HYPOVITAMINOSIS D AND LOW URINARY TRACT SYMPTOMS IN A FEMALE POPULATION

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An increasing number of studies have suggested a key role for low levels of vitamin D in the development of several chronic diseases and bacterial infections. In particular, its role in acute respiratory infection has been clarified, while the potential role of vitamin D for susceptibility to urinary tract infections still remains unexplored. Since the typical symptoms associated with urinary infections or with other conditions, like overactive bladder, include dysuria, urgency and frequency, the aim of this study was to investigate the association between these symptoms and vitamin D status. We conducted a retrospective study on 233 women who, in the previous year, had their serum levels of vitamin D measured. The subjects were queried about the presence of urinary symptoms and their frequency over the previous year. Women with low serum levels of vitamin D had a higher prevalence of symptoms than those with normal levels of vitamin D ($p<0.001$). In particular, women who reported high frequency of symptoms had a mean vitamin D level of ~ 27 ng/mL, those with low frequency had a mean vitamin D level of ~ 24 ng/mL, while asymptomatic women had mean levels of ~ 37 ng/mL ($p=0.004$ among group). In this study hypovitaminosis D is associated with urinary symptoms in a population of women, and it may suggest a key role of this vitamin in the development of infections or other conditions affecting the urinary tract.

An increasing number of observational studies have suggested a key role for the low vitamin D status in the development of several chronic diseases such as cardiovascular diseases, metabolic disorders and cancer (1-5). Furthermore, there is convincing evidence that vitamin D insufficiency is an important risk factor for bacterial infections (6-8). In particular, a number of investigations on the role of vitamin D for the prevention of acute respiratory infections have been conducted (6). Vitamin D receptor (VDR) and the enzyme required for the conversion of vitamin D into its active form, that is 1α-hydroxylase, are both present in the cells of the immune system (9, 10), suggesting an immunomodulatory action by vitamin D. To date, only one study performed on the VDR gene polymorphisms in children has suggested the potential role of vitamin D in the susceptibility to urinary tract infections (UTIs) (11). Symptomatic UTIs cause significant discomfort in affected patients, and could predispose individuals to complications, as well as to the abuse of antibiotic therapy. Women are significantly more likely to experience UTIs

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than men (12) and often an empiric antibiotic therapy is started on the basis of the presence of low urinary tract symptoms (LUTS) alone (13-16). Full comprehension of the factors influencing LUTS and susceptibility to UTIs, in particular in the female sex, requires additional research. In addition, alternative low-cost interventions protecting from UTIs are needed. For these reasons, the aim of this study was to investigate the association between LUTS and vitamin D status in a population of women.

MATERIALS AND METHODS

We conducted a retrospective study using the database of the Clinical Nutrition Unit of the University of Catanzaro. The database included 250 sexually active women who, over the previous year, had their serum levels of vitamin D measured once for research purposes (study protocol number 2011.48 approved by the ethics committee of the Policlinico Universitario Mater Domini, Catanzaro).

We consecutively re-called the women, to complete the collection of the clinical data through a medical exam at our Unit. For this purpose, The subjects were asked to fill in a structured questionnaire, to be completed in approximately 20 minutes. Furthermore, a physical examination by the urologist was carried out to check for any abnormalities affecting the urinary system. The subjects were queried about morbidities and the types of urinary symptoms in line with recommended standards from the International Continence Society (17). LUTS included storage, voiding, and post micturition symptoms (17). Storage symptoms included:

•*Increased daytime frequency*/pollakisuria (when individuals emptied the bladder too often by day)

•*Nocturia* (when individual had to wake up at night one or more times to void).

•*Urgency* (when a sudden compelling desire to pass urine, which is difficult to defer, emerged)

•*Urinary incontinence* (when any involuntary leakage of urine emerged).

Voiding symptoms included:

•*Slow stream* (when reduced urine flow was perceived)

•*Splitting or spraying* of the urine

•*Intermittency* (when the urine flow had stops and starts during micturition)

•*Hesitancy* (when the individual described difficulty in initiating micturition)

•*Straining* (when individuals used muscular effort to initiate, maintain or improve the urinary stream)

•*Terminal dribble* (when the individual described a prolonged final part of micturition)

Post micturition symptoms included:

•*Feeling of incomplete emptying* after passing urine

•*Post micturition dribble* (when the individual described the involuntary loss of urine immediately after rising from the toilet).

In this population LUTS were considered present if at least one of the above symptoms were reported during the previous year. Patients were also asked about the frequency of LUTS during the year and all types of antimicrobial or other agents use during the symptomatic episodes. If a symptom lasted for a long time, it was considered as a single event to avoid overestimation of the number of symptoms.

Postmenopausal status was defined as no natural menses for at least 1 year and serum FSH levels higher than 40 IU/l, or no menses for at least 2 years, when FSH levels were not available.

Exclusion criteria included pregnancy, diabetes, hypertension, neurogenic abnormality affecting micturition function and severe anatomical deformation of the lower urinary tract. Furthermore, we excluded women with a history of surgery on the lower urinary tract, or urinary infection diseases or regular use of a catheter for urine drainage and those with renal disease, liver insufficiency (a condition often associated with low vitamin D status) and other conditions that involve long-term use of antibiotics or immunosuppressive agents or the taking of vitamin D supplements (current users and those who had used them during the previous year). Consequently, a total of 17 women were excluded from the study.

The investigation conforms to the principles outlined in the Declaration of Helsinki. All patients provided informed consent to participate in our research and to use their data.

Biochemical evaluation

Venous blood was collected after fasting overnight into vacutainer tubes (Becton & Dickinson) and centrifuged within 4 h. Serum glucose, creatinine and calcium were measured with Enzymatic colorimetric test. 25-hydroxy vitaminD [(25OH)D] was measured by radioimmunoassay (normal value 30-100 ng/mL) (18, 19). Quality control was assessed daily for all determinations.

Anthropometric and dietetic measurements

Body weight was measured before breakfast with the subjects lightly dressed, subtracting the weight of clothes. Body weight was measured with a calibrated scale and height measured with a wall-mounted stadiometer. BMI was calculated based on the following equation: weight (kg) /height (m) ^{2}(20).

The proportion of the total energy intake derived from

macronutrients was calculated by the nutritional software MetaDieta 3.0.1, (Meteda srl, S. Benedetto del Tronto, Italy).

Statistical analysis

Data are reported as mean ± S.D. The *t*-test and ANOVA were used to compare the means between groups. The *chi*-square-test was used to compare the prevalence among the groups. For these purposes, we divided the population according to the frequency of symptoms during one year, categorizing women as asymptomatic (A), High Frequency (HF) if the frequency of at least one symptom was 6 times or more per year, and Low Frequency (LF) if the frequency of at least one symptom was less than 6 times per year. In addition, we also divided the population according to vitamin D quartiles. Furthermore, a logistic regression analysis was used, considering the presence of LUTS as the dependent variable, and including vitamin D levels as independent variables (since vitamin D was significantly different between groups, and considering it as categorical variables – normal/low) as well as traditional variables that could be associated with LUTS (such as glucose, calcium, age, and creatinin). Significant differences were assumed to be present at $P < 0.05$. All comparisons were performed using the SPSS 20.0 for Windows (Chicago, USA).

RESULTS

In this study a total of 233 women were enrolled of whom 54.5% (129 individuals) had low levels of vitamin D (<30 ng/mL). Postmenopausal status was present in more than 70% of women. In the overall population the prevalence of subjects with at least one symptom during the previous year was 36.5% (85 women). All subjects reported the use of an antimicrobial agent or urinary disinfectants or other agents for alleviating LUTS during the symptomatic episodes. All women adhered to a diet containing at least 50% of food energy from a variety of carbohydrate sources.

Table I shows the characteristics of the population according to vitamin D status (normal/low) (Table I). Women with low levels of vitamin D had the higher prevalence of LUTS in comparison to those with normal levels (75.3% *vs* 24.7%; p <0.001; Table I). In a subgroup of 80 participants, insulin and PCR were assessed with the higher PCR value in low levels of the vitamin D group (p=0.03 between groups). In Table II the characteristics of the population according to the frequency of LUTS are shown. There were no differences in other biochemical, anthropometric and demographic features between the two groups. Women with HF had mean levels of vitamin D of ~ 27 ng/mL and asymptomatic women (A) had mean levels of vitamin D of ~ 37 ng/mL (p=0.004). Women with LF had the lowest vitamin

Table I. *Characteristics of the population according to vitamin D levels.*

Variables **Means** ± SD *(t-test)*	**Normal vitamin D (n=104) (52.3 ± 16 ng/mL)**	**Low vitamin D (n=129) (17.7 ± 7 ng/mL)**	**p**
Age (ys)	58.25±8	59.07±11	0.52
BMI	31.13±5	30.7±7	0.64
WC (cm)	101.9±15	98.4±12	0.12
Creatinin(mg/dl)	0.72±0.1	0.70±0.1	0.20
Glucose(mg/dl)	96.2±18	97.4±19	0.67
Calcium (mg/dl)	8.9±0.4	8.9±0.5	0.81
Insulin*(μU/ml)	16.6±9	14.1±6	0.27
PCR* (mg/dl)	3.6±3	6.0±6	0.03
Prevalence (chi-square test)			
Menopausal status	68%	72%	0.34
LUTS (at least one symptoms)	24.7%	75.3%	<0.001

**In a subgroup of 80 participants (a total of 42 and 38 women, respectively)*

Table II. *Characteristic of the population with and without LUTS, according to frequency/year.*

Means ± SD (ANOVA test)	**A**	**LF**	**HF**	**p**	**Post-hoc test**
Age (ys)	59±9	58.1±12	58.4±9	0.97	_
BMI	30.4±6	33.2±8	31.3±7	0.27	_
WC (cm)	98.9±2	103.4±8	101.3±15	0.42	_
Creatinin(mg/dl)	0.72±0.1	0.68±0.1	0.69±0.1	0.35	_
Glucose(mg/dl)	95.9±15	103.5±32	97.5±20	0.40	_
Calcium (mg/dl)	8.9±0.5	8.6±0.5	9±0.4	0.05	A vs LF= 0.027 LF vs HF= 0.020
Insulin* (μU/ml)	17±9	17±8	11.6±5	0.18	_
PCR*(mg/dl)	4.9±4	7.2±12	4.4±3	0.55	_
Vitamin D (ng/mL)	37.2±21	25.7±22	27.7±19	0.004	A vs LF=0.048 A vs HF= 0.003
Prevalence (chi -square test)					
Menopausal status (%): physiological/ surgical	71.9/12.4	62.5/18.8	66.2/19.1	0.71	
LUTS (%): (none, < 6 times/ys, and ≥ 6 times/ys respectively)	64.6	6.8	28.7	<0.001	

* *In a subgroup of 80 participants only (a total of 49, 5 and 26 women among groups, respectively)*

Table III. *Logistic regression analysis with LUTS as dependent variable.*

Dependent variable: LUTS	**B**	**p**	**Exp(B)**
Independent variables			
glucose	-0.015	0.395	0.985
creatinin	-21.125	0.999	0.000
calcium	0.239	0.622	1.270
Vitamin D status	-1.490	0.005	0.225
age	0.024	0.306	1.024

D (~ 25 ng/mL) and, as expected, the lowest calcium levels (Table II, post-hoc test) in comparison to the other groups. Fig. 1 shows the distribution of vitamin D values in the whole population in relation to the frequency of LUTS. The logistic regression analysis confirmed the association between vitamin D status and LUTS (Table III). All the other variables (glucose, calcium, age, and creatinin) were not

Table IV. *LUTS prevalence; Chi-square according to vitamin D quartiles.*

LUTS prevalence (*Chi*-square test)	**I quartile (n=58)**	**II quartile (n=58)**	**III quartile (n=58)**	**IV quartile (n=59)**	p
A %	46.6	55.4	79.3	74.6	0.004
LF %	12.1	7.1	3.4	3.4	
HF %	41.4	37.5	17.2	22	
Mean Vitamin D among quartiles (ANOVA)					
Vitamin D (ng/ml)	10.6±4	22.3±2	35.2±5	62.3±11	p<0.001

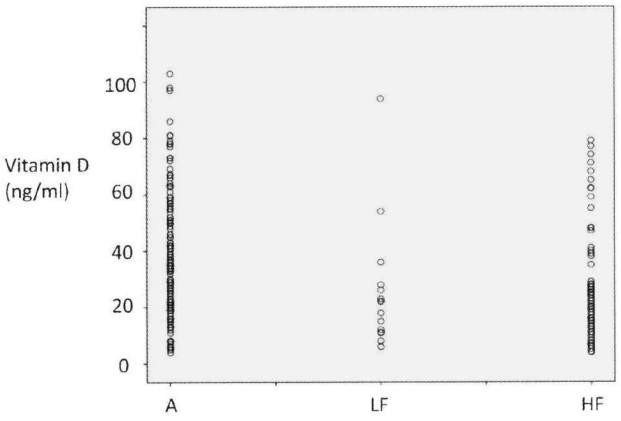

Fig. 1. *Vitamin D values according to LUTS frequency.*

associated with LUTS.

Fig. 2 shows the prevalence of LUTS (considered on the basis of their frequency) among vitamin D quartiles (I quartile with the lowest levels; IV quartile with the highest levels) , showing a higher prevalence of LUTS in the I quartile than in the IV quartile. In Table IV the *Chi*-square test between vitamin D quartiles is shown in detail, as well as the mean vitamin D value among quartiles by ANOVA test.

DISCUSSION

The main finding of our study was that women having low serum levels of vitamin D had the higher prevalence of LUTS in comparison to those with normal levels (Fig. 2). This is an original finding, never investigated to date. In fact, some studies have evaluated the role of vitamin D in the development of and for the prevention of acute respiratory infections (6, 7) while studies on the relationship between serum levels of vitamin D and LUTS or UTIs are lacking.

This issue is relevant since acute uncomplicated UTIs are the most common bacterial infections among women, with an annual incidence of 7% for all ages (21). Typical symptoms associated with UTIs include the triad of dysuria, urgency and frequency (13). Consequently, the results of this study may have many important clinical implications due to the possibility to reduce the prescription of unnecessary antibiotics by restoring the recommended levels of vitamin D, particularly in women with recurrent episodes. Indeed, the antimicrobial actions of vitamin D are already known. It has been shown that cathelicidins, defensins and other antimicrobial peptides are key components of the innate immune system, playing an important role in combating infections (22-24). Sufficient levels of circulating 25(OH)D3, the precursor of the active form 1,25(OH)2D3, are required to induce the human cathelicidin antimicrobial peptide (CAMP) gene expression as well as the cathelicidin expression to combat infection (25, 26). Furthermore, it has been demonstrated that Human b-defensin 2 (HBD-2) expression, induced by inflammatory stimuli, was also induced by 1,25(OH)2D3 (27). In addition, treatment of normal human keratinocytes with

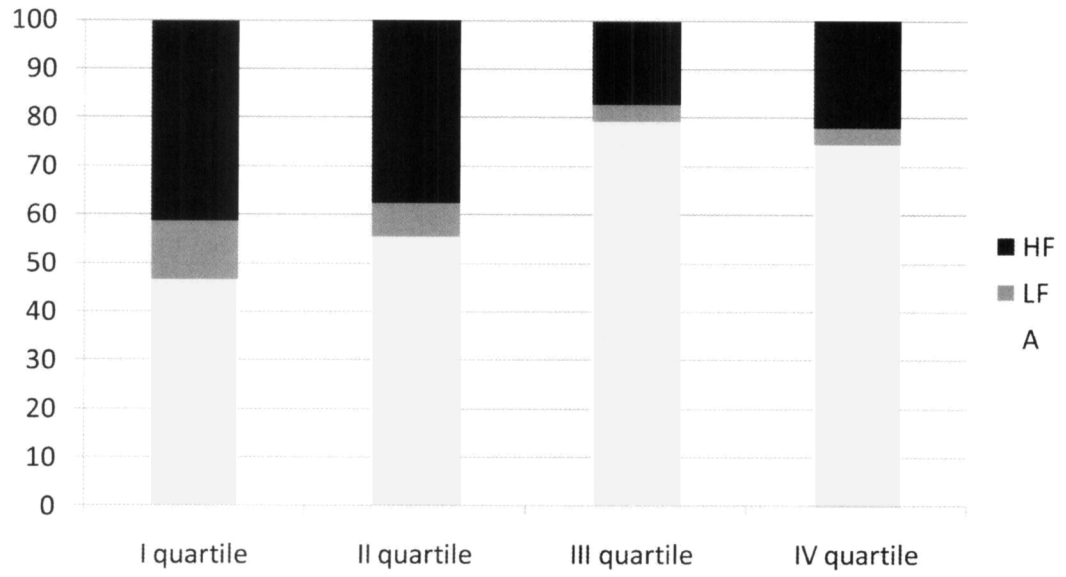

Fig. 2. *LUTS Prevalence among vitamin D quartiles.*

1,25(OH)2D3 increased HBD-3 mRNA levels in a dose-dependent manner (28). Moreover, it is well accepted that LUTS may include symptoms of the overactive bladder (17). It has been suggested that vitamin D receptor agonist has a role in regulating Ca^{2+} effects in bladder smooth muscle contractions (29). Moreover, muscle wasting is a symptom of osteomalacia, the condition resulting from vitamin D deficiency in adults, and muscle atrophy has been described in Type II fibres (30). Consequently, the potential role of vitamin D status in the function of the detrusor muscle also needs to be considered.

Of course, in our study the causal role of the low levels of vitamin D in the LUTS may be only hypothesized, and the evidence remains inconclusive. Future studies are needed to refine the diagnosis of UTIs or overactive bladder utilizing information on severity and duration of symptoms, alone, or in combination with vitamin D levels.

There are certain limitations to this study that need to be addressed. The first is related to the collection of the symptoms by a non-validated questionnaire. However, we provided information regarding the symptoms reflecting the individual's perspective, (patient-generated), on the genuine experience of the patients over a period of time. A second limitation of this study is that we did not confirm our clinical suspects with dipstick tests or urodynamic investigations, which could give strength to this study. Moreover, in these women we did not carry out any accurate nutritional intake investigation to evaluate vitamin D intake, but it is well accepted that the amount of vitamin D taken with food is very negligible (28). In contrast, sun exposure is the key to maintaining normal levels of vitamin D, since it is phytochemically produced in the skin by exposure to UV light, and our population comes from the same geographical area, exposed to the sun throughout the year (31).

Several investigations have found that 25(OH) D levels vary moderately within individuals over time, with variation coefficients ranging from 14 to 18%. Additional study is needed to better understand the intraindividual variation of vitamin D over periods of years. However, there is moderate intraindividual variation in 25(OH)D concentrations over approximately five years in women (32), thus, these results based on a one-time measurement of vitamin D levels could be considered of interest.

In conclusion, hypovitaminosis D is associated with LUTS in a population of women, and it may play a key role in the development of UTIs or other urinary tract diseases, as confirmed by several studies (6, 7, 25-30). Further studies are necessary to test

whether the recurrence of LUTS may be prevented by the administration of vitamin D, at least in women with low serum levels. This fact could reduce the use of antibiotic therapy, thereby obtaining a reduction of the costs, as well as a reduction of the discomfort in patients with overactive bladder.

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