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The relationship between serum 25-hydroxyvitamin D and urinary incontinence in Iranian reproductive-aged women: a cross-sectional study

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A – Study Design, **B** – Data Collection, **C** – Statistical Analysis, **D** – Data Interpretation, **E** – Manuscript Preparation, **F** – Literature Search, **G** – Funds Collection

Summary **Background.** Urinary incontinence is one a lower urinary tract disorder prevalent in women of reproductive age. The role of vitamin D in urinary incontinence has not been proven, despite extensive study.
Objectives. This study aimed to investigate the relationship between serum 25(OH)D levels with urinary incontinence in Iranian reproductive-aged women.
Material and methods. This cross-sectional study was conducted on 437 samples of women aged 15–49 years who complained about any urinary tract disease and were referred to two comprehensive health centres in Izeh, Iran, from May to September 2017. Serum 25(OH)D was assessed using High-Performance Liquid Chromatography after 8–12 hours of fasting. The severity of urinary incontinence was measured using the International Consultation on Incontinence Questionnaire-Short Form (ICIQ-UISF) and was confirmed by a gynaecologist.
Results. The serum levels of 25(OH)D in UI patients were significantly lower than the comparative group ($p < 0.05$). The association between vitamin D levels and UI subgroups was significantly positive ($p = 0.001$). Vitamin D also had a negative coefficient of correlation (-0.096) with the severity of urinary incontinence ($p = 0.001$).
Conclusions. There was a significant relationship between serum 25(OH)D levels and the severity of urinary incontinence among Iranian reproductive-aged women. A low serum 25(OH)D level is also significantly related to UI subgroups.
Key words: vitamin D, urinary incontinence, Iranian women.

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Background

Urinary incontinence (UI) is a common disorder of the lower urinary tract and pelvic floor. According to the definition of the US International Urinary Continence Society (2005), UI includes a set of mental symptoms and objective indications that result from lower activity of the pelvic floor muscles [1]. Additionally, UI is one of the most common medical and social problems of women in all age groups [2]. The actual prevalence of UI is unclear for cultural and social reasons [3] and is reported as being about 5% to 70%, with varying degrees, in studies conducted in different parts of the world, and most studies estimate a prevalence of 25–45% for subgroups of UI [4].

In a study conducted in the US by Hagan et al. (2018), it was reported that 39% of women aged 39–56 years had slight urinary incontinence, 45% had moderate urinary incontinence, and 17% had severe urinary incontinence [5]. In Germany and Denmark, the prevalence rate of UI was 48.3% and 46.4%, respectively [6]. In general, half of women with UI complain of stress incontinence, 10–20% of urgency incontinence, and 30–40% of a mixed pattern of UI [7]. In a study in Pakistan (2013), the overall prevalence of UI was 44.6%, stress incontinence was 31%, urgent incontinence was 47.4%, and mixed incontinence was 33.1% [8]. Based on a systematic review, the overall prevalence of UI in Iranian women was 46%, stress UI was 34%, urge UI was 19%, and mixed UI was 24% [9]. The most common form of UI is stress incontinence, in which the urine is released by increasing intra-abdominal pressure [3].

The predisposing factors for pelvic floor muscle relaxation include age, gender, history of hysterectomy, pregnancy and childbirth, diabetes, smoking, constipation, chronic cough, menopause, tobacco consumption and race [10]. UI can cause many problems for the patients and their families, as well as the community, in different physical, psychological, social and economic areas. Treatment of UI includes surgical and non-surgical treatments, such as lifestyle changes, behavioural therapy and bladder training, vaginal and urethral devices and chemical drugs [7].

Nowadays, considerable attention has been paid to certain therapies, such as supplements for the prevention of several diseases. Vitamin D is a fat-soluble vitamin, which not only plays a role as a vitamin but also as a hormone. Vitamin D is formed in the skin under the influence of sunlight. Further metabolism of vitamin D to its major circulating form (25(OH)D) takes place in the liver and to its hormonal form (1,25(OH)2D) in the kidney, but in other tissues where the 1,25(OH)2D produced serves as a paracrine or autocrine function [11]. Furthermore, this vitamin plays a crucial role in homeostasis of calcium, which is essential for optimal health of the body. A deficiency of this vitamin is also associated with osteoporosis. Vitamin D deficiency is common worldwide, with 78% of adults in the United States [12] and 80% of women of reproductive age having insufficient levels of vitamin D [13]. Factors affecting levels of vitamin D include ethnicity (especially among people with darker skin and African Americans [14], vitamin D supplementation, obesity, metabolic syndrome, seasons (lower levels after winter) [15, 16] and area of residence (lower levels in higher geographic areas and less sun). In recent years, increasing attention has been paid to the role of vitamin D as a cause of musculoskeletal diseases, cardiovascular disease, diabetes, asthma [17] and preeclampsia [18]. Ghanbari et al. (2019), in a meta-analysis study that was aimed at summarising data demonstrating the association between vitamin D levels and pelvic floor disorders (PFD) using published observational studies, revealed that the serum vitamin D levels in women with PFD were significantly lower than healthy women (SMD -0.60; 95% CI, -1.06, -0.13; p = 0.01). Additional prospective studies regarding the association between vitamin D status and PFD were suggested to confirm the findings [19]. A study by Elshazly et al. in the United States showed that men who were older than 50 and with lower urinary tract symptoms have lower levels of vitamin D compared to men without lower urinary tract symptoms [20].

Vitamin D plays an important role in calcium and phosphate homeostasis and normal mineralisation of bone [21]. Recent data has also demonstrated that vitamin D is related to many urological diseases, such as male lower urinary tract symptoms and as a potential marker of benign prostatic hyperplasia [22], urological cancers [23] and erectile dysfunction [24]. There is also evidence that pelvic floor diseases are related to low vitamin D levels [25]. Vitamin D is assumed to have specific pathways in females which affect UI. An immunohistological study showed that the 1,25-dihydroxy vitamin D3 receptor was located in the skeletal muscle of humans [26]. Additionally, a randomised clinical trial of 122 elderly women showed that the musculoskeletal function in a group receiving 1,200 mg calcium plus 800 IU vitamin D compared to a group receiving calcium only improved considerably [27]. It was hypothesised that a low level of vitamin D may facilitate the development of UI by inducing a weakening of the muscles in the pelvic floor.

In particular, muscle weakness in the pelvic floor may prevent incontinent women from closing the urethra effectively during periods of high intra-abdominal pressure, resulting in UI stress [28]. A second potential pathophysiological reason for a link between vitamin D and UI is that vitamin D insufficiency will influence the wall of the detrusor and thus lead to overactive bladder symptoms and urge UI [28]. Vitamin D receptors have been established in both the urothelium and the smooth muscle of the detrusor wall, as evidenced in human and rat bladders [29]. Thus, from a pathophysiological viewpoint, there is a shortage of evidence to confirm the association between low levels of vitamins D and UI.

Objectives

This study proposed to investigate the relationship between serum 25(OH)D levels and UI in reproductive-aged women.

Material and methods

Study design

This was a cross-sectional study conducted in Izeh City, located in the southwest of Iran, from May to September 2017.

Participants

In the present study, 437 reproductive-aged women (15–49 years) who were married, non-pregnant, non-breastfeeding and not within the postpartum period were included using an easy sampling method. The exclusion criteria for the women included: inflammatory bowel disease, a history of gastric bypass surgery, chronic renal disease, musculoskeletal degenerative disease, brain vessel disease, spinal cord injury, diabetes, vascular involvement, multiple sclerosis, smoking, alcohol consumption, urinary infection and renal disease in the last 4 weeks, neurological diseases or cognitive impairment, consumption of a diuretic, cardio-pulmonary diseases, took vitamin D supplement over the past 6 months.

Sampling method

The study was carried out in two comprehensive health centres with a gynaecologist in attendance, where patients were referred for gynaecological problems or counselling. Prior to the collection of data, the aim of this study was explained, and informed consent was obtained from all participants. Data was gathered using demographic and reproductive questionnaires, and urinary incontinence data was compiled using the Urinary Incontinence Short Form (ICIQ-UISF) questionnaire [30]. A gynaecologist then tested the women whose UI had been identified by the questionnaire to validate the diagnosis.

Sample size determination

Sampling continued until the completion of the sample size. The sample size was calculated to be 437 women using the previous study [10] and the following assumptions: $Z_{1-\alpha/2}$ = 1/96, p = 0/24, p - 1 = 0.76, d = 0/1P, confidence interval = 95% of the formula:

$$n = \frac{z^2_{1-\frac{\alpha}{2}}}{d^2}(p-1)$$ [31].

Finally, a total of 437 women were selected, considering the inclusion/exclusion criteria.

Diagnosis of urinary incontinence

UI was assessed using the score of the ICIQ-UISF questionnaire and was based on reports made by women about any forms of urinary leakage and confirmation by a gynaecologist.

Urinary stress incontinence is the most common form of UI in young women and is defined urine leakage with increased intra-abdominal pressure, such as through sneezing, coughing or activity [30]. In this study, this was diagnosed by the answer of given by the women to question number 6 of the ICIQ-UISF questionnaire.

Urgency UI is defined as uncontrolled urine leakage with the feeling of urgent urination which comes with it or immediately before it. In other words, it is an urge to urinate which will be difficult to postpone [30]. In this research, a diagnosis was performed according to the response given to the other item of question number 6 of the ICIQ-UISF questionnaire. In mixed-type incontinence, there are symptoms of stress incontinence and urge incontinence together, and this was diagnosed according to the type of response to all items in question number 6 of the ICIQ-UISF questionnaire.

The severity of urinary incontinence is defined as the amount of urine leakage, and in this research, this was diagnosed based on the total score obtained from the ICIQ-UISF questionnaire and was categorised as: mild (1–5); moderate (6–12); severe (13–18); very intense (19–21) incontinence. The women were also examined by an experienced specialist for UI.

BMI calcification

The BMI was calculated by dividing weight (kg) with squared height (metres) and reported in kg/m^2 and was categorised as normal when BMI was < 25 kg/m^2, overweight when BMI was 25.1–29.9 kg/m^2, and obese when BMI was ≤ 30 kg/m^2 [32].

Laboratory analyses

After 8–12 h of fasting, a 2cc serum sample was taken from all participants in the morning, and all the samples were sent frozen to the same laboratory at the end of the sampling. Serum 25(OH)D, the most stable circulating form of this molecule, was assessed using High-Performance Liquid Chromatography (HPLC). Vitamin D (25(OH) levels) were categorised as severely deficient when it was < 10 ng/mL; deficient when it was 10–20 ng/mL; insufficient when it was 21–29, and sufficient when it was ≥ 30 ng/mL [25].

Statistical analysis

All statistical analyses were performed using SPSS version 22 (IBM Corp., Armonk, NY, USA). Quantitative variables were described by the mean and standard deviation (SD), and categorical variables were also provided by frequency (percentage).

Using the Chi-square test, categorical variables between women with urinary incontinence and healthy women were evaluated. Moreover, the Mann-Whitney U test was implemented to compare quantitative variables between the two mentioned groups. Spearman's correlation coefficient was also used to determine the correlation of 25(OH)D levels with the severity of UI, and the relationship between the serum 25(OH) level and severity of UI was investigated by using the Chi-square test. A *p*-value < 0.05 was considered statistically significant.

Ethical considerations

This paper is a part of a research project registered under no. RHPRC-9604. This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the Ahvaz Jundishapur University of Medical Sciences (No. IR.AJUMS.REC.1396.183). All participants granted their permission by signing the informed consent form before entering into the study. All participants were allowed to be excluded at any time during the study if they did not wish to continue in the research.

Results

Among 2,668 women referring to the health centres, 233 women with urinary incontinence and 201 women without urinary incontinence were included (Fig. 1).

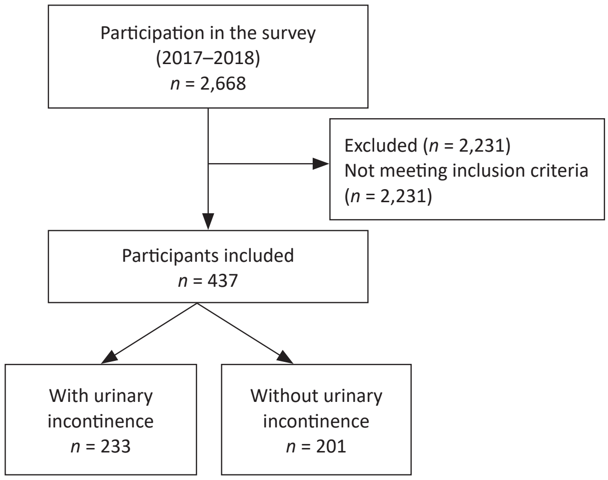

Figure 1. Flowchart of the cross-sectional study

The average age of participants was 32.99 ± 7.8 years. The mean 25(OH)D serum in UI group, 21.59 (14.37), was significantly lower than the healthy women group, 23.02 (12.46), (p = 0.008). Also, 21.5% of women in the UI group versus 12.46% in the healthy women group had a severe Vitamin D deficiency (Table 1).

Table 1. Socio-demographic and reproductive characteristics of participants in women with urinary incontinence and healthy women

Characteristics	Urinary incontinence	Healthy women	*p*
	n = 233	*n* = 204	
	M ± SD/*n* (%)		
Age (years)*	38.13 ± 7.45	27.07 ± 6.40	< 0.001
Age of first childbirth (year)*	22.31 ± 4.79	24.95 ± 3.62	< 0.001
[a] BMI (kg/m^2) *	26.14 ± 4.12	23.97 ± 3.15	< 0.001
New-born weight (g) ≥ 4 kg	62 (28.6)	11 (9.6)	< 0.001
Education			
illiterate	59 (25.3)	3 (1.5)	
primary	93 (39.9)	53 (26.0)	
secondary	38 (16.3)	54 (26.5)	< 0.001
diploma	43 (18.5)	94 (46.1)	
Gravidity (*n*)**	4 (1–12)	2 (1–8)	< 0.001
Parity (*n*)**	4 (1–11)	1 (1–6)	< 0.001
Breastfeeding	64 (29.5)	46 (40)	0.053
History of twin pregnancy	18 (8.3)	4 (3.5)	0.09
Delivery type			
normal vaginal delivery	147 (72.1)	37 (50.0)	
caesarean section	34 (16.7)	34 (45.9)	< 0.001
both	23 (11.3)	3 (4.1)	

Table 1. Socio-demographic and reproductive characteristics of participants in women with urinary incontinence and healthy women

Characteristics	Urinary incontinence	Healthy women	*p*
	n = 233	*n* = 204	
	M ± SD/*n* (%)		
[d] UTI	104 (44.6)	40 (19.6)	< 0.001
History of UTI in family members	86 (36.9)	20 (9.8)	< 0.001
Diabetes	14 (6)	0 (0)	< 0.001
Depression	3 (1.2)	2 (1)	0.76
Nervous system disease	5 (2.1)	0 (0)	0.06
Constipation	49 (21)	34 (16.7)	0.25
[c]25(OH)D status (ng/ml)	21.59 (14.37)	23.02 (12.46)	0.008
severe deficient	50 (21.5)	8 (3.9)	
deficiency	108 (46.4)	85 (41.7)	
insufficient	40 (17.2)	56 (27.5)	< 0.001
sufficient	35 (15)	55 (27)	

Abbreviations: [a] BMI, Body mass index; [b, c] 25(OH)D, 25-hydroxyvitamin D; [d] UTI, urinary tract infection.
* Mann-Whitney U test.
** Median (min–max), Mann-Whitney U test.

Table 2. Correlation coefficient of reproductive characteristics and urinary incontinence

Urinary incontinence severity		
Characteristics	[γ] Correlation coefficient	*p*
25(OH) Vit D level	- 0.096	0.045*
Age of first childbirth (year)	0.65	< 0.001
Gravidity	0.54	< 0.001*
Parity	0.57	< 0.001*

* $p < 0.05$ as significant.
[γ] Spearman's Rank Correlation Tests.

Table 3. Relation between subtypes of Urinary Incontinence and different levels of 25(OH)D (*n* = 233)

25(OH)D serum levels (ng/Ml) / [a] UI Subtypes Frequency (%)	*n* (%)	Severe deficiency (< 10) *n* (%)	Deficiency (10–20) *n* (%)	Insufficient (21–29) *n* (%)	Sufficient (≥ 30) *n* (%)	*p*
Stress UI	131 (56.22)	25 (19.1)	57 (45.51)	26 (19.84)	23 (17.55)	0.001*
Urgent UI	50 (21.45)	7 (14)	27 (54)	5 (10)	11 (22)	0.001*
Mixed UI	52 (22.31)	19 (36.35)	24 (46.15)	8 (15.38)	1 (1.94)	0.001*
UI severity status						
very severe	27	13 (48.1)	9 (33.3)	2 (7.4)	3 (11.1)	0.001
severe	77	23 (29.9)	30 (39)	12 (15.6)	12 (15.6)	
moderate	114	13 (11.4)	58 (50.9)	24 (21.1)	19 (16.7)	
mild	15	1 (6.7)	11 (73.3)	2 (13.3)	1 (6.7)	
normal	204	8 (3.9)	85 (41.7)	56 (27.5)	55 (27)	

* *p*-values less than 0.05 were considered statistically significant using the Chi-square test.
[a] UI – urinary incontinence.

Vitamin D had a negative correlation coefficient (-0.096) with the severity of UI ($p = 0.045$), but there was a positive correlation between the age of first childbirth, gravidity and parity (Table 2).

Among 56.22% of the women who had stress UI, 64.61% had a vitamin D deficiency, and the results of the Chi-square test showed that vitamin D levels affect stress UI ($p = 0.001$). Furthermore, among 21.45% of samples who had urgent UI, 68% had vitamin D deficiency, and the results of the Chi-square test showed that vitamin D levels affect urgent UI ($p = 0.001$). Finally, 22.31% of the samples who had a mixed UI had an 82.5% vitamin D deficiency. The Chi-square test showed that vitamin D levels affect mixed UI ($p = 0.001$). UI severity status also had a significant relationship with the different levels of 25(OH)D ($p = 0.001$) (Table 3).

Discussion

The purpose of the present study was to evaluate the relationship between 25(OH)D serum levels and UI in reproductive-aged women. The data revealed a significant negative relationship between levels of vitamin D and UI. The average amount of 25(OH)D serum level in the UI group was significantly lower than the comparative group (21.59 (14.37) vs 23.02 (12.46), $p = 0.008$). These results are in line with previous studies. For example, a retrospective study carried out on 394 American women demonstrated that women with a pelvic floor disorder had significantly lower vitamin D levels than healthy women (29.3 ± 115 vs 35.0 ± 14.1 ng/ml, $p < 0.001$). A higher score on the Incontinence Impact Questionaire-7 was independently associated with an insufficiency of vitamin D ($p < 0.001$) [19]. Our result is also in line with the results of a study that investigated the correlation between the serum levels of vitamin D and urogenital disorders, including UI [33].

In contrast, Barat et al. (2019) indicated in their case-control study that the highest levels of vitamin D (24.58 ± 20.75 ng/mL) were found in the UI group, especially in the stress UI and urgent UI groups, compared to the control group (15.53 ± 13.11 ng/mL). The author mentioned that this differences in levels of vitamin D dependend on diet, place of residence and other factors [34].

In their research, Lee et al. (2019) have suggested that low serum levels of vitamin D in Korean women were independently correlated with UI [35]. They discussed using the dataset as a limitation.

The finding of the present study confirmed the hypothesis that a low level of vitamin D could promote the development of UI by causing muscle weakness in the pelvic floor. The muscle weakness may play the role of a barrier in effectively closing the urethra under increased intra-abdominal pressure, resulting in urinary stress incontinence [19].

The findings of this analysis also revealed that only 15% of the participants in the UI group vs 27% in the comparative group had a sufficient level of vitamin D.

Such observations are consistent with the results of an earlier report on Iranian women [9]. The underlying causes of low serum levels of vitamin D in Iranian women are dietary habits, as well as women's clothing, which restricts access to sun light. Sunshine impacts vitamin D synthesis [36]. These factors could be effective indicators for the low serum levels of vitamin D among reproductive-aged women in the present study.

Limitations of the study

This research has some limitations. The cross-sectional nature of this analysis may restrict the investigation of the association between serum 25(OH)D concentrations and UI status and does not permit causal inferences relative to prospective longitudinal cohort studies. The strength of the study lies in selecting patients from the two comprehensive health centres, and a specialist also examined all the women for confirmation of UI subgroups.

Conclusions

This study confirmed that low serum levels of 25(OH)D are correlated with the high severity of UI in reproductive-aged women. A low serum 25(OH)D level is significantly related to UI subgroups.

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