It recorded (r = 1.000**, p-value 0.005) for *E. coli*; (r = 1.000*, p-value 0.010) for *K. pneumonia* and (r = 1.000**, p-value 0.004) for *P. mirabilis*, this correlation is highly significant because the p-value is less than 0.05. On the other hand, Pearson's correlation coefficient between 25-hydroxyvitamin D and the frequency of *Staphylococcus aureus* non-significant (p-value > 0.05).

Table (5): Pearson's correlation between 25-hydroxyvitamin D level and the frequency of isolated pathogens in male children

		25-hydroxyvitamin D level	Frequency of Isolated Pathogens
Escherichia Coli	**Vitamin D level** Pearson's Correlation Sig. (2 tailed) N	 1 3	 1.000(**) 0.005 3
	Frequency *Escherichia Coli* Pearson's Correlation Sig. (2 tailed) N	 1.000(**) 0.005 3	 1 3
K. pneumonia	**Vitamin D level** Pearson's Correlation Sig. (2 tailed) N	 1 3	 1.000(*) 0.010 3
	Frequency *Klebsiella pneumonia* Pearson's Correlation Sig. (2 tailed) N	1.000(*) 0.010 3	1 3

		25-hydroxyvitamin D level	Frequency of Isolated Pathogens
Proteus mirabilis	**Vitamin D level** Pearson's Correlation Sig. (2 tailed) N	1 3	1.000(**) 0.004 3
	Frequency *Proteus mirabilis* Pearson's Correlation Sig. (2 tailed) N	1.000(**) 0.004 3	1 3
Staph. aureus	**Vitamin D level** Pearson's Correlation Sig. (2 tailed) N	1 3	0.994 0.069 3
	Frequency *Staphylococcus aureus* Pearson's Correlation Sig. (2 tailed) N	0.994 0.069 3	1 3

**** Correlation is significant at the 0.01 level (2-tailed).**
*** Correlation is significant at the 0.05 level (2-tailed).**

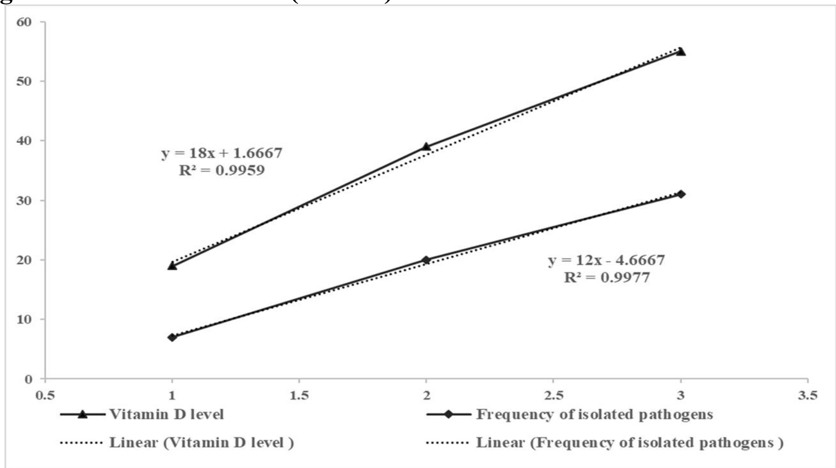

Fig 3: Correlation between 25-hydroxyvitamin D levels and the frequency of *Escherichia Coli* isolates in male children (r = 1.000, p = 0.005).**

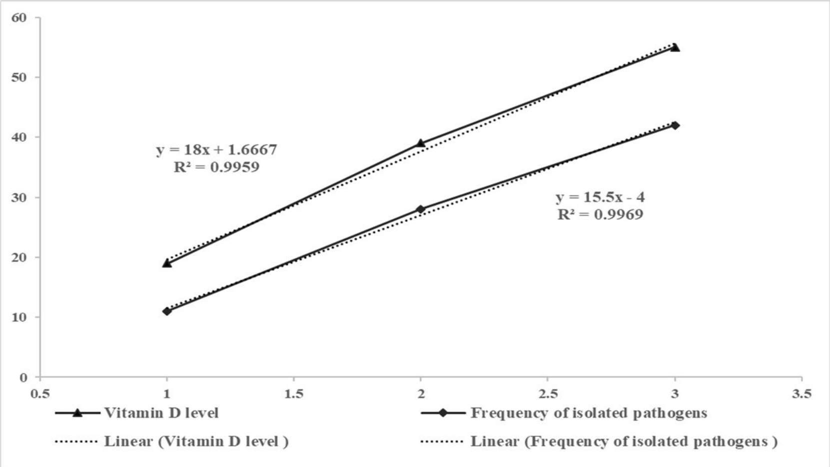

Fig 4: Correlation between 25-hydroxyvitamin D levels and the frequency of *Klebsiella pneumonia* isolates in male children (r = 1.000*, p = 0.010).

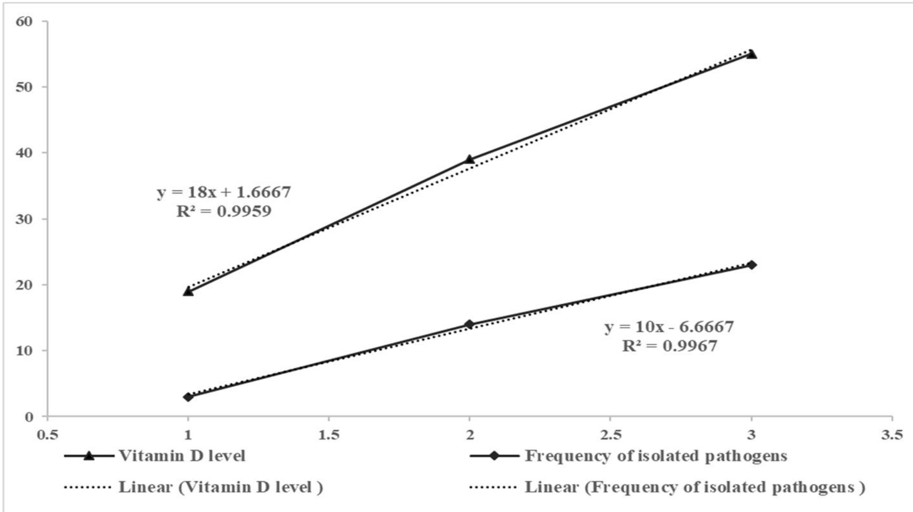

Fig 5: Correlation between 25-hydroxyvitamin D levels and the frequency of *Proteus mirabilis* isolates in male children (r = 1.000, p = 0.004).**

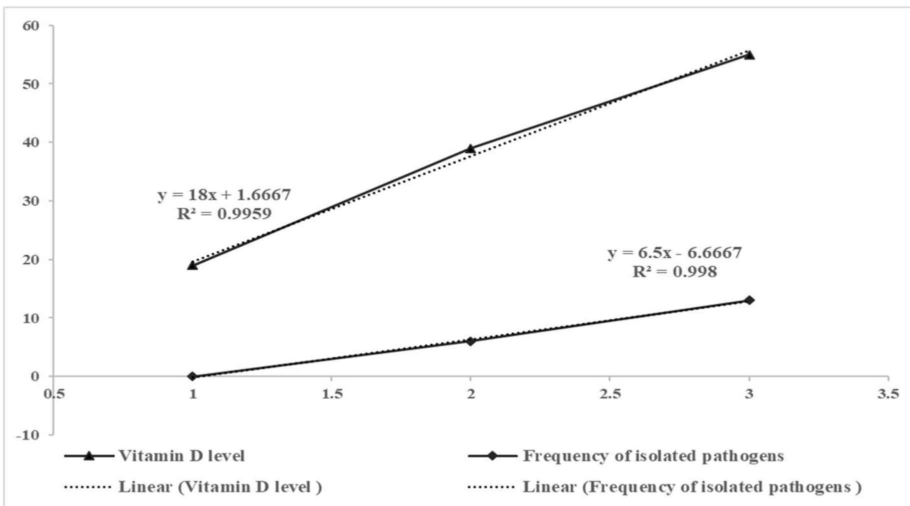

Fig 6: Correlation between 25-hydroxyvitamin D levels and the frequency of *Staphylococcus aureus* isolates in male children (r = 0.994, p = 0.069).

3.6. The association between 25-hydroxyvitamin D level and the frequency of isolated pathogen in female children

Table (6), represents the association between 25-hydroxyvitamin D level and the frequency of isolated pathogen in female children. The frequency of *Escherichia coli* isolate recorded the higher percentage between enrolled female children which have deficient 25-hydroxyvitamin D level in compared to sufficient 25-hydroxyvitamin D level. It recorded 47 (50.54%) and 14 (15.05%), respectively. Followed by *Klebsiella pneumonia* and *Proteus mirabilis* which recorded also a higher percentage between deficient 25-hydroxyvitamin levels in enrolled female children in compared to sufficient 25-hydroxyvitamin D level. They recorded 41 (53.95%), 9 (11.84%) and 33 (56.90%), 5 (8.62%), respectively. While the frequency of *Pseudomonas aeruginosa* recorded the lowest values in compared to the frequency of *Escherichia coli*, *Klebsiella pneumonia* and *Proteus mirabilis.* It recorded 19 (65.52%) and zero in deficient and sufficient 25-hydroxyvitamin D level groups, respectively. Statistically, there is a higher significant different between 25-hydroxyvitamin D deficiency and the frequency of *Escherichia coli*, *Klebsiella pneumonia* and *Proteus mirabilis*, *Streptococcus faecalis* and *Pseudomonas aeruginosa* (p-value < 0.05).

Table (6): The association between 25-hydroxyvitamin D level and the frequency of isolated pathogen in female children

Isolated pathogens	25-hydroxyvitamin D level (No.) %		Frequency of isolated pathogens (No.) %	P-value
Escherichia coli	Sufficient	21 (17.50)	14 (15.05)	0.004
	Insufficient	41 (34.16)	32 (34.41)	
	Deficient	58 (48.33)	47 (50.54)	
Klebsiella pneumonia	Sufficient	21 (17.50)	9 (11.84)	0.007
	Insufficient	41 (34.16)	26 (34.21)	
	Deficient	58 (48.33)	41 (53.95)	
Proteus mirabilis	Sufficient	21 (17.50)	5 (8.62)	0.004
	Insufficient	41 (34.16)	20 (34.48)	
	Deficient	58 (48.33)	33 (56.90)	
Streptococcus faecalis	Sufficient	21 (17.50)	2 (4.55)	0.015
	Insufficient	41 (34.16)	15 (34.09)	
	Deficient	58 (48.33)	27 (61.36)	
Pseudomonas aeruginosa	Sufficient	21 (17.50)	0	0.010
	Insufficient	41 (34.16)	10 (34.48)	
	Deficient	58 (48.33)	19 (65.52)	

3.7. Pearson's correlation between 25-hydroxyvitamin D level and the frequency of isolated pathogens in female children

Table (7) & Fig (7, 8, 9, 10, 11), showed a higher positive significant correlation between 25-hydroxyvitamin D levels and the frequency of *Escherichia coli*, *Klebsiella pneumonia* and *Proteus mirabilis*, *Streptococcus faecalis* and *Pseudomonas aeruginosa* (p-value < 0.05). Pearson's correlation coefficient between 25-hydroxyvitamin D and the frequency of isolated pathogens recorded a higher positive significant value. It recorded (r = 1.000**, p-value 0.004), for *E. coli*; (r = 1.000**, p-value 0.007) for *K. pneumonia*; (r = 1.000**, p-value 0.004) for *P. mirabilis*; (r = 1.000*, p-value 0.015) for *Streptococcus faecalis*; (r = 1.000*, p-value 0.010) for *P. aeruginosa*, this correlation is highly significant because the p-value < 0.05.

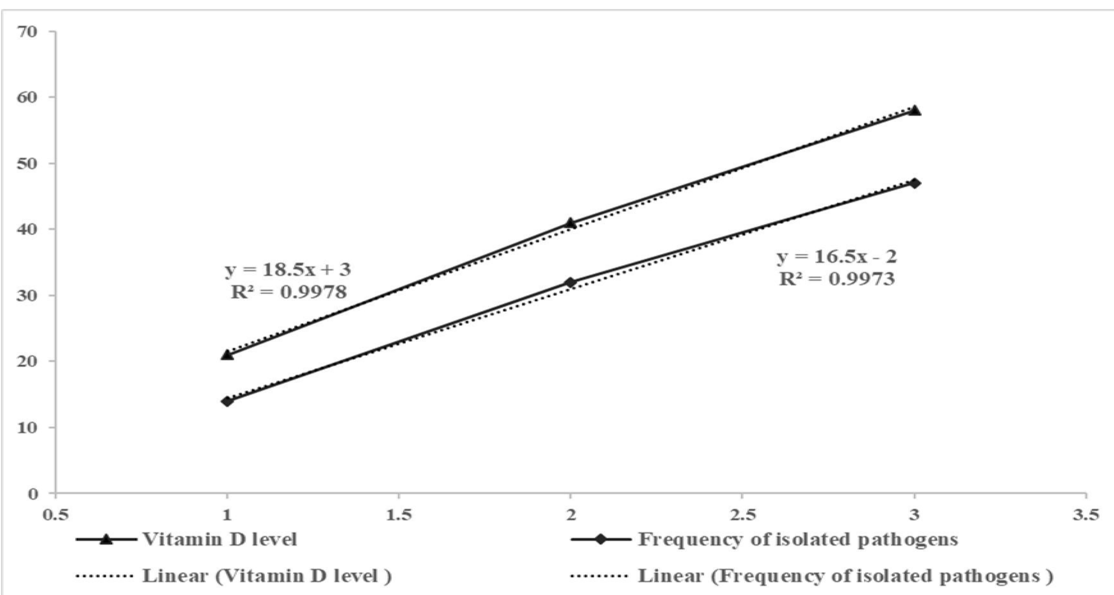

Fig 7: Correlation between 25-hydroxyvitamin D levels and the frequency of *Escherichia Coli* isolates in female children (r = 1.000, p = 0.004).**

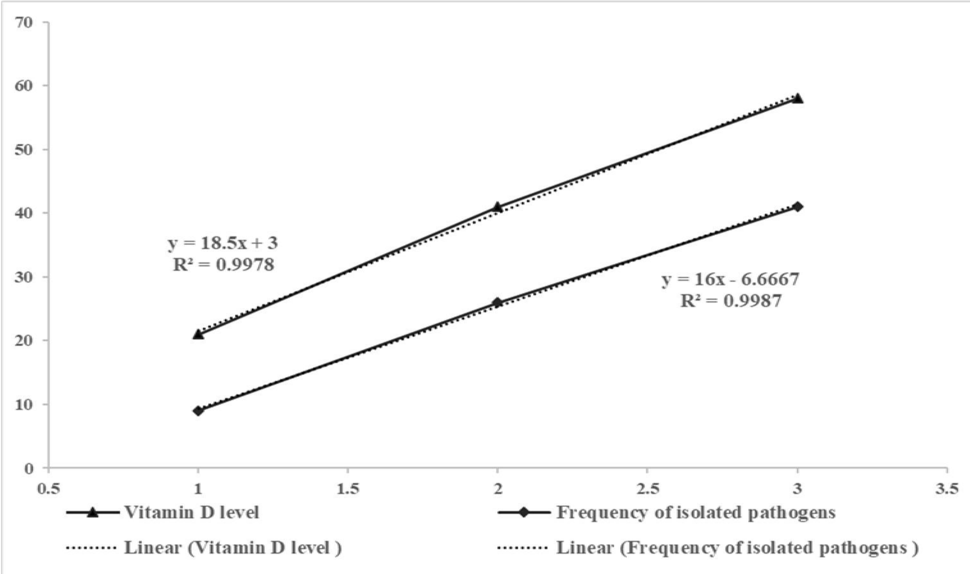

Fig 8: Correlation between 25-hydroxyvitamin D levels and the frequency of *Klebsiella pneumonia* isolates in female children (r = 1.000, p = 0.007).**

Table (7): Pearson's correlation between 25-hydroxyvitamin D level and the frequency of isolated pathogens in female children

		25-hydroxyvitamin D level	Frequency of Isolated Pathogens
Escherichia Coli	**Vitamin D level** Pearson's Correlation Sig. (2 tailed) N	 1 3	 1.000(**) 0.004 3
	Frequency *Escherichia Coli* Pearson's Correlation Sig. (2 tailed) N	 1.000(**) 0.004 3	 1 3
K. pneumonia	**Vitamin D level** Pearson's Correlation Sig. (2 tailed) N	 1 3	 1.000(**) 0.007 3
	Frequency *Klebsiella pneumonia* Pearson's Correlation Sig. (2 tailed) N	 1.000(**) 0.007 3	 1 3
Proteus mirabilis	**Vitamin D level** Pearson's Correlation Sig. (2 tailed) N	 1 3	 1.000(**) 0.004 3
	Frequency *Proteus mirabilis* Pearson's Correlation Sig. (2 tailed) N	 1.000(**) 0.004 3	 1 3
Strept. faecalis	**Vitamin D level** Pearson's Correlation Sig. (2 tailed) N	 1 3	 1.000(*) 0.015 3

	Frequency *Streptococcus faecalis* Pearson's Correlation Sig. (2 tailed) N	 1.000(*) 0.015 3	 1 3
P. aeruginosa	**Vitamin D level** Pearson's Correlation Sig. (2 tailed) N	 1 3	 1.000(*) 0.010 3
	Frequency *Pseudomonas aeruginosa* Pearson's Correlation Sig. (2 tailed) N	1.000(*) 0.010 3	1 3

**** Correlation is significant at the 0.01 level (2-tailed).**
*** Correlation is significant at the 0.05 level (2-tailed).**

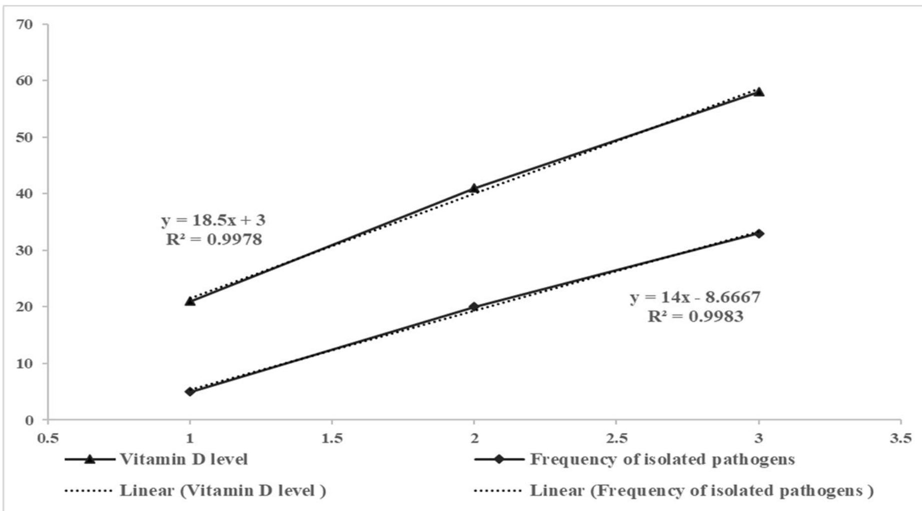

Fig 9: Correlation between 25-hydroxyvitamin D levels and the frequency of *Proteus mirabilis* isolates in female children (r = 1.000, p = 0.004).**

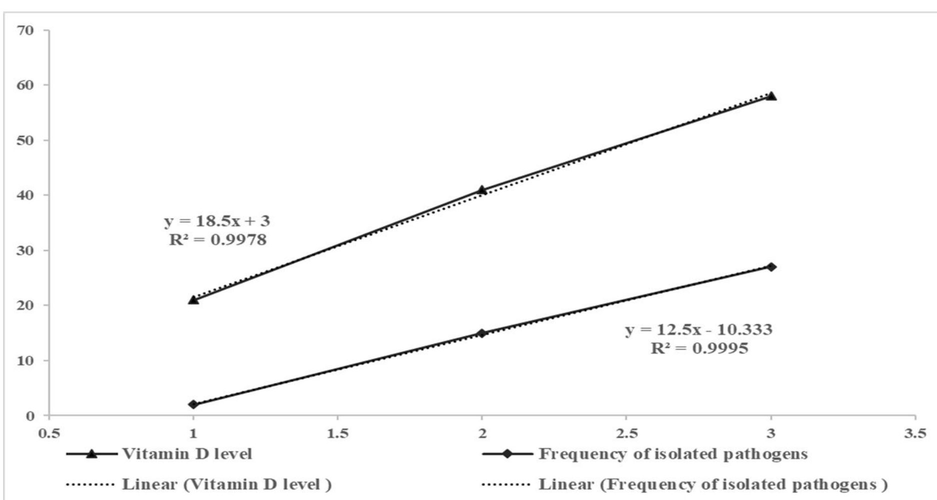

Fig 10: Correlation between 25-hydroxyvitamin D levels and the frequency of *Streptococcus faecalis* isolates in female children (r = 1.000*, p = 0.015).

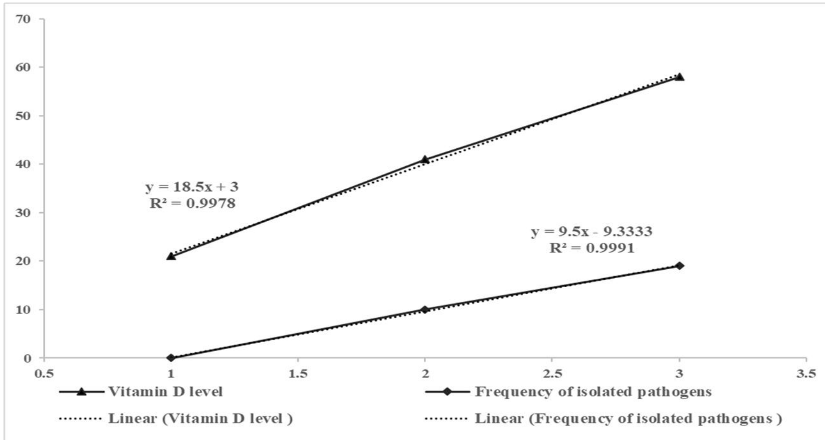

Fig 11: Correlation between 25-hydroxyvitamin D levels and the frequency of *Pseudomonas aeruginosa* isolates in female children (r = 1.000*, p = 0.010).

4. Discussion

Urinary tract infection (UTI) is one of the most commonly acquired bacterial infections in childhood. It affects 10% of children and causes significant morbidity (Craig, 2001). *Escherichia coli* (*E. coli*) is the most predominant pathogen in childhood UTI found in 90% of girls and in 80% of boys at the primary UTI. These infections have been reported to be one of the most infections that cause serious health problems affecting millions of people both in the community and hospital setting and may cause permanent kidney damage (Abdulhadi *et al*., 2008; Becknell *et al*., 2015; Hay *et al*., 2016).

Vitamin D deficiency and insufficiency is a global issue (Holick and Chen, 2008). It is well known that vitamin D is involved in classical calcium homeostasis. More recent data from a variety of sources indicate that vitamin D has a broad spectrum of actions against autoimmune diseases and infections (Arnson e*t al*., 2007; Borella *et al*., 2014). The present study was undertaken to assessment the association between serum 25-Hydroxyvitamin D level and urinary tract infections (UTIs) in both male and female children. Out of the 233 patients 120 female (51.5%) and 113 males (48.5%) with an average age 10.33 ± 3.68, participated in this study. Mostly (46.35%) had light brown skin color, approximately one-third had dark brown skin color (27.03%) and few (9.87%) had black skin color. Most of the study participants were from the intermediate school level (47.63%), and the rest were distributed between the primary school level (18.02%) and secondary school level (34.33%).

Furthermore, more than 50% of participants were of high economic level (54.93%), 32.61% were of intermediate economic level, and 12.44% were of low economic level. Statistically, there is a highly significant difference between the different age, skin colors and different economic levels between all enrolled participants, p-value < 0.05. The frequency of isolated pathogens and its relation to urine analysis in both male and female children showed higher incidence of *Escherichia coli, Klebsiella pneumonia, Proteus mirabilis.* In male *Escherichia coli,* recorded 81 (40.91%) followed by *Klebsiella pneumonia* 58 (29.29%) and *Proteus mirabilis* 40 (20.20%), while the incidence of *Staphylococcus aureus* was 19 (9.60%). Also, frequency of isolated pathogens in female children showed higher incidence of *Escherichia coli* 93 (31.00%) followed by *Klebsiella pneumonia* 76 (25.33%) and *Proteus mirabilis* 58 (19.33%), while the incidence of *Pseudomonas aeruginosa* recorded the lowest value. It recorded 29 (9.67%). Our data showed that the incidence of isolated pathogens recorded a higher incidence in female than in male children. Statistically our result showed higher significant bacteriuria in all infected urine samples (*P*-value = 0.000). Similar to our study, Kehinde *et al*., (2017), found that, out of 280 urine samples analyzed, 91(32.5 %) samples were positive to urine culture 61(38.1%) for females and 30 (25%) for males and *Escherichia coli* have the highest (33%) occurrence.

The association between 25-hydroxyvitamin D level and the frequency of isolated pathogens in male children showed that, frequency of *Escherichia coli* isolate recorded the higher percentage between enrolled male children which have deficient 25-hydroxyvitamin D level in compared to sufficient 25-hydroxyvitamin D level. It recorded 42 (51.85%) and 11 (13.58%), respectively. Followed by *Klebsiella pneumonia* and *Proteus mirabilis* which recorded also a higher percentage between deficient 25-hydroxyvitamin levels in enrolled male children in compared to sufficient 25-hydroxyvitamin D level.

They recorded 31 (53.45%), 7 (12.07%) and 23 (57.50%), 3 (7.50%), respectively. Pearson's correlation coefficient recorded a higher positive significant value. It recorded (r = 1.000**, p-value 0.004), for *E. coli*; (r = 1.000**, p-value 0.007) for *K. pneumonia*; (r = 1.000**, p-value 0.004) for *P. mirabilis*; (r = 1.000*, p-value 0.015) for *Streptococcus faecalis*; (r = 1.000*, p-value 0.010) for *P. aeruginosa*, this correlation is highly significant because the p-value < 0.05. Our findings were in agreement to that reported by Shalaby *et al.*, (2018), they showed that the serum level of 25(OH)D3 in children with UTI was significantly lower than in healthy children ($p < 0.001$). Insufficient levels of (≤ 25 nmol/l) were significantly more frequent (38%) in patients with UTI, compared with controls (12%) (p = 0.031).

Also, the association between 25-hydroxyvitamin D level and the frequency of isolated pathogens in female children showed a higher frequency of *Escherichia coli* between enrolled female children which have deficient 25-hydroxyvitamin D level in compared to sufficient 25-hydroxyvitamin D level. It recorded 47 (50.54%) and 14 (15.05%), respectively. Followed by *Klebsiella pneumonia* and *Proteus mirabilis* which recorded also a higher percentage between deficient 25-hydroxyvitamin levels in enrolled female children in compared to sufficient 25-hydroxyvitamin D level. They recorded 41 (53.95%), 9 (11.84%) and 33 (56.90%), 5 (8.62%), respectively. While the frequency of *Pseudomonas aeruginosa* recorded lowest values in compared to the frequency of *Escherichia coli*, *Klebsiella pneumonia* and *Proteus mirabilis.* It recorded 19 (65.52%) and zero in deficient and sufficient 25-hydroxyvitamin D level groups, respectively. Pearson's correlation coefficient recorded a higher positive significant value. It recorded (r = 1.000**, p-value 0.004), for *E. coli*; (r = 1.000**, p-value 0.007) for *K. pneumonia*; (r = 1.000**, p-value 0.004) for *P. mirabilis*; (r = 1.000*, p-value 0.015) for *Streptococcus faecalis*; (r = 1.000*, p-value 0.010) for *P. aeruginosa*, this correlation is highly significant because the p-value < 0.05.

Our findings were similar to that reported by (Nseir *et al.*, 2013; Nielsen *et al.*, 2014; Kwon *et al.*, 2015; Tekin *et al.*, 2015; Yang *et al.,* 2016; Mahyar *et al.*, 2018; Georgieva *et al.*, 2018) they showed that there was a relationship between serum 25-hydroxyvitamin D levels and UTI in children. They concluded that the risk of UTI is high in children with vitamin D deficiency. Furthermore, our study was in agreement with, a recently published Turkish study of 82 children experiencing a first episode of UTI, with no risk factors for UTI, and 64 healthy control children showed that a serum level of 25(OH)D3 < 20 nmol/l is associated with UTI (OR = 3.503, 95% CI: 1.621–7.571; $p = 0.001$). Children with serum levels of 25(OH)D3 < 20 nmol/l are 3.5 times more likely to develop UTI than those with normal levels (Tekin *et al.*, 2018). On the other hand, Haghighi *et al.*, (2017) showed that, serum level of 25-hydroxyvitamin D was significantly lower in patients with asymptomatic bacteriuria, compared to women without asymptomatic bacteriuria. Also, Ryu *et al.*, (2019) found that, vitamin D deficiency is correlated with hematuria in women, particularly after menopause.

Bodin *et al.*, (2019), found that, multiple infections were significantly more frequent among girls with the lowest vitamin D levels. This is in accordance with reports of increased risk for hospitalization due to respiratory tract infections among adults with 25(OH) D levels below 37 nmol/L (Jovanovich *et al.*, 2014; Mamani *et al.*, 2017). Furthermore, intervention studies addressing vitamin D supplementation show decreased respiratory infections at serum levels above 50 nmol/L (Martineau *et al.*, 2017; Li *et al.*, 2018), as well as the prevention of seasonal influenza among school children in Japan (Urashima *et al.*, 2010; Arihiro *et al.*, 2019). Also, our finding was in agreement with Mahmoudzadeh *et al.*, (2020), they found that, 25-hydroxyvitamin D levels were lower in the UTI group [14.5 ng/mL (9.4–18.8)] than in the controls [27 ng/mL (22.4–39.0)] ($p < 0.001$). In addition, the prevalence of 25-hydroxyvitamin D levels <20 ng/mL was higher in the children with UTI than in the controls (68% *vs* 18%) ($p < 0.001$).

5. Conclusion

Escherichia coli, Klebsiella pneumonia, and *Proteus mirabilis* are the most common bacterial isolates causes urinary tract infections (UTIs) in both male and female children but their incidence recoded a higher frequency in female than in male children. Also, there is a higher positive significant correlation between vitamin D deficiency and the incidence of UTIs between male and female children.

Acknowledgment

The author wishes to thank the participants who took part in the study.

Corresponding Author:
Dr. Mohamed Abdelhameid Fareid Alekhtaby
Botany and Microbiology Department
Faculty of Science, Al-Azhar University, Nasr City, Cairo 11884, Egypt.
Present address: Basic Science Dept., Prep Year Deanship, University of Ha'il, Saudi Kingdom.
E-mail: mohamedfareid73@yahoo.com

References

1. Abdulhadi SK, Yashua AH and Uba A (2008): Organisms Causing Urinary Tract Infections in Paediatric Patients. *Journal of Biomedical Health Sciences*, 4, 165-167.
2. Adams JS and Hewison M (2010): Update in vitamin D. *J Clin Endocrinol Metab*., 95: 471-478.
3. Alladi YR and Gopal J (2017): Vitamin-D Deficiency and Relation to Recurrent Respiratory Tract Infection in children less than 5 years. *Journal of Dental and Medical Sciences* (IOSR-JDMS). 16 (8): 88-96.
4. Allamin IA, Machina IB, Ibn Abbas M and Angale KI (2015): Prevalence of Urinary Tract Infection among Children Attending Babban Mutum Science Primary School Shekal, Bauchi State, Nigeria. *Open Access Library Journal*,2: e1596.
5. Arihiro S, Nakashima A, Matsuoka M, Suto S, Uchiyama K, Kato T, Mitobe J, Komoike N, Itagaki M and Miyakawa Y (2019): Randomized Trial of Vitamin D Supplementation to Prevent Seasonal Influenza and Upper Respiratory Infection in Patients with Inflammatory Bowel Disease. *Inflamm. Bowel Dis.* 25(6):1088-1095.
6. Arnson Y, Amital H, and Shoenfeld Y (2007): Vitamin D and autoimmunity: new etiological and therapeutic consideration. *Ann Rheum Dis*; 66:1137-1142.
7. Aydin S, Aslan I, Yildiz I, Agachan B, Toptas B and Toprak S (2011): Vitamin D levels in children with recurrent tonsillitis. *Int J Pediatr Otorhinolaryngol*., 75:364-367.
8. Becknell B, Schober M, Korbel L and Spencer JD (2015): The Diagnosis, Evaluation and Treatment of Acute and Recurrent Pediatric Urinary Tract Infections. *Expert Rev Anti Infect Ther*.,13(1): 81–90.
9. Berry DJ, Hesketh K, Power C and Hypponen E (2011): Vitamin D status has a linear association with seasonal infections and lung function in British adults. *Br J Nutr*., 106(9): 1433-1440.
10. Bodin J, Mihret A, Holm-Hansen C, Dembinski CJ, Trieu M, Tessema B, Tarekegne A, Yimer SA, Cox R, Aseffa A, Haneberg B and Mjaaland S (2019): Vitamin D Deficiency is Associated with Increased Use of Antimicrobials among Preschool Girls in Ethiopia. *Nutrients*, 11(3), 575.
11. Borella E, Nesher G, Israeli E and Shoenfeld Y (2014): Vitamin D: a new anti-infective agent. *Ann N Y Acad Sci;* 1317:76-83.
12. Brandström P and Hansson S (2015): Long-term, low-dose prophylaxis against urinary tract infections in young children. *Pediatr Nephrol*; 30:425-432.
13. Buchanan RE and Gribbons NE (1974): Bergey's Manual of Determinative Bacteriology (8th edition). Williams & Wilkins Co. Baltimore USA.
14. Cannell JJ, Vieth R, Umhau JC, Holick MF, Grant WB and Madronich S (2006): Epidemic influenza and vitamin D. *Epidemiol Infect*., 134:1129-1140.
15. Cheesbrough M (2006): District Laboratory Practice in Tropical Countries. Part II. Cambridge University Press, New York. Pp. 105-114.
16. Collak A, Bozaykut A, Demirel B, Sezer R G, Seren LP and Dogru M (2014): Serum vitamin D levels in children with recurrent tonsillopharyngitis. *North Clin Istanbul – NCI*; 1(1):13-18.
17. Craig JC (2001): Urinary tract infection: new prospective on a common disease. *Curr Opin Infect Dis*; 14:309-313.
18. Georgieva V, Kamolvit W, Herthelius M, P Luthje P, A Brauner A and Chromek M (2018): Association between vitamin D, antimicrobial peptides and urinary tract infection in infants and young children. *Acta Paediatrica.* 108, pp. 551-556.
19. Guillot X, Semerano L, Saidenberg-Kermanac'h N, Falgarone G and Boissier MC (2010): Vitamin D and inflammation. *Joint Bone Spine*; 77:552-557.
20. Haghighi M, Shoaee S, Moghaddam MA, Mohammad Sistanizad M and Nikbakht M (2017): The Association Between Serum Level of Vitamin D and Asymptomatic Bacteriuria in Pre- and Postmenopausal Women Evaluated During 2011-2016. *Arch Clin Infect Dis.* 2017 July; 12(3): e62134.
21. Hay A, Sterne J and Hood K (2016): Improving the Diagnosis and Treatment of Urinary Tract Infection in Young Children in Primary Care: Results from the DUTY Perspective Diagnostic Cohort Study. *Ann Fam Med*., 14:325-336.
22. Hewison M (2011): Antibacterial effects of vitamin D. *Nat Rev Endocrinol*; 7:337-435.
23. Holick MF (2007): Vitamin D deficiency. *N Engl J Med*; 357:266-281.
24. Holick MF (2010): Vitamin D: Extra-skeletal health. *Endocrinol Metab Clin North Am*; 39:381-400.
25. Holick MF and Chen TC (2008): Vitamin D deficiency: a worldwide problem with health consequences. *Am J Clin Nutr*; 87:1080-1086.
26. Jian Z, Juan D, Leting H, Youcheng W, Yimei S and Hailong L (2018): Preventive Effects of Vitamin D on Seasonal Influenza A in Infants: A Multicenter, Randomized, Open, Controlled

Clinical Trial. *The Pediatric Infectious Disease Journal*. 37 (8): 749-754.
27. Jovanovich AJ, Ginde AA, Holmen J, Jablonski K, Allyn RL, Kendrick J and Chonchol M (2014): Vitamin D level and risk of community-acquired pneumonia and sepsis. *Nutrients*, 6, 2196-2205.
28. Karlowsky JA, Hoban DJ, Decorby MR, Laing NM and Zhanel GG (2006): Fluoroquinolone-resistant urinary isolates of *Escherichia coli* from outpatients are frequently multi-drug: Results from the North American urinary tract infection collaborative alliance-quinolone resistance study. *Antimicrobial Agents and Chemotherapy*, 50: 2251-2254.
29. Kehinde JA, Oladipupo AJA, Adewale AO and Tope TA (2017): Prevalence of Urinary Tract Infection (UTI) and Concomitant Urinary Schistosomiasis among Primary School Children in Remo North Local Government, Ogun State, Nigeria. *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, 16(11): 68-73.
30. Kolawole AS, Kolawole OM, Kandaki- Olukemi, YT, Babatunde SK, Durowade KA and Kolawole CF (2009): Prevalence of urinary tract infections (UTIs) among patients attending Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State, Nigeria. *Int. J. Medicinal Med. Sci.*, 1(5):163-167.
31. Kwon YE, Kim H, Oh HJ, Park JT, Han SH and Ryu DR (2015): Vitamin D deficiency is an independent risk factor for urinary tract infections after renal transplants. *Medicine (Baltimore)*; 94: e594.
32. Laaksi I, Ruohola JP, Touhimaa P, Auvinen A, Haataja R and Pihlajamaki H (2007): An association of serum vitamin D concentration < 40 nmol/L with acute respiratory tract infection in young Finnish men. *Am J Clin Nutr*., 86:714-717.
33. Lagishetty V, Liu NQ and Hewison M (2011): Vitamin D metabolism and innate immunity. *Mol Cell Endocrinol*; 347:97-105.
34. Li W, Cheng X, Guo L, Li H, Sun C, Cui X, Zhang Q and Song G (2018): Association between serum 25-hydroxyvitamin D concentration and pulmonary infection in children. *Medicine (Baltimore)*, 97, e9060.
35. Mahyar A, Ayazi p, Safari S, Dalirani R, Javadi A and Esmaeily S (2018): Association between vitamin D and urinary tract infection in children. *Korean J. Pediatr*. 61(3): 90-94.
36. Mahmoudzadeh H, Nikibakhsh AA, Pashapour S and Ghasemnejad-Berenji M (2020): Relationship between low serum vitamin D status and urinary tract infection in children: a case–control study. *Pediatrics and international child health*.
37. Doi.org/10.1080/20469047.2020.1771244.
38. Mamani M, Muceli N, Ghasemi Basir HR, Vasheghani M and Poorolajal J (2017): Association between serum concentration of 25-hydroxyvitamin D and community-acquired pneumonia: A case-control study. *Int. J. Gen. Med.*, 10, 423-429.
39. Martineau AR, Jolliffe DA, Hooper RL, Greenberg L, Aloia JF, Bergman P, Dubnov-Raz G, Esposito S, Ganmaa D and Ginde AA (2017): Vitamin D supplementation to prevent acute respiratory tract infections: Systematic review and meta-analysis of individual participant data. *BMJ*, 356, i6583.
40. Nielsen KL, Dynesen P, Larsen P, Jakobsen L, Andersen PS and Frimodt-Møller N (2014): Role of urinary cathelicidin LL-37 and human β-defensin 1 in uncomplicated Escherichia coli urinary tract infections. *Infect Immun*; 82:1572-1578.
41. Nseir W, Mograbi J, Abu-Rahmeh Z, Mahamid M, Abu-Elheja O and Shalata A (2012): The association between vitamin D levels and recurrent group A streptococcal tonsillopharyngitis in adults. *Int J Infect Dis*., 16(10): 735-738.
42. Nseir W, Taha M, Nemarny H and Mograbi J (2013): The association between serum levels of vitamin D and recurrent urinary tract infections in premenopausal women. *Int J Infect Dis*; 17:1121-1124.
43. Prescott M, Harley P and Klein A (2008): Microbiology 7th. edition. McGraw-Hill, New York. pp. 124-126.
44. Rosen CJ, Abrams SA, Aloia JF, Brannon PM, Clinton SK and Durazo-Arvizu RA (2012): IOM committee members respond to Endocrine Society vitamin D guideline. *J Clin Endocrinol Metab*., 97: 1146-1152.
45. Ross AC, Taylor CL, Yaktine AL and Del Valle HB (2011): Dietary reference intakes for calcium and vitamin D. Washington (DC): National Academies Press (US).
46. Ryu H, Cho H, Oh YK, Joo KW, Kim YS, Ahn C and Han SS (2019): Association between vitamin D level and hematuria from a dipstick test in a large-scale population-based study: Korean National Health and nutrition examination survey. *BMC Nephrology*. 20(187): 1-9.Doi.org/10.1186/s12882-019-1369-z
47. Sabetta JR, DePetrillo P, Cipriani RJ, Smardin J, Burns LA and Landry ML (2010): Serum 25-hydroxyvitamin D and the incidence of acute

viral respiratory tract infections in healthy adults. *PLoS One*., 5(6): e11088.

48. Science M, Maguire JL, Russell ML, Smieja M, Walter SD and Loeb M (2013): Low serum 25-hydroxyvitamin D level and risk of upper respiratory tract infection in children and adolescents. *Clin Infect Dis*., 57: 392-397.
49. Shaikh N, Ewing AL, Bhatnagar S and Hoberman A (2010): Risk of renal scarring in children with a first urinary tract infection: a systematic review. *Pediatrics*; 126: 1084-1091.
50. Shalaby SA, Handoka NM and Amin RE (2018): Vitamin D deficiency is associated with urinary tract infection in children. *Arch Med Sci*., 14, 1: 115-121.
51. Smith, P. J., Morris, A. J. and Reller, L. B. (2003): Predicting urine culture results by dipstick testing and phase contrast microscopy. *Pathology*, 35: 161-165.
52. Solberg OO, Ajiboye R and Riley LW (2006): Origin of class 1 and 2 integron and gene cassettes in a population-based sample of Uropathogenic Escherichia coli. *Journal of Clinical Microbiology*, 44: 1347-1351.
53. Tekin M, Konca C, Celik V, Almis H, Kahramaner Z, Erdemir A, Gulyuz A, Uckardes F and Turgut M (2015): The Association between Vitamin D Levels and Urinary Tract Infection in Children. *Horm Res Paediatr*; 83:198-203.
54. Urashima M, Segawa T, Okazaki M, Kurihara M, Wada Y and Ida H (2010): Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. *Am. J. Clin. Nutr.*, 91: 1255-1260.
55. Wilkinson RJ, Llewelyn M, Toossi Z, Patel P, Pasvol G and Lalvani A (2000): Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis among Gujarati Asians in west London: a case-control study. *Lancet*; 355:618-621.
56. Yang J, Chen G, Wang D, Chen M, Xing C and Wang B (2016): Low serum 25- hydroxyvitamin D level and risk of urinary tract infection in infants. *Medicine (Baltimore)*;95: e4137.
57. Yildiz I, Unuvar E, Zeybek U, Toptas B, Cacina C and Toprak S (2012): The role of vitamin D in children with recurrent tonsillopharyngitis. *Ital J Pediatr*., 38(25): 1-6.

9/1/2020