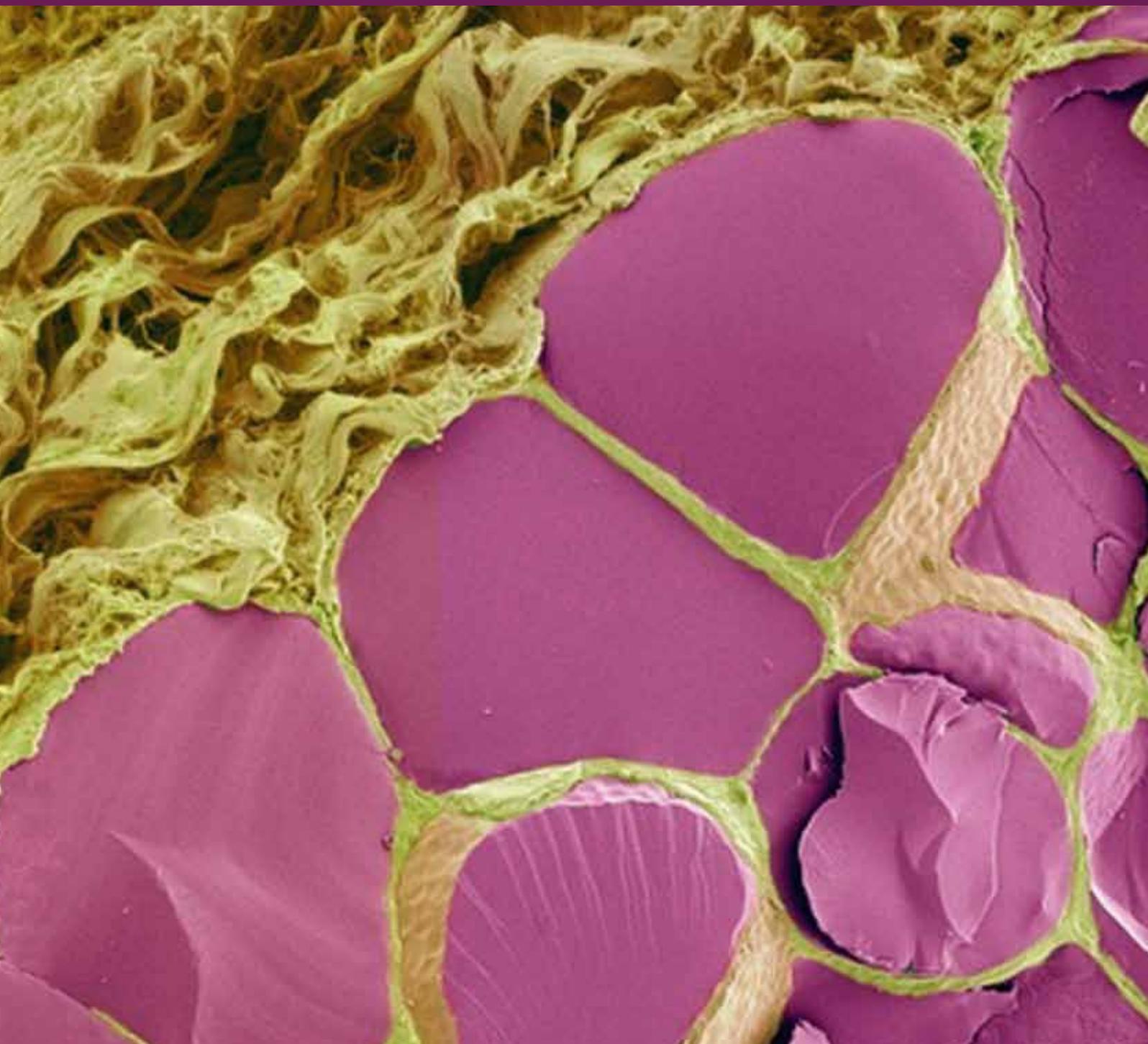


Vitamin D and Kidney

Guest Editors: Hulya Taskapan, Ibrahim Sahin, Paul Tam,
and Tabo Sikaneta





Vitamin D and Kidney

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Editorial

Vitamin D and Kidney

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After absorption from the gut or entry into the circulation from the skin, vitamin D is hydroxylated to 25(OH)D by 25-hydroxylase enzyme in the liver and then to $1\alpha,25(\text{OH})_2\text{D}_3$ (or calcitriol) by 1α -hydroxylase enzyme in the kidney and in many cells.

The hormonal or active form of vitamin D, that is, $1,25(\text{OH})_2\text{D}_3$ acts through a nuclear receptor (VDR) to carry out its many functions. The receptor has been recognized in a wide variety of tissues such as in osteoblasts, distal renal tubular cells, parathyroid gland cells, skin keratinocytes, lymphocytes, enterocytes, prostate, colon, pituitary gland, and ovaries. Many tissues in the body, *express 1-hydroxylase* and synthesize $1,25(\text{OH})_2\text{D}_3$ locally.

Vitamin D has at least 2 distinct groups of functions. One is classically endocrine functions regulating PTH secretion and hence its own production. The other is autocrine functions (or paracrine): the cells or tissues concerned make and degrade active vitamin D locally to regulate their own proliferation and differentiation. Vitamin D supplementation can affect many aspects of health because *function*.

Many epidemiologic studies have shown that chronic vitamin D deficiency is associated with an increased risk of diseases such as dementia, hypertension, multiple sclerosis, rheumatoid arthritis, cancer of the colon, prostate, breast, and ovary, and types 1 and 2 diabetes.

In the general population and especially in patients with chronic kidney disease (CKD) has been reported. The reasons for hypovitaminosis D in CKD are not known but likely include reduced oral intake of vitamin D because of dietary restrictions and/or anorexia.

In this special issue about vitamin D and kidney, Nordenström evaluated vitamin D status in patients operated for primary hyperparathyroidism (PHPT) and compared the

patients from southern and northern Europe. These authors found that postmenopausal women with PHPT from Spain had lower preoperative levels of 25(OH)D and more severe PHPT Swedish *patients*, and patients with PHPT and vitamin D deficiency gained more bone density at some sites one year after parathyroidectomy.

The effects of vitamin D on *gentamicin-induced* acute kidney injury in an experimental rat model were investigated by Hur et al. The authors reported that vitamin D does not seem to affect histological findings although some beneficial effects via Renin Angiotensin System and a promising effect on antioxidant system.

Cardiometabolic risk factors related to vitamin D and adiponectin in obese children and adolescents were evaluated by Kardas et al. The authors showed that lower vitamin D and adiponectin levels were strongly associated with metabolic risk factors and obesity in children and adolescents.

Kidney disease was found to be a major risk factor for vitamin D deficiency population study of both *hospitalized and non-admitted patients*. Significant independent predictors of vitamin D deficiency in inpatients and outpatients of a nephrology unit were retrospectively studied by Bentli. These authors showed that vitamin D deficiency is an important problem in both inpatients and outpatients of nephrology. Monitoring serum 25(OH)D concentrations regularly and replacement of vitamin D are important.

Finally, Yildirim et al. report their findings on the association between inflammatory markers (C-reactive protein, erythrocyte sedimentation rate, and leukocyte count) and the presence of CKD in vitamin D-deficient patients sedimentation rate, and leukocyte count, in vitamin D deficient patients with and without chronic kidney disease.

Acknowledgment

The guest editors wish to thank all authors for their valuable contributions. Without their efforts, this special issue would not have been possible.

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Clinical Study

Vitamin D Status in Patients Operated for Primary Hyperparathyroidism: Comparison of Patients from Southern and Northern Europe

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Aim. The interaction between vitamin D deficiency and primary hyperparathyroidism (PHPT) is not fully understood. The aim of this study was to investigate whether patients with PHPT from Spain and Sweden differed in vitamin D status and PHPT disease activity before and after surgery. **Methods.** We compared two cohorts of postmenopausal women from Spain ($n = 126$) and Sweden ($n = 128$) that had first-time surgery for sporadic, uniglandular PHPT. Biochemical variables reflecting bone metabolism and disease activity, including levels of 25-hydroxy vitamin D₃ (25(OH)D) and bone mineral density, BMD, were measured pre- and one year postoperatively. **Results.** Median preoperative 25(OH)D levels were lower, and adenoma weight, PTH, and urinary calcium levels were higher in the Spanish cohort. The Spanish patients had higher preoperative levels of PTH (13.5 versus 11.0 pmol/L, $P < 0.001$), urinary calcium (7.3 versus 4.1 mmol/L, $P < 0.001$), and heavier adenomas (620 versus 500 g, $P < 0.001$). The mean increase in BMD was higher in patients from Spain and in patients with vitamin D deficiency one year after surgery. **Conclusion.** Postmenopausal women with PHPT from Spain had a more advanced disease and lower vitamin 25(OH)D levels. Improvement in bone density one year after surgery was higher in patients with preoperative vitamin D deficiency.

1. Introduction

Primary hyperparathyroidism (PHPT) is a common endocrine disorder affecting about 2-3% of all postmenopausal women [1] in Europe. PHPT and vitamin D deficiency coexist in elderly patients [2, 3]. The association between vitamin D and PHPT is complex and not fully understood [4–6]. It is not known if vitamin D deficiency could trigger PHPT or if vitamin D deficiency is caused or worsened by PHPT. Patients with PHPT and low levels of 25-hydroxyvitamin D (25(OH)D) have a more severe bone disease [7–9]. Previous data has also suggested that suboptimal vitamin D nutrition is linked to parathyroid adenoma growth and that improved vitamin D nutrition might decrease PHPT disease activity [10].

Vitamin D status in general is influenced by sun exposure and nutrition, and data from previous studies have shown that vitamin D deficiency is common among elderly people both in northern and southern Europe [11, 12].

One study has actually shown that vitamin D deficiency is more common in southern than northern Europe indicating that not only the number of sun hours per year of a country is important in the context of vitamin D deficiency [13]. Comparisons between different cohorts of patients with PHPT from different part of Europe with assumed different sun exposure and/or different vitamin D nutrition are lacking. PHPT is associated with increased bone turnover and increased fracture risk [14, 15].

Thus, on average, BMD increases after successful surgery for pHPT, but not all patients experience improvement in BMD. One year after parathyroidectomy about half of the patients will have a significant remineralisation [16, 17]. Factors like the severity of primary hyperparathyroidism, age, and renal function has been found to influence bone recovery after surgery.

The aim of this study was to investigate if two cohorts of postmenopausal women with primary hyperparathyroidism with assumed low sun exposure (Sweden) and high sun exposure (Spain) differed preoperatively in vitamin D status, disease activity, and outcome after surgery measured as change in BMD and biochemical variables one year after surgery.

2. Methods

Data collected prospectively in two cohorts of patients with primary hyperparathyroidism from Spain and Sweden, respectively, were compared preoperatively, and at one year follow-up.

Informed consent for anonymous use of data was obtained from all patients.

All patients were postmenopausal and had successful first time surgery for sporadic PHPT.

The diagnosis of PHPT was histologically proven in all patients. Part of the Swedish cohort has been described in a previous paper [16], and data from the Spanish cohort was recently published [17].

2.1. Clinical and Biochemical Variables. Demographic data and history of fractures up to 10 years before surgery were recorded. Serum levels of calcium, phosphate, alkaline phosphatase, creatinine, intact PTH, 25(OH)D and 1,25(OH)₂D₃, urinary calcium output over 24 h, and GFR were determined before and 1 year after parathyroidectomy. In the Swedish population high performance liquid chromatography, HPLC, was used for assessment of the concentration of serum 25(OH)D (reference range >75 nmol/L), and 1,25(OH)₂D₃ (reference range 10–60 ng/L) was measured with a radio-receptor assay (Incstar, Stillwater, MN, USA). Concentration of plasma parathyroid hormone (PTH) was analysed by an immunoradiometric assay for intact PTH (Hitachi Modular-E), (reference range 1.6–6.9 pmol/L). The analysis has a total CV of 5% at 7 pmol/L and 5% at 100 pmol/L.

In the Spanish group intact PTH was analyzed by an immunoradiometric assay, (reference range 14–55 pg/mL), 25(OH)D with a chemiluminescence immunoassay, CLI, reference range 8–80 ng/mL and 1,25(OH)₂D₃ with a radioimmunoassay, (reference range 15–56 pg/mL). To convert from the conventional unit to the SI unit, we multiplied by the conversion factor 0.105 for PTH, 2.496 for 25(OH)D, and with 2.6 for 1,25(OH)₂D₃. In the Spanish cohort GFR was assessed according to the Modification of Diet in Renal Disease formula and in the Swedish cohort the iothexol clearance method was used [18]. Vitamin D deficiency was defined as 25(OH)D < 50 nmol/L [12, 18].

2.2. Bone Mineral Density. BMD was measured by dual-energy absorptiometry (DEXA) at three sites, the femoral neck, total hip, and lumbar spine. In the Spanish cohort the QDR 4500 SL (Hologic, Waltham, Massachusetts, USA) machine was used. This technique has an *in vivo* coefficient of variation, CV, of 1 per cent at the lumbar spine and 1.6 per cent at the femoral neck. In the Swedish cohort the Lunar Expert XL (Lunar Corp, Madison, Wis, USA) equipment was used, with an *in vivo* CV of 1 per cent. Results were expressed as grams per square centimetre and as age and gender specific standard deviations (*z* scores). BMD was measured preoperatively and one year after parathyroidectomy. A significant increase in BMD for an individual patient was calculated according to the formula $1.96 \times \sqrt{2} \times CV$. This formula generates the value that separates two independent samples with 95 per cent confidence. For an individual in the Spanish cohort, the BMD should increase by at least 3.7 per cent to be significant and for an individual in the Swedish cohort the corresponding figure was 2.8 per cent. Unfortunately *t* score was not assessed in all the Swedish patients and therefore not shown.

2.3. Surgery and Followup. Bilateral neck exploration or selective parathyroidectomy depending on preoperative localization studies was performed under general anaesthesia. All adenomas were confirmed by typical histological features. All patients were seen 2–6 weeks and 1 year after parathyroidectomy. At this time, BMD and biochemical variables were reassessed. In the Spanish cohort one gram of elemental calcium per day was prescribed during the follow-up period. In the Swedish cohort prescription of calcium and vitamin D was done on demand.

2.4. Statistics. Results are expressed as medians (interquartile range, IQR). Wilcoxon and Mann-Whitney *U* tests were used when comparing continuous variables between groups. For categorical data statistical significance was analysed by the chi squared test (χ^2). Statistical significance was set at $P < 0.050$. Data were analysed with STATA 11.0 (Stata corp., Texas, USA).

3. Results

3.1. Preoperative Data. 254 patients with a median age of 65 (57–73) years were included in the study. The median serum calcium level was 2.74 (2.65–2.85) mmol/L, and the median serum intact PTH level was 12.0 (9.3–16.2) pmol/L. The median adenoma weight in all patients was 546 (300–1200) mg. 19 patients in the Spanish and 29 in the Swedish cohort had a history of fragility fracture before parathyroidectomy. 63 per cent of the Spanish and 39 per cent of the Swedish patients had biochemical vitamin D insufficiency preoperatively ($P = 0.007$). There was no difference in age at surgery or preoperative calcium levels, but median preoperative levels of PTH, phosphate, urinary calcium, ALP, GFR, creatinine, 1,25(OH)₂D₃, and adenoma weight were all higher, and median preoperative 25(OH)D lower, in the Spanish cohort (see Table 1).

TABLE 1: Pre- and perioperative biochemical and clinical variables in patients operated on for primary hyperparathyroidism. Median, (IQR). Groups compared with Kruskal-wallis and chi square where appropriate.

Variable	All	Spanish cohort <i>n</i> = 126	Swedish cohort <i>n</i> = 128	<i>P</i>
Age (years)	65 (57–73)	64 (57–72)	66 (58–74)	0.33
p-Ca (mmol/L)	2.74 (2.65–2.85)	2.74 (2.65–2.85)	2.74 (2.66–2.84)	0.73
PTH (pmol/L)	12 (9.3–16.2)	13.5 (10.5–20.4)	11.0 (8.4–14.0)	<0.001
25-OH-D (nmol/L)	44 (26–64)	40 (25–58)	51 (28–66)	0.01
1,25-OH-D (pmol/L)	55 (98–162)	142 (99–198)	56 (28–66)	<0.001
Phosphate (mmol/L)	0.85 (0.77–0.96)	0.90 (0.80–1.0)	0.82 (0.72–0.90)	<0.001
ALP (unit)	2.6 (1.6–3.8)	3.0 (1.7–4.3)	2.3 (1.5–3.4)	0.002
GFR (mL/min)	75 (63–89)	70 (58–85)	76 (65–93)	0.01
Creatinine (umol/L)	71 (62–81)	80 (71–88)	65 (56–73)	<0.001
Urinary calcium (unit)	5.9 (3.6–9)	7.3 (3.9–10.0)	4.1 (2.8–5.9)	<0.001
Fracture (yes/no)	48/195	19/107	29/99	0.002
Vitamin D deficiency (yes/no)	130/96	80/42	50/54	0.007
Adenoma weight (mg)	546 (300–1200)	620 (290–1400)	500 (320–1030)	<0.001

3.2. *Followup One Year after Surgery.* At one year after surgery, all patients in both cohorts remained normocalcemic. However, median levels of 1,25(OH)₂D₃ were higher, and median levels of calcium and 25(OH)D were lower, in the Spanish cohort as compared to the Swedish (see Table 2).

BMD increased one year after surgery as compared to preoperatively at all sites in both cohorts, both in absolute terms (g/cm²) and as *z*-scores (see Table 3). Preoperatively, BMD was higher in the Swedish cohort for lumbar spine and femoral neck in absolute terms, but not as *z*-scores.

When the two cohorts were compared regarding change in BMD, the Spanish cohort improved more at the lumbar spine, in absolute terms, and at the femoral neck, as *z*-score. For the other measurements, there was no difference between the two cohorts (see Table 4).

3.3. *Vitamin D Status.* Bone remineralisation after operation in patients with or without vitamin D deficiency (25(OH)D < 50 nmol/L) are shown in Table 5. Patients with vitamin D deficiency had higher remineralization one year after surgery at total hip compared to patients with sufficient vitamin D levels, but not at the lumbar spine and/or femoral neck.

4. Discussion

In this study comparing postmenopausal Swedish and Spanish patients operated on for PHPT, the Spanish cohort had lower median levels of 25(OH)D whereas median PTH and urinary calcium levels were higher before surgery, compared to the Swedish group of patients. Median adenoma weight was also higher in the Spanish cohort, but preoperative calcium levels and age were similar in the two cohorts.

One year after surgery, we found no difference in median PTH levels between the two cohorts, but median levels of 25(OH)D were still lower in the Spanish than in the Swedish cohort, even though median levels of 25(OH)D were higher after surgery as compared to preoperatively in both cohorts.

A potential explanation for these findings could be a selection bias, that is, that patients in Spain are referred

and accepted for surgery in a more advanced stage of the disease. If this is the case, the lower levels of 25(OH)D in the Spanish cohort preoperatively might be due to increased conversion of 25(OH)D into 1,25(OH)₂D₃ by PTH-mediated renal 1 α -hydroxylation [19]. This phenomenon is supported by the fact that PTH and 25(OH)D are inversely correlated; thus, in a more severe PHPT disease higher PTH and lower 25(OH)D are expected [17]. Patients with PHPT might also have low levels of 25(OH)D because of poor nutrition and/or less sun exposure [20]. Whether the high serum calcium and PTH levels may interfere with the conversion of 7-dehydrocholesterol in the skin by UV-B to previtamin D, or with the hydroxylation of vitamin D in the liver to 25(OH)D remains to be determined.

We assumed that patients from Spain with PHPT had higher sun exposure than their Swedish counterparts [8]. However, sun exposure is not the only determinant of 25(OH)D levels. Hence, previous studies have shown a north-south gradient regarding serum 25(OH)D, with higher levels of 25(OH)D in Scandinavia and lower levels in Italy and Spain countries. This points to other determinants than sunshine, for example, nutrition, food fortification, and supplement use [13]. Unfortunately, information about nutrition was lacking in the present study. Thus, we cannot rule out that the Swedish patients were more inclined to take vitamin D supplementation and had a higher nutritional intake of vitamin D.

On the other hand the HELENA study has demonstrated that the winter half-year adolescents in Southern Europe have higher serum 25OHD levels compared to adolescents in Central Europe [21], so data about vitamin D status in different populations even without PHPT is conflicting.

Few studies have compared differences in preoperative status and outcome after PHPT surgery in two cohorts living in two countries that differ in distance from the equator, that is, different sun exposure and difference in preoperative D vitamin status [22, 23].

Silverberg compared two cohorts of patients with PHPT from New York City and Beijing, China. Patients from China

TABLE 2: Biochemical variables one year after surgery for primary hyperparathyroidism.

Variable	Spanish cohort <i>n</i> = 126	Swedish cohort <i>n</i> = 128	<i>P</i>
p-Ca (mmol/L)	2.32 (2.24–2.42)	2.37 (2.30–2.48)	0.002
PTH (pmol/L)	4.7 (3.8–6.9)	4.7 (3.7–6)	0.51
25-OH-D (nmol/L)	45 (24–65)	62 (49–84)	<0.001
1,25-OH-D (pmol/L)	84 (0–120)	31 (38–50)	<0.001
ALP	1.2 (0.9–2.5)	1.3 (1.0–2.1)	0.35

TABLE 3: Bone mineral density before and one year after surgery for primary hyperparathyroidism. Medians (IQR) Wilcoxon's signed rank test.

BMD site	Spanish cohort Before surgery	Spanish cohort One year postop	<i>P</i>	Swedish cohort Before surgery	Swedish cohort One year postop	<i>P</i>	<i>P</i> diff Spanish-swedish preop
Lumbar spine, absolute	0.79 (0.70–0.88)	0.83 (0.74–0.93)	<0.001	1.00 (0.83–1.11)	1.03 (0.88–1.14)	<0.001	<0.001
Lumbar spine, z-score	-0.7 (-1.6–0.3)	0.7 (0.4–1.3)	<0.001	-0.4 (-1.6–0.6)	0.7 (0.4–1.38)	<0.001	0.75
Femoral neck, absolute	0.62 (0.56–0.69)	0.64 (0.57–0.70)	<0.001	0.76 (0.69–0.85)	0.79 (0.70–0.88)	<0.001	<0.001
Femoral neck, z-score	-0.7 (-1.3 to -0.1)	-0.4 (-1.1–0.2)	<0.001	-0.5 (-1.1–0.1)	-0.5 (-0.9–0.2)	<0.001	0.11
Total hip, absolute	0.76 (0.68–0.85)	0.76 (0.70–0.87)	<0.001	0.77 (0.56–0.88)	0.84 (0.75–0.95)	<0.001	0.40
Total hip, z-score	-0.5 (-0.9–0.3)	-0.2 (-0.8–0.5)	<0.001	-0.4 (1.0–0.4)	0 (-0.7–0.7)	<0.001	0.91

TABLE 4: Change in bone mineral density (Δ) after surgery for primary hyperparathyroidism. Change in absolute values in percent. Change in z-score in arithmetic difference. Medians (IQR). Kruskal-Wallis test.

BMD site	Spanish cohort	Swedish cohort	<i>P</i>
Δ Lumbar spine, absolute	4.2 (1.0–6.9)	1.3 (-0.9–6.8)	0.009
Δ Lumbar spine, z-score	0.4 (0.2–0.6)	0.2 (0.0–0.6)	0.18
Δ Femoral neck, absolute	2.8 (-0.9–7.3)	2.1 (-1.1–5.8)	0.46
Δ Femoral neck, z-score	0.3 (0.0–0.5)	0.1 (-0.1–0.4)	0.004
Δ Total hip, absolute	2.8 (-0.3–5.7)	1.9 (0.6–4.2)	0.09
Δ Total hip z-score	0.3 (0.1–0.5)	0.2 (0.1–0.4)	0.05

had a more advanced PHPT disease and a more profound Vitamin D deficiency [23]. This could reflect the same findings we found between Swedish and Spanish patients, that is, patients from Spain and China come to surgery in a more advanced state of the PHPT disease.

However, in our study these mechanisms cannot account for the observed difference in 25(OH)D levels between the two cohorts after surgery, since at this point, both cohorts had similar levels of PTH and were normocalcemic, that is, cured of PHPT. Another explanation might instead be that low levels of 25(OH)D causes or at least worsens PHPT. One study suggests that low levels of 25(OH)D stimulate parathyroid adenoma growth [10].

It seems that it is very hard to define the mechanisms of interaction between vitamin D and PHPT exactly. We believe that the PHPT disease itself could worsen vitamin D deficiency but also the reverse situation, that is, vitamin D deficiency could worsen the PHPT disease.

Patients from Spain had preoperatively lower bone mineral density in lumbar spine and femoral neck probably because Spanish patients had a more advanced PHPT disease. The Spanish patients also gained more from the operation in terms of bone density suggesting that the remineralisation response is more pronounced in patients with lower BMD before parathyroidectomy [18].

Patients with vitamin D deficiency had higher increase in bone density in total hip. Previous studies have shown that patients with PHPT and vitamin D deficiency gain more in bone density after PHPT surgery [23]. This highlights the possible benefit of vitamin D supplementation in PHPT disease regarding gain in bone density. So far no convincing study has shown if postoperative vitamin D supplementation enhances increase in bone density after parathyroidectomy [9], and further studies are warranted.

The cohorts in the present study were homogeneous, and data was recorded in a prospective and consecutive manner. A limitation is that 25(OH)D was measured with different methods in Sweden and Spain, respectively. Previous results suggest that 25(OH)D levels measured with CLI, that is, the Spanish method in the present study yields lower levels than HPLC, that is, the Swedish method [24]. Thus, the difference in 25(OH)D between the cohorts might be spurious and caused by the use of different methods. However, the other biochemical findings and bone density measurements support the conclusion that the Spanish patients suffered from a more severe PHPT disease. Another potential limitation is that we did not record season of blood sampling before PHPT surgery. However, there was no reason to suspect that distribution of season of operation differed between cohorts. Unfortunately, we did not have information about nutrition in the two cohorts.

TABLE 5: Bone remineralisation after operation for primary hyperparathyroidism in patients with or without vitamin D deficiency (25-OH-D < 50 nmol/L). Change in absolute values in percent. Change in z-score in arithmetic difference. Medians (IQR). Kruskal-Wallis test.

BMD site	Vitamin D-deficiency (25-OH-D < 50 nmol/L)	Vitamin D-sufficiency (25-OH-D ≥ 50 nmol/L)	P
ΔLumbar spine, absolute	3.5 (−0.5–7.0)	2.2 (−2.0–6.6)	0.12
ΔLumbar spine, z-score	0.4 (0.1–0.6)	0.3 (0.0–0.6)	0.40
ΔFemoral neck, absolute	2.8 (−0.8–7.3)	2.5 (−1.9–6.0)	0.47
ΔFemoral neck, z-score	0.3 (0–0.5)	0.2 (−0.1–0.3)	0.05
ΔTotal hip, absolute	2.7 (0–5.7)	1.00 (−1.4–4.8)	0.03
ΔTotal hip, z-score	0.3 (0.1–0.5)	0.2 (0.0–0.4)	0.01

In conclusion we found that postmenopausal women with PHPT from Spain had lower preoperative levels of 25(OH)D and more severe PHPT compared to Swedish patients, and patients with PHPT and vitamin D deficiency gained more in bone density at some sites one year after parathyroidectomy.

Disclosure

This work was presented orally at the 5th biennial ESES Congress in Gothenburg May 24–26, 2012.

Conflict of Interests

There is no conflict of interests that could be perceived as prejudicing the impartiality of the research reported.

Authors' Contribution

Erik Nordenström and Antonio Sitges-Serra designed research. Erik Nordenström, Antonio Sithes-Serra, Joan Sancho, Mark Their, and Martin Almqvist conducted research. Erik Nordenström, Antonio Sitges-Serra, and Martin Almqvist analyzed data. Erik Nordenström, Antonio Sitges-Serra, Joan Sancho, Mark Their and Martin Almqvist wrote paper.

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Research Article

Cardiometabolic Risk Factors Related to Vitamin D and Adiponectin in Obese Children and Adolescents

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Obesity-related diseases are becoming the most important causes of mortality worldwide. Several studies have suggested an association between low levels of vitamin D and obesity. In addition, plasma adiponectin levels have been found to be lower in obese subjects. We evaluated the association of metabolic risk factors with both adiponectin and vitamin D levels and that between adiponectin and vitamin D levels. The study consisted of 114 obese and healthy subjects. 25-Hydroxy vitamin D [25(OH)D] levels were positively correlated with adiponectin and HDL-cholesterol (HDL-C) and inversely correlated with body mass index (BMI), LDL-cholesterol (LDL-C), total cholesterol (T-C), triglyceride (TG), fasting glucose, homeostasis model assessment of insulin resistance (HOMA index), systolic blood pressure (SBP), and diastolic blood pressure (DBP). The mean 25(OH)D levels in the obese and nonobese groups were 22.5 ± 5.7 and 32.3 ± 5.8 ng/mL, respectively ($P < 0.0001$). The mean adiponectin level in the obese group was lower than that in the nonobese group ($P < 0.0001$). Lower vitamin D and adiponectin levels were strongly associated with metabolic risk factors and obesity in Turkish children and adolescents.

1. Introduction

Obesity is a growing health concern worldwide and is a major cause of morbidity and mortality. Recent studies have suggested that vitamin D deficiency is associated with cardiometabolic risk factors, including obesity, autoimmune diseases, cancer, and insulin resistance [1, 2]. Furthermore, low 25(OH)D levels have been shown to be associated with higher rates of myocardial infarction and diabetes [3–5], and the incidence of hypertension has been found to increase in association with low vitamin D levels [6].

On the basis of current evidence of an inverse association between vitamin D levels and obesity, we performed the first investigation to elucidate the association of obesity with 25(OH)D and adiponectin levels in the Turkish children.

The adiponectin protein is exclusively secreted by adipose tissue into the bloodstream [7] and is abundant in plasma compared to other hormones. Furthermore, low adiponectin levels have been confirmed in patients with diabetes [8], and body fat percentage has been found to be negatively

associated with adiponectin levels in adults [7]. Serum adiponectin levels showed an inverse association with hypertension and the homeostasis model assessment of insulin resistance (HOMA index) [9].

However, there are few studies regarding the association of vitamin D and adiponectin levels with cardiometabolic risk factors in obese and healthy children, and to the best of our knowledge, this is the first study to assess these parameters in obese children and adolescents in Turkey.

2. Materials and Methods

2.1. Participants. We enrolled a total of 114 children and adolescents (age, 10–16 years) who were admitted to the Unit of Paediatric Metabolism of the Child Hospital of Erciyes University Medical Faculty (Kayseri Province, Turkey) from March to May 2011. The study population was divided into 2 groups (obese and nonobese) by body mass index (BMI) (kg/m^2), which was percentile-specific for gender and age of Turkish children and adolescents [10].

The number of adolescents in the obese (28/63; 44.5%) and nonobese (24/51; 47%) groups was similar.

Healthy, age- and gender-matched subjects were selected from local schools. Obesity was defined as a BMI >90 (kg/m²) according to the reference BMI curves for Turkish children [10].

Anthropometric measurements including weight in kilograms (kg), height in centimetres (cm), and BMI for each participant were performed by the same trained nurse using standard devices. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice using a mercury sphygmomanometer, after the subject rested for at least 20 min.

2.2. Biological Parameters. After overnight fasting, venous blood samples were collected. All samples were obtained during the spring (March to May 2011). Within 3 h of venipuncture, whole blood samples were centrifuged and separated, and serum portions were frozen at -80°C for future adiponectin analysis. Biochemical parameters, such as LDL-cholesterol (LDL-C) (normal range, 100–130 mg/dL), total cholesterol (T-C) (normal range, 160–200 mg/dL), HDL-cholesterol (HDL-C) (normal range, 35–80 mg/dL), triglyceride (TG) (normal range, 40–140 mg/dL), and fasting glucose (normal range, 65–105 mg/dL) levels, were analyzed immediately using standard assay kits (Abbott GmbH & Co. KG, Wiesbaden, Germany). Adiponectin levels were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (BioVendor GmbH, Heidelberg, Germany). Plasma 25(OH)D levels were measured by high-pressure liquid chromatography (HPLC) using Clin-Rep kits (IRIS Technologies International GmbH, Cursdorf, Germany). Insulin levels were measured using an immunoradiometric assay kit.

The HOMA index to determine insulin resistance was calculated using the formula $[\text{fasting insulin } (\mu\text{U/mL}) \times \text{fasting glucose (mmol/liter)}] / 22.5$ [11]. Vitamin D deficiency was defined as vitamin D levels of <20 ng/mL and vitamin D insufficiency as vitamin D levels of 21–29 ng/mL [12].

2.3. Statistical Analysis. Data analysis was performed using SPSS version 17.0 (SPSS, Inc., Chicago, IL, USA). The results were expressed as mean \pm SD. The Kolmogorov-Smirnov test was used to determine the normality of the data. Differences between groups were analysed using the Student's *t*-test. Discrete variables were compared using the Pearson χ^2 test. The Pearson correlation test was used to determine the correlations among the variables.

3. Results

The obese group consisted of 63 subjects (32 males, 31 females), and nonobese group consisted of 51 subjects (26 males, 25 females). The mean ages of the study population, obese group, and nonobese group were 13.5 ± 1.6 , 13.5 ± 1.7 , and 13.4 ± 1.6 years, respectively.

SBP, DBP, T-C, LDL-C, TG, and fasting glucose were higher in the obese group than those in nonobese group

TABLE 1: Adiponectin and 25(OH)D levels according to gender.

Parameters	Male (<i>n</i> = 58)	Female (<i>n</i> = 56)	<i>P</i> value
25(OH)D (ng/mL)	27.2 \pm 7.5	26.6 \pm 7.4	0.662
Adiponectin ($\mu\text{g/mL}$)	4.58 \pm 1.91	4.45 \pm 1.64	0.688

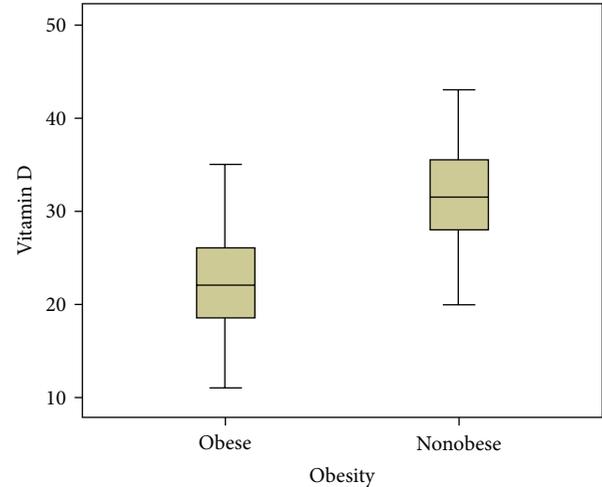


FIGURE 1: Serum 25(OH)D levels in obese and nonobese subjects.

($P < 0.01$ for all variables), whereas 25(OH)D, adiponectin, and HDL-C levels were lower in the obese group ($P < 0.01$ for all variables).

The mean 25(OH)D levels in the overall population, males, and females were 26.9 ± 7.4 , 27.2 ± 7.5 , and 26.6 ± 7.4 ng/mL, respectively. There was no significant difference in 25(OH)D levels according to gender in the overall population ($P > 0.05$) (Table 1).

All samples were collected during the spring season; therefore, the 25(OH)D reference values were the same for all participants. The descriptive characteristics of the study population (clinical and biological parameters) are shown in Table 2.

Association of 25(OH)D levels with obesity is as follows: mean 25(OH)D levels in the total study, obese group, and nonobese group were 26.9 ± 7.4 , 22.5 ± 5.7 , and 32.3 ± 5.8 ng/mL, respectively. There was a significant difference in 25(OH)D levels between the groups ($P < 0.0001$). Serum 25(OH)D levels are shown in Figure 1.

25(OH)D levels in relation to biochemical and clinical parameters were as follows: 25(OH)D levels showed a positive correlation with adiponectin and HDL-C levels and an inverse correlation with BMI, TG, T-C, LDL-C, fasting glucose levels, HOMA index, SBP, and DBP (Table 3).

The mean adiponectin level in the obese group (3.3 ± 0.89 ng/mL) was lower than that in the nonobese group (6.0 ± 1.4 ng/mL) ($P < 0.0001$) (Table 1). Adiponectin levels according to the groups are shown in Figure 2. BMI, TG, T-C, LDL-C, and fasting glucose levels, HOMA index, SBP, and DBP showed an inverse correlation with adiponectin levels. However, 25(OH)D and HDL-C levels showed a positive correlation with adiponectin levels (Table 4). There was no

TABLE 2: Baseline clinical and biological parameters of study subjects.

Parameters	Total (<i>n</i> = 114)	Obese group (<i>n</i> = 63)	Nonobese group (<i>n</i> = 51)	<i>P</i> value ^a
Age (years)	13.5 ± 1.6	13.5 ± 1.6	13.4 ± 1.7	0.835
BMI (kg/m ²)	24.5 ± 5.4	28.5 ± 2.7	19.6 ± 3.6	<0.0001
SBP (mmHg)	119.7 ± 9.1	122.6 ± 9.6	116.2 ± 7.0	0.001
DBP (mmHg)	66.9 ± 8.9	71.0 ± 8.9	61.9 ± 5.7	<0.0001
HOMA index	1.8 ± 0.55	1.9 ± 0.64	1.6 ± 0.31	0.001
TG (mg/dL)	113.3 ± 25.6	128.7 ± 22.9	94.3 ± 13.1	<0.0001
LDL-C (mg/dL)	94.6 ± 27.5	111.9 ± 24.1	73.3 ± 12.5	<0.0001
T-C (mg/dL)	160.1 ± 27.8	177.6 ± 24.3	138.6 ± 12.4	<0.0001
HDL-C (mg/dL)	44.6 ± 5.9	40.7 ± 3.4	49.4 ± 4.8	<0.0001
25(OH)D (ng/mL)	26.9 ± 7.4	22.5 ± 5.7	32.3 ± 5.8	<0.0001
Adiponectin (μg/mL)	4.5 ± 1.7	3.3 ± 0.89	6.0 ± 1.4	<0.0001
Fasting glucose (mg/dL)	85.2 ± 12.8	89.0 ± 15.0	80.5 ± 7.2	0.003

^a*P* values between obese and nonobese groups.

TABLE 3: Correlations of 25(OH)D with other parameters of the total study, obese group, and nonobese group.

Parameters	Obese (<i>n</i> = 63)		Nonobese (<i>n</i> = 51)		Total population	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Adiponectin (μg/mL)	-0.147	0.251	-0.23	0.105	0.360	0.000
BMI (kg/m ²)	-0.199	0.118	-0.073	0.612	-0.553	0.000
TG (mg/dL)	0.199	0.117	0.094	0.513	-0.306	0.001
T-C (mg/dL)	0.167	0.190	-0.35	0.808	-0.360	0.000
HDL-C (mg/dL)	-0.111	0.385	-0.20	0.890	0.404	0.000
LDL-C (mg/dL)	0.179	0.161	0.048	0.736	-0.343	0.000
Fasting glucose (mg/dL)	0.112	0.381	-0.144	0.313	-0.369	0.031
HOMA index	0.075	0.558	-0.147	0.304	-0.480	0.035
SBP (mmHg)	0.33	0.796	0.013	0.926	-0.190	0.043
DBP (mmHg)	-0.66	0.608	-0.252	0.75	-0.392	0.000

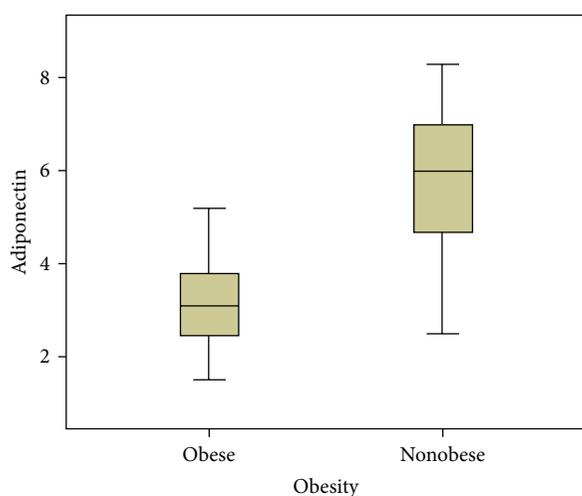


FIGURE 2: Serum adiponectin levels in obese and nonobese subjects.

difference in adiponectin levels according to gender in the total study population (Table 1).

4. Discussion

Here we investigated the association of obesity with several metabolic risk factors and both vitamin D and adiponectin levels in children and adolescents. Recent studies suggested that vitamin D levels were lower and vitamin deficiency was more common in obese patients [13, 14]. Furthermore, decreased vitamin D levels in obese patients have been reported due to minimal sun exposure from a sedentary lifestyle, sequestration in fat tissue, and low dietary vitamin D intake because of poor dietary habits [13, 15]. Although we did not discriminate between vitamin D deficient subjects, vitamin D levels were significantly lower in the obese group ($P < 0.0001$). While most findings regarding obesity and vitamin D level come from adult studies, Lenders et al. [16] reported that lower vitamin D level was associated with higher body fat index among obese adolescents.

In this study, we observed an inverse correlation between vitamin D and T-C, LDL-C, and TG levels and a positive correlation between vitamin D and HDL-C levels, which was consistent with previous studies [17–20]. In a study by Gannagé-Yared et al. [19], vitamin D was positively correlated

TABLE 4: Correlations between adiponectin levels and other parameters in the total study, obese group, and nonobese group.

Parameters	Obese ($n = 63$)		Nonobese ($n = 51$)		Total population	
	r	P	r	P	r	P
25(OH)D (ng/mL)	-0.147	0.251	-0.23	0.105	0.360	0.000
BMI (kg/m ²)	0.288	0.022	0.095	0.506	-0.556	0.000
TG (mg/dL)	0.143	0.265	0.239	0.091	-0.429	0.000
T-C (mg/dL)	0.110	0.391	0.249	0.078	-0.463	0.000
HDL-C (mg/dL)	-0.145	0.256	-0.013	0.927	0.525	0.000
LDL-C (mg/dL)	0.095	0.459	0.137	0.338	-0.485	0.000
Fasting glucose (mg/dL)	0.110	0.389	-0.041	0.774	-0.224	0.016
HOMA index	0.023	0.861	-0.154	0.282	-0.264	0.004
SBP (mmHg)	0.059	0.645	0.147	0.304	-0.206	0.028
DBP (mmHg)	0.010	0.941	-0.049	0.734	-0.394	0.000

with HDL-C levels in 381 young adults, and a second recent study demonstrated that vitamin D deficiency increased peripheral insulin resistance and thereby altered the lipid profile [21].

Many studies have reported that vitamin D levels were negatively associated with type 1 diabetes and insulin resistance in children and adults [13, 19, 22, 23]. Results of our study demonstrated that lower vitamin D levels were significantly correlated with higher fasting glucose levels and HOMA index ($r = -0.369$, $P = 0.031$ and $r = -0.480$, $P = 0.035$). Furthermore, our study suggested that obesity was a risk factor for lower vitamin D levels, which probably worsened insulin resistance.

Reportedly, the incidence of hypertension increases during the winter months [1, 24], and increased hypertension rates in non-Hispanic blacks with lower vitamin D levels, compared to whites, suggest an association between hypertension and vitamin D levels [25]. Here we found an inverse correlation between vitamin D levels and both SBP and DBP; there was some evidence of a role of vitamin D in hypertension regulation. One is the inhibition of renin gene expression by 1,25(OH)₂D, which is an active metabolite of 25(OH)D, and also vitamin D inhibits the renin-angiotensin-aldosterone system [26]. Another proposed mechanism involves the direct vascular effects of vitamin D as mediated by the α -hydroxylase enzyme, in the conversion of 25(OH)D to 1,25(OH)₂D, which is expressed in vascular smooth muscle cells [27]. Furthermore, Sun and Zemel [28] suggested that the adiponectin gene expression may be upregulated by vitamin D and reported that adipokine synthesis in visceral adipose tissue was regulated by 1,25-hydroxyvitamin D₃. Tumour necrosis factor alpha affects adiponectin expression, and 1,25-hydroxyvitamin D₃ regulates tumour necrosis factor alpha gene [29, 30]. Therefore, we propose that the interaction between vitamin D and adiponectin levels may be an indicator of cardiometabolic risk factors of diseases such as atherosclerosis, because we know that adiponectin protects against atherosclerosis [31]. Nonetheless, further experimental and clinical observations are required to elucidate this interaction. Importantly, we found a positive correlation

between 25(OH)D and adiponectin levels in the total cohort of Turkish subjects ($P < 0.001$, $r = 0.360$).

Our results showed that adiponectin levels were lower in obese group ($P < 0.001$) and were inversely correlated ($r = -0.556$, $P < 0.001$) with BMI in accordance with previous reports on adults. However, the association in children and adolescents remains unclear. Adiponectin levels in diabetic patients were found to be lower than those in healthy subjects [7, 8]. In a study reported by Lindsay et al. [32], plasma adiponectin levels were lower in Pima Indians, a group with a high prevalence of obesity and diabetes, whereas a second study demonstrated that adiponectin was strongly associated with insulin sensitivity [33]. Our results showed that plasma adiponectin levels were strongly correlated with fasting glucose levels and HOMA indexes ($r = -0.224$, $P = 0.016$ and $r = -0.264$, $P = 0.004$, resp.), suggesting that hypoadiponectinemia plays a crucial role in insulin resistance and in the development of diabetes. Furthermore, a recent study reported that low serum adiponectin levels were negatively associated with hypertension [9], which confirms our finding of an association between obese and nonobese groups when compared for SBP and DBP ($P = 0.001$, $P < 0.0001$, resp.). Lastly, our study demonstrated a negative correlation between plasma adiponectin levels and both SBP and DBP ($r = -0.206$, $P = 0.028$ and $r = -0.394$, $P = 0.000$, resp.).

5. Conclusion

This is the first study to evaluate the association of multiple metabolic risk factors with both adiponectin and vitamin D levels in Turkish subjects. In this study, we found strong associations between obesity-related parameters, and vitamin D and adiponectin levels in children and adolescents. However, further studies are needed to confirm our findings in larger populations.

Conflict of Interests

The authors declare that they have no conflict of interests.

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Research Article

Inflammatory Markers: C-Reactive Protein, Erythrocyte Sedimentation Rate, and Leukocyte Count in Vitamin D Deficient Patients with and without Chronic Kidney Disease

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Although some studies revealed a positive relationship between vitamin D₃ deficiency and inflammatory markers, there have been also many studies that failed to find this relationship. The aim of this large scaled study is to determine the association between the level of plasma 25 hydroxy vitamin D₃ [25-(OH) D₃] and inflammatory markers in the general population without chronic kidney disease (CKD) and in patients with CKD. Participants with simultaneously measured inflammatory markers and 25-(OH) D₃ levels were retrospectively analyzed ($n = 1897$). The incidence of all-cause inflammation infection, hospitalization, chronic renal failure, and vitamin B12 deficiency was evaluated. The medians of serum creatinine levels in subjects without renal failure were lower in 25-(OH) D₃ deficient group. Patients with CKD were more likely to have vitamin D₃ deficiency compared with normal GFR. 25-(OH) D₃ levels were associated with a greater incidence of all-cause hospitalization, hypoalbuminemia, and vitamin B12 deficiency. However, there was no relationship between inflammatory markers and vitamin D₃ levels. In 25-(OH) D₃ deficient patients, inflammatory markers can be related to other inflammatory and infectious status such as malnutrition and cachexia. We believed that there must be a relationship between vitamin deficiency and inflammatory markers due to other causes than low 25-(OH) D₃ status.

1. Introduction

The deficiency of vitamin D₃ is commonly associated with chronic kidney disease (CKD), and the prevalence of this hypovitaminosis increases as kidney function declines [1, 2]. Several factors, such as aging, loss of appetite, and other factors affecting cutaneous synthesis, such as low sun exposure and skin pigmentations [3], have consistently been associated with low 25-hydroxyvitamin D [25-(OH) D₃] levels in the general population. Therefore, it is common in the elderly, malnourished individuals, and some societies [4].

Even though there is growing evidence to suggest that vitamin D₃ status is associated with the development and progression of cardiovascular disease [5, 6], diabetes [7], and immune system disorders [8], there is limited information

about the association of 25-(OH) D₃ deficiency and inflammation in the general population without CKD and in patients with CKD.

Studies examining the association between low 25-(OH) D₃ levels and inflammation infection are still popular. There are studies suggesting a relationship between a lack of vitamin D₃ and morbidity and also mortality as well [9–12]. The results of these studies were contradictory and confusing. Randomized controlled trials of vitamin D₃ supplementation have shown incompatible results, with some trials suggesting a decrease [13, 14] and other studies concluding no effect on inflammatory biomarkers [15].

The potential relationship between the deficiency of vitamin D₃ and infection-inflammation remains poorly understood. Therefore, the aim of present study is to examine the

TABLE 1: Demographical data.

	<i>n</i>
Patients with CKD	340
Gender (M/F)	1897 (377/1520)
Outpatient (%)	1717 (90.5)
Hospitalized patient (all-cause) (%)	180 (9.5)
The elderly group	
65–74 years	320
75–84 years	209
>85 years	13

CKD: chronic kidney disease; M: male; F: female; *n*: the number of participants.

association between the level of plasma 25-(OH) D₃ and inflammatory markers in the general population without chronic kidney disease and in patients with CKD.

2. Methods

2.1. Study Population. Present study was conducted between January 1, 2008 and April 25, 2012 in Bulent Ecevit University Hospital and 1897 patients with 25-(OH) D₃ levels and inflammatory markers measured simultaneously were included. Patients whose age under 18 years, patients with primary hyperparathyroidism and hypoparathyroidism, were excluded. The study participants' age, gender, and hospitalization data were recorded. The clinical and laboratory data are shown in Tables 1 and 2. The relationship between 25-(OH) D₃ levels and serum creatinine, parathormone (PTH), sensitive C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), leucocyte count, platelet count, and hemoglobin concentrations were evaluated as retrospectively in this study population.

Serum 25-(OH) D₃ levels vary depending on season; we categorized patients into two groups according to serum 25-(OH) D₃ levels. Group 1 was composed of vitamin D₃ deficient (<10 µg/L) population, and Group 2 was composed of vitamin D₃ normal group (>21 µg/L). Patients with vitamin D₃ levels between 10 µg/L and 20 µg/L were excluded from the analysis in order to avoid the effects of seasonal changes. So this intermediate group was not used in this study (Figure 1).

Patients with known levels of CRP were grouped categorically as normal (CRP < 6 mg/L; there was no inflammation or infection) and as abnormal (CRP > 30 mg/L; there was important inflammatory or infectious status). Likewise, to examine the relationship between renal failure and 25-(OH) D₃ levels, participants were divided into categorical groups: the patients with and without renal failure. CKD was defined according to serum creatinine levels. Study cases with serum creatinine levels above 1.3 mg/dL for more than 3 months were considered as patients with CKD.

Moreover, the participants in this study were also divided into two groups: ambulatory patients and hospitalized patients.

TABLE 2: Clinical and laboratory data.

Variable (<i>n</i>)	Mean ± SD (Min–Max)
Age (year) (1897)	55 ± 15 (18–90)
25-(OH) D ₃ (µg/L) (1897)	16 ± 13 (3–201)
Vit B12 (pg/mL) (443)	401.9 (84–2001)
PTH (pg/mL) (1162)	112 ± 184 (2–2500)
CRP (mg/L) (996)	12.9 ± 26.7 (2–219)
ESR (mm/h) (1314)	29 ± 21.7 (1–141)
WBC (10 ³ /µg/L) (1451)	7.7 ± 3.5 (2.6–100)
Hemoglobin (gr/dL) (1001)	12.5 ± 1.7 (6.6–17.8)
Platelet (10 ³ /µg/L) (1003)	264 ± 82 (9–765)
Albumin (gr/dL) (577)	4.13 ± 0.58 (1.6–5.0)

Vit B12: vitamin B12; PTH: parathormone; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; WBC: white blood cells; SD: standard deviation; Min: minimum; Max: maximum.

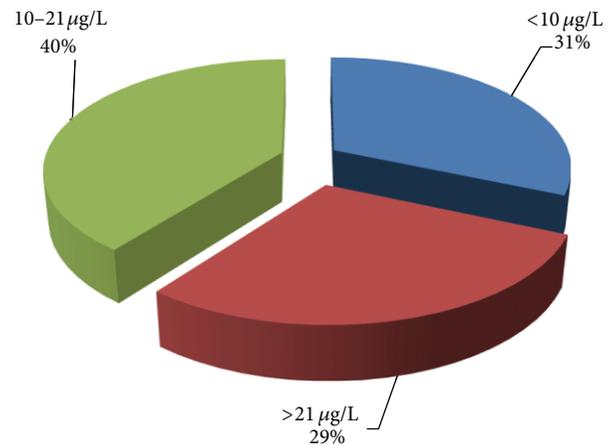


FIGURE 1: Distribution of 25-(OH) vitamin D₃ levels in study population.

Finally, vitamin B12 levels were measured in vitamin D₃ deficient and vitamin D₃ normal group, and then these two groups were divided into subgroups of their own.

Primary endpoints are as follows:

- (1) determining the 25-(OH) D₃ level in the general population and in patients with CKD;
- (2) comparing the clinical and laboratory data regarding inflammation with levels of 25-(OH) D₃;
- (3) evaluation of whether low and normal 25-(OH) D₃ levels and inflammation could explain this potential association;

2.2. Biochemical Analysis. 25-Hydroxyvitamin D₃ levels were measured by high performance liquid chromatographic analysis performed with using a Zivak HPLC system (Gebze, Turkey) using a commercial 25-OH vitamin D₃ kit (Recipe, Munich, Germany). The reference values were 10–50 µg/L for winter, 20–120 µg/L for summer seasons. A deficiency in 25-(OH) D₃ level was considered as below 10 µg/L.

TABLE 3: Vitamin D₃ deficient and normal group medians in all outpatients participants.

(Total number)	25-(OH) D ₃ < 9.9 µg/L		25-(OH) D ₃ > 21 µg/L		P
	n	Median (Min–Max)	n	Median (Min–Max)	
Age (n = 1032)	520	54 (18–88)	512	56 (18–87)	0.139
Albumin (n = 292)	163	4.3 (2.4–5)	129	4.4 (3–5)	0.071*
CRP (n = 554)	262	3.6 (3–204)	283	3.5 (2–156)	0.943
Creatinine (n = 770)	386	0.9 (0.3–10)	384	0.9 (0.4–12)	0.018
PTH (n = 655)	335	72.8 (3–1675)	320	57 (4–2269)	0.000
ESR (n = 716)	353	23 (2–131)	363	23 (2–109)	0.204*
WBC (n = 767)	392	7.2 (3.2–22.4)	375	7.1 (3.1–16.3)	0.780
Vitamin B12 (n = 254)	138	301 (84–2001)	116	336 (111–1454)	0.115

CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; WBC: white blood cells; Min: minimum; Max: maximum. * When hospitalized patients included in the analysis; albumin and ESR gained significance ($P = 0.001$, $P = 0.024$, resp.).

Serum vitamin B12 and plasma PTH levels were measured with chemiluminescence method by Immulite 2000 (Diagnostic Products Corp., LA, USA).

PTH was measured by chemiluminescence with reference values of 16–87 µg/L.

C-reactive protein was assayed with Dade Behring BN ProSpec System using a nephelometric method.

Serum albumin levels and ESR were measured by routine laboratory methods.

Leukocyte count, platelet count, and hemoglobin concentrations were measured by Beckman Coulter LH 780 hematology analyzer.

2.3. Statistical Analysis. Statistical analyses were performed by SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Distribution of data was determined by Kolmogorov-Smirnov test. Continuous variables were expressed as median (minimum-maximum) and categorical variables as frequency and percent. Continuous variables were compared with the Mann-Whitney *U* test and categorical variables were compared using Pearson's Chi-square test. Linear relation between two continuous variables was evaluated by Spearman correlation analysis. *P* value of less than 0.05 was considered statistically significant for all tests.

3. Results

A total of 1897 subjects were included in this retrospective study. Patients that measured 25-(OH) D₃ levels under 10 µg/L were 598 (31.5%), the number of those between 10 and 21 µg/L was 751 (39.5%), and the number of those over 21 µg/L was 550 (28.9%), respectively, in the study group (Figure 1).

The difference between male and female in 25-(OH) D₃ levels was statistically significant ($P < 0.001$), and 25-(OH) D₃ levels were significantly lower in female [16.1 ± 12.8 (3–121)] than in male [19.2 ± 12.9 (3–201)]. For this reason, male and female patients were divided into groups according to the presence of renal failure. The results are summarized in Tables 3 and 4.

There was no significant correlation between age and vitamin D₃ deficiency in our study population. There were lower serum albumin levels in patients with vitamin D₃ deficiency,

but this was not statistically significant (Table 3). Median serum creatinine levels were less in patients with vitamin D₃ deficiency without renal failure than in participants with normal vitamin D₃ levels without renal failure (Table 4).

Serum albumin, CRP, ESR, and WBC levels had no significant relationship in groups that vitamin D₃ deficiency and vitamin D₃ normal in male and female patients without renal failure (Tables 1–4). There was no difference in the levels of albumin, CRP, ESR, and WBC in women with renal insufficiency, but there was significant difference between levels of serum albumin and ESR in male patients.

The inflammatory status measured by CRP showed no difference with respect to the 25-(OH) D₃ ($P = 0.318$).

In CRP variable that was categorized as <6 mg/L and ≥6 mg/L, there was no significant difference between CRP categories and 25-(OH) D₃ levels ($P = 0.728$). The results are summarized in Table 5.

In CRP variable that was categorized as <6 mg/L and ≥30 mg/L, there was no significant difference between these CRP categories and 25-(OH) D₃ levels ($P = 0.635$) (Table 6).

Then, all participants were included in the study, both 25-(OH) D₃ and CRP variables taken as a numerical, and correlation analysis was performed. There was no correlation between the two groups ($r = -0.03$, $P = 0.335$).

The difference between men and women CRP levels was statistically significant ($P = 0.013$).

CRP and vitamin D₃ levels in men and women were different; therefore, similar analyses were repeated in men and women groups. Median CRP in vitamin D₃ deficient group and vitamin D₃ normal group showed no significant difference in the male and female patients.

There was a weak positive correlation between age and CRP ($r = 0.21$, $P < 0.001$).

When 25-(OH) D₃ and CRP variables are taken as categorical variables and analyzing the relationships between variables, no significant correlation was found ($P = 1.000$).

The prevalence of vitamin D₃ deficiency in patients with renal failure had a higher number ($P = 0.000$) (Table 6). This frequency were not statistically significant in male patients except advanced-stage renal failure (creatinine < 3.8 mg/dL), ($P = 0.148$).

The frequency of vitamin D deficiency was evaluated between outpatient and hospitalized patients groups in this

TABLE 4: (a) Vitamin D₃ deficient and normal group medians in subjects without renal failure; (b) vitamin D₃ deficient and normal group medians in subjects with renal failure.

	25-(OH) vitamin D ₃ < 9.9 µg/L		25-(OH) vitamin D ₃ > 21 µg/L		<i>P</i>
	<i>n</i>	Median (Min–max)	<i>n</i>	Median (Min–Max)	
Age (<i>n</i> = 706)	348	54 (18–85)	358	55 (18–87)	0.373
Albumin (<i>n</i> = 227)	125	4.4 (2.4–5)	102	4.4 (3–5)	0.355
CRP (<i>n</i> = 401)	186	3.5 (3–158)	215	3.5 (2–105)	0.722
Creatinine (<i>n</i> = 706)	348	0.8 (0.3–1.3)	358	0.9 (0.4–1.3)	0.000
PTH (<i>n</i> = 426)	216	72.9 (3–1013)	210	58 (16–118)	0.000
ESR (<i>n</i> = 543)	260	23 (2–107)	283	23 (2–109)	0.639
WBC (<i>n</i> = 605)	299	7.15 (3.2–22.4)	306	7.1 (3.1–16.1)	0.990

CRP: C-reactive protein; PTH: parathormone; ESR: erythrocyte sedimentation rate; WBC: white blood cells; Min: minimum; Max: maximum.

	25-(OH) D ₃ < 9.9 µg/L		25-(OH) D ₃ > 21 µg/L		<i>P</i>
	<i>n</i>	Median	<i>n</i>	Median	
Age (<i>n</i> = 178)	114	69 (22–90)	64	62.5 (18–83)	0.017
Albumin (<i>n</i> = 109)	81	3.4 (1.6–4.9)	28	4.05 (2.7–4.8)	0.001
CRP (<i>n</i> = 102)	61	15 (3–218)	41	6 (3–205)	0.067
Creatinine (<i>n</i> = 178)	115	2.1 (1.4–13.6)	63	1.75 (1.4–12.4)	0.289
PTH (<i>n</i> = 121)	77	194.5 (6–2166)	44	141 (2–2269)	0.161
ESR (<i>n</i> = 106)	63	48 (2–131)	43	33 (8–141)	0.003
WBC (<i>n</i> = 144)	97	8.4 (0.1–44.4)	47	7.6 (3.7–21.7)	0.641

CRP: C-reactive protein; PTH: parathormone; ESR: erythrocyte sedimentation rate; WBC: white blood cells; SD: standard deviation; Min: minimum; Max: maximum.

TABLE 5: Vitamin D₃ levels in patients with inflammation and without inflammation.

	<i>n</i>	25-(OH) D ₃ Median (Min–Max)	25-(OH) D ₃ (Mean ± SD)
CRP < 6 mg/dL	605	15 (3–79)	17.42 ± 12.21
CRP > 6 mg/dL	390	15 (3–201)	17.47 ± 15.65
CRP total	995	15 (3–201)	17.44 ± 13.65

CRP: C-reactive protein; SD: standard deviation.

study population. The incidence of vitamin D₃ deficiency in all-cause hospitalized patients was more frequent (*P* = 0.000). The prevalence of vitamin D₃ deficiency in outpatients was 46.3% (*n* = 236) and in hospitalized patients was 67.5% (*n* = 77). The number of subjects with normal vitamin D₃ was 53.7% for outpatients (*n* = 274) and for hospitalized patients was 32.4% (*n* = 37), respectively (Table 6). Moreover age, ESR, WBC, and CRP medians had higher levels in hospitalized patients.

Finally, B12 levels were measured in 254 patients. The prevalence of low vitamin B12 (<160 pg/mL) was 68% (*n* = 30), and the prevalence of normal vitamin B12 level (>160 pg/mL) was 32% (*n* = 14) in the group with vitamin D₃ deficiency, whereas the prevalence of low vitamin B12 was 51.4% (*n* = 108), and the prevalence of normal vitamin B12 (Number 30) was 48.6% (*n* = 102) in the normal vitamin D₃

TABLE 6: The frequency of CRP, vitamin B12, hospitalization, and renal failure in 25-(OH) vitamin D₃ deficiency.

	25-(OH) D ₃ < 9.99 µg/L, <i>n</i> (%)	25-(OH) D ₃ > 21 µg/L, <i>n</i> (%)	<i>P</i>
CRP < 6 mg/dL	180 (84.5)	193 (90.2)	
CRP > 30 mg/dL	33 (15.5)	21 (9.8)	
Total	213	214	0.770
Vit B12 < 160 pg/mL	33 (21.7)	15 (12.4)	
Vit B12 > 160 pg/mL	119 (78.3)	106 (87.6)	
Total	152	211	0.043
Albumin < 3.5 g/dL	46 (21)	11 (7.6)	
Albumin > 3.5 g/dL	173 (79)	133 (92.4)	
Total	219	144	0.001
Outpatients	520 (87.1)	513 (93.3)	
Inpatients	77 (12.9)	37 (6.7)	
Total	597	550	0.000
With CKD	115 (24.8)	63 (15)	
Without CKD	348 (75.2)	358 (85)	
Total	463	421	0.000

CRP: C-reactive protein; CKD: chronic kidney disease.

group. Vitamin B12 deficiency was more frequently seen in patients with vitamin D₃ deficiency (*P* = 0.043) (Table 6).

4. Discussion

Present study did not reflect the true incidence of vitamin D₃ deficiency because patients who are thought to lack of vitamin D₃ were included in this study. A limited number of studies conducted in Turkey have shown that vitamin D₃ deficiency is a common issue during the fall and winter in individuals, particularly for elderly. The deficiency of vitamin D₃ is seen in 70–75% of women in our country. Vitamin D₃ deficiency rates are 80–84% in the Middle East, 60–65% in Asia, 50–55% in Europe, and 50% in Latin America [16–18]. Female constitutes the majority of patients may be due to less exposure to the sun and the higher prevalence of osteoporosis.

There was no significant correlation between age and vitamin D deficiency and that may be due to individual characteristics of the studied population. This relationship is shown in some other studies [11, 19]. But many studies did not mentioned the relationship between age and vitamin D levels.

In present study we did not find a relationship between vitamin D₃ deficiency and inflammatory markers, such as CRP, ESR, and leukocyte counts. Some other studies measured CRP was found the relationship but in these studies the relatively small number of participants were the limiting factor [20, 21]. In several studies were unknown accompanying diseases, and hospitalization rates [9, 22]. There were no studies evaluating ESR, and leukocyte counts were evaluated in 25-(OH) D₃ deficiency.

Sensitive CRP that was not measured is the limitation of the study. To resolve this drawback was categorized patients according to the levels of CRP. Therefore we divided our study populations into subsets according to CRP levels. Firstly, we counted the number of patients with and without vitamin D₃ deficiency in CRP normal group. We found no significant difference between two subgroups. Secondly, we separated the study population into CRP normal and significantly high CRP groups. We found no significant difference between the last subgroups again. The reason for this classification was to evaluate the frequency of vitamin D₃ deficiency in outpatients with important high level of CRP. Finally, we applied correlation analysis between the level of CRP and 25-(OH) D₃. But a relationship between the level of CRP and 25-(OH) D₃ was not found in all our analyses. In other words, we did not observe an association between vitamin D deficiency and CRP levels anyway.

Patients' age, serum albumin, CRP, and ESR levels, leukocyte counts, and creatinine values were significantly different between ambulatory and hospitalized patients. The medians of inflammatory markers of hospitalized patients were higher compared to those of ambulatory patients except albumin levels. In addition, the frequency of 25-(OH) D₃ deficiency was higher once again in hospitalized patients. These also mean that 25-(OH) D₃ deficiency aggravates all-cause diseases, which is associated with the course of inflammation and infection but not CRP levels.

The prevalence of vitamin D₃ deficiency in patients with CKD was more common at all stages in female patients; however, it was more common at advanced stage in male patients. This could be explained by a combination of factors, such as poor nutrition or a lack of skin synthesis due to low

sun exposure [23]. In CKD patients, dietary restriction and loss of appetite due to uremia or high levels of fibroblast growth factor 23 may be stronger determining factors for 25-(OH) D₃ deficiency.

In groups without renal failure, creatinine values of vitamin D₃ deficient patients were lower than vitamin D₃ normal subjects. Vitamin D₃ deficient patients had higher PTH values. Higher PTH values were known and expected to be higher among the vitamin D₃ deficient patients [23]. However, the low level of creatinine was not been described previously, and this difference was statistically significant.

In our study population, the levels of albumin were lower in vitamin D₃ deficient patients than in vitamin D₃ normal participations. However, this state did not reach statistical significance. This also pointed out other studies [24]. It has been reported decreased level of albumin in a large scaled study of Melamed et al. [9].

In groups without renal failure, low creatinine and albumin levels might be associated with a nutritional disorder or other comorbid inflammatory-infectious status. It is known that deficiency of vitamin D₃ and malnutrition were related to each other. Some studies demonstrated that the replacement of vitamin D₃ did not correct mortality [19]. Patients with high mortality despite treatment with vitamin D₃ could have other disorders. To clarify this state we evaluated another vitamin such as vitamin B12. Vitamin B12 deficiency was more common in vitamin D₃ deficient patients. Multivitamin deficiency was common in malnourished and elderly patients, but there was no study that tested vitamin B12 levels in vitamin D₃ deficient patient in the literature.

For more accurate assessment it is necessary to know other factors that trigger inflammation and infection in studies examining the relationship between vitamin D and inflammatory markers. For example, when hospitalized patients are included in our analysis; all inflammatory markers gained significance statistically.

In addition, the reason for the deficiency of 25-(OH) D₃ should be known in similar studies. However, there may be no relationship in encountered 25-(OH) D₃ deficiency due to low sunlight exposure, and it could be expected in patients with 25-(OH) D₃ deficiency due to malnutrition.

According to the results of our study, high levels of CRP in vitamin D deficient patients might be related to other factors such as infectious, inflammatory status, malnutrition, cachexia, or multivitamin deficiency. These factors and others may affect high morbidity and mortality in patients with vitamin D₃ deficiency. Therefore, replacement of vitamin D alone could be corrected only in patients with vitamin D deficient patients in the foreground.

Conflict of Interests

The authors declare that they have no competing interests.

Authors' Contribution

Yildirim Ibrahim participated in concept, design, data collection, data analysis, data interpretation, and writing. Hur

Ender participated in data interpretation and writing. Kokturk Furuzan participated in data analysis.

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Research Article

The Effects of Vitamin D on Gentamicin-Induced Acute Kidney Injury in Experimental Rat Model

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Introduction. Acute kidney injury (AKI) pathogenesis is complex. Findings of gentamicin nephrotoxicity are seen in 30% of the AKI patients. Vitamin D has proven to be effective on renin expression, inflammatory response, oxidative stress, apoptosis, and atherosclerosis. We aimed to investigate the effect of vitamin D in an experimental rat model of gentamicin-induced AKI. **Methods.** Thirty nonuremic Wistar albino rats were divided into 3 groups: Control group, 1 mL saline intramuscular (im) daily; Genta group, gentamicin 100 mg/kg/day (im); Genta + vitamin D, gentamicin 100 mg/kg/day (im) in addition to $1\alpha, 25(\text{OH})_2\text{D}_3$ 0.4 mcg/kg/day subcutaneously for 8 days. Blood pressures and 24-hour urine were measured. Blood urea and creatinine levels and urine tubular injury markers were measured. Renal histology was semiquantitatively assessed. **Results.** Urea, creatinine and urine neutrophil gelatinase-associated lipocalin, and kidney injury molecule-1 were all increased in Genta group indicating AKI model. Systolic blood pressure decreased, but urine volume and glutathione increased in Genta + Vit D group compared to Control group. Histological scores indicating tubular injury increased in Genta and Genta + Vit D groups. **Conclusions.** Vitamin D does not seem to be effective on histological findings although it has some beneficial effects via RAS system and a promising effect on antioxidant system.

1. Introduction

Acute kidney injury (AKI) pathogenesis is complex, and promoting events may be completely different (ischemia or toxins are major factors that precipitate in the injury), but similar pathways may be involved in subsequent injury responses. For this, reason to study AKI models, various methods were defined for each specific situation.

Gentamicin derived from gram-positive bacteria called *Micromonospora purpurea* present in soil and water having potential in treating aerobic gram-negative bacteria. Accumulation of gentamicin in proximal renal tubules may cause nephrotoxicity which leads to brush border network

damage [1]. The nephrotoxicity involves renal free radical production and accumulation, consumption of antioxidant defense mechanisms, glomerular congestion, and acute tubular necrosis [2–5], leading to diminished creatinine clearance and renal dysfunction. The pathological mechanisms also involve elevation of endothelin-1, upregulation of transforming growth factor-beta (TGF- β), significant increase in monocyte/macrophage infiltration into the renal cortex and medulla, augmentation of oxidative stress, and apoptosis and also necrosis [6–9].

Vitamin D is a pleiotropic hormone that affects classical and nonclassical tissues. Its primary sites of action are still considered to be the intestine, bone, and kidneys [10]. A

number of studies have shown positive therapeutic efficacy of vitamin D and analogs to reduce proteinuria [11–13]. Recently, a large randomized placebo-controlled clinical trial (the VITAL Study, $n = 281$) confirmed that paricalcitol was able to reduce albuminuria and blood pressure in patients with diabetic nephropathy who were already on renin-angiotensin system inhibitor therapy [14]. Together, these clinical data provide a strong case to argue for the use of vitamin D analogs as a complementary therapy for treatment of proteinuria. Given the importance of podocytes in the regulation of glomerular filtration, it is speculated that podocytes are important antiproteinuric targets of vitamin D [15] although tubular effect of vitamin D is still debate.

Inflammation and reactive oxygen substances play an important role on acute kidney injury pathophysiology. Vitamin D has already known antiinflammatory and immunomodulatory effects. In the present study, the aim was to investigate whether vitamin D might be a useful therapeutic agent for gentamicin-induced AKI model in rats. Up to now vitamin D related protection mechanisms on AKI remain to be fully proven. Given the complexity of the disease and the pleiotropic nature of the agent activity, the protective effect would be expected and be of a multifactorial nature.

2. Methods

2.1. Study Protocol. Thirty nonuremic Wistar albino male rats ($n = 30$; weight 180–220 g) were divided into three equal groups. They were housed in polycarbonate cages under 24°C room temperature with a 12-hour light/dark cycle and fed a standard laboratory diet. The Animal Ethics Committee of Ege University Hospital approved the study design. The three groups of rats consisted of the following: Control group, 1 mL saline intramuscular (im) daily; Genta group, gentamicin 100 mg/kg/day (im); Genta + vitamin D, gentamicin 100 mg/kg/day (im) in addition to $1\alpha, 25(\text{OH})_2\text{D}_3$ 0.4 mcg/kg/day subcutaneously for 8 days.

Systolic blood pressure was measured in conscious rats by the indirect tail cuff method, using an electrophygmomanometer and pneumatic pulse transducer (MAY NIBP200-A, Ankara, Turkey), and 24-hour urine was collected in metabolic cages. After 1 hour, ketamine HCL anesthesia (60 mL/kg body weight) was applied, and immediately, blood samples were collected through direct cardiac puncture in sacrificed rats. Semiquantitative assessment of kidneys was carried out by the same pathologist who was unaware of the groups.

2.2. Functional Parameters. Serum urea and creatinine levels and urine gamma glutamine transferase (GGT) activity were determined using commercial available kits spectrophotometrically (Biolabo Reagents, Maizy France). Urine (Lipocalin-2)-Neutrophil gelatinase-associated lipocalin (NGAL) (Boster Biological Technology Co, Ltd.), and kidney injury molecule-1 (KIM-1) (Adipo Bioscience, Inco, USA) levels were measured with ELISA kits. Urine glutathione (GSH) levels were determined by a commercially

available kit (Cayman Chemical Company, Ann Arbor, MI, USA).

2.3. Structural Parameters. Four percent formalin was used for fixation of kidney samples which were embedded into paraffin wax. Paraffin blocks were divided into sections 5 μm in thickness, and hematoxylin-eosin and Masson's were trichrome used for staining. All samples were examined by the same pathologist who was unaware of which samples originated from which group. Tubular degeneration, necrosis, tubule interstitial nephritis, and total histological scores were evaluated semiquantitatively from 0 to 3.

Tubular degeneration (TD): in the cytoplasm of the proximal tubule epithelial cells, stained bodies of various sizes and vacuolization containing acidophilus were considered as TD.

Scoring:

Absence of TD; 0

Mild TD: small and a few focus TD in immediately beneath the capsule (0%–10); 1

Moderate TD: for a few focal focus TD and along the tubular segment (10%–25); 2

Severe TD: diffuse and significant TD along the tubular segment (% 25–50); 3

Very severe TD: TD was greater than 50%; 4

Tubular necrosis (TN): defined as loss of epithelial cells of the nucleus, dark acidophilic cytoplasm, loss of tubular epithelial cells into tubular lumen, and acellular sections of tubules.

Scoring:

Absence of TN; 0

Mild TN: small and a few focus TN in immediately beneath the capsule (0%–10); 1

Moderate TN: for a few focal focus TN and along the tubular segment (10%–25); 2

Severe TD: diffuse and significant TN along the tubular segment (% 25–50); 3

Very severe TN: TN was greater than 50%; 4

Tubulointerstitial inflammation (TIN): defined as infiltration of inflammatory cells in perivascular and interstitial areas.

Scoring:

Absence of TIN; 0

Mild TIN: a few pieces of infiltration concentrated on perivascular area (0–5%); 1

Moderate TIN: usually infiltrations involved in cortical interstitial and many focal areas (5–10%); 2

Severe TIN: diffuse and significant infiltration areas (15–25%); 3

Very severe TIN: TIN was greater than 50%; 4

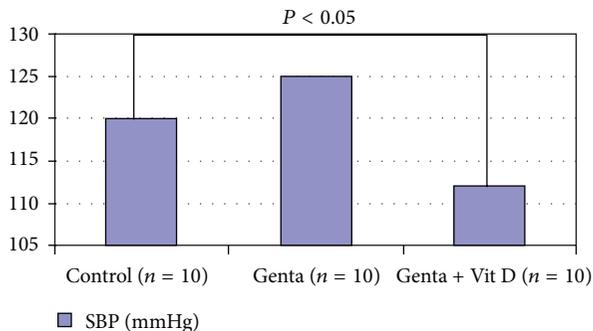


FIGURE 1: Blood pressures.

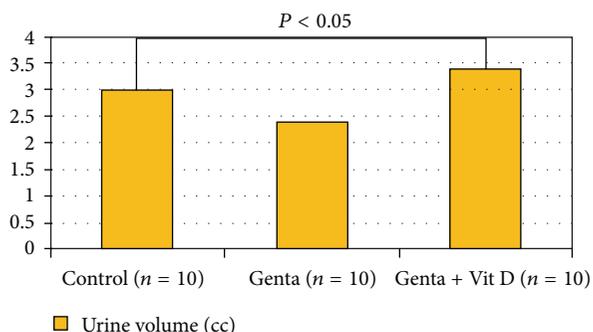


FIGURE 2: Urine Volumes.

Total histologic score (THS): $TD/2 + TN + TIN/2$, respectively.

- Normal THS: 0–2
- Mild THS: 2–5
- Moderate THS: 5–8
- Severe TH: >8

2.4. Statistical Analysis. Study results are presented as mean ± standard error of the mean (SEM). Nonparametric tests (Kruskal Wallis, Man-Whitney *U* test) were performed as statistical evaluation, and $P < 0.05$ was considered as significant.

3. Results

Systolic blood pressure in Control group was 120 ± 6 and decreased to 112 ± 13 mmHg (Figure 1), and urine volume increased in Genta + Vit D group (3.4 ± 0.5 cc) compared to Control group (3 ± 0.5 cc) ($P < 0.05$) (Figure 2). In Control group, urea and creatinine levels were 91 ± 6 and 0.74 ± 0.03 and increased to 137 ± 6 mg/dL and 1.1 ± 0.1 mg/dL in Genta group, also 149 ± 5 mg/dL and 1.6 ± 0.3 mg/dL in Genta + Vit D, indicating acute kidney injury (Figure 3). Neutrophil gelatinase-associated lipocalin (NGAL) was 49.5 ± 7 significantly and increased to 390 ± 143 ng/mL in Genta group and decreased to 247 ± 112 ng/mL in Genta + Vit D group. Glutathione and gamma glutamine transferase were 0.4 ± 0.13 and 1.3 ± 0.35 and increased to 0.6 ± 0.1 nmol/mL and 59 ± 19 U/L in Genta + Vit D group (Figure 4). Kidney injury

TABLE 1: Clinical and laboratory findings.

	Control, n = 10	Genta, n = 10	Genta + Vit D, n = 10
SBP (mmHg)	120 ± 6	125 ± 10	112 ± 13^a
DBP (mmHg)	68 ± 4	75 ± 7	74 ± 8
Urine volume (cc)	3 ± 0.5	2.4 ± 0.4	3.4 ± 0.5^a
Urea (mg/dL)	91 ± 6	137 ± 6^a	149 ± 5^a
Creatinine (mg/dL)	0.74 ± 0.03	1.1 ± 0.1^a	1.6 ± 0.3^a
NGAL (ng/mL)	49.5 ± 7	390 ± 143^a	247 ± 112
GSH (nmol/mL)	0.4 ± 0.13	0.3 ± 0.04	0.6 ± 0.1^a
GGT (U/L)	1.3 ± 0.35	38 ± 37	59 ± 19^a
KIM-1 (ng/mL)	0.64 ± 0.05	4.7 ± 0.6^a	6 ± 0.5^a
TD	0.9 ± 0.1	2 ± 0^a	2.3 ± 0.2^a
TN	0	1.3 ± 0.2^a	1.2 ± 0.2^a
TIN	0.4 ± 0.2	1.4 ± 0.2^a	2 ± 0.1^{ab}
THS	0.75 ± 0.15	3.4 ± 0.4^a	6.3 ± 0.7^{ab}

SBP: systolic blood pressure; DBP: diastolic blood pressure; NGAL: neutrophil gelatinase-associated lipocalin; GSH: glutathione; KIM: kidney injury molecule; TD: tubular degeneration; TN: tubular necrosis; TIN: tubulointerstitial nephritis; THS: total histological score; $P < 0.05$, ^aGroup versus Control, ^bGroup versus Genta.

molecule 1 (KIM-1) level was 0.64 ± 0.05 in Control group and increased significantly in both Genta and Genta + Vit D groups (4.7 ± 0.6 and 6 ± 0.5 ng/mL) (Table 1).

Histological scores of tubular degeneration (TD), tubular necrosis (TN), tubulointerstitial nephritis (TIN), and total histological score (THS) all increased significantly in Genta and Genta + Vit D groups compared to Control group (Figures 5 and 6). TIN and THS scores also significantly were higher in Genta + Vit D group compared to Genta group (Table 1).

4. Discussion

Gentamicin is a positively charged chemical that strongly binds to the acidic phosphoinositide components of the brush border membrane which is a negatively charged portion of the proximal tubule, and they mainly act on the cationic drug receptor, megalin, located deeply at the base of the brush border villi. The receptor-drug complex thus formed is rapidly internalized by a pinocytosis process and checked up by lysosomes, where lysosomal phospholipidosis occurs that disrupts a number of renal intracellular processes [16, 17].

Renin angiotensin system (RAS) in the kidney is a mandatory mediator of renal injury. Vitamin D hormone has a negative regulatory effect on RAS by suppressing renin expression [18, 19]. It is shown that vitamin D receptor-absent mutant mice develop more severe renal damage (e.g., interstitial fibrosis, increased albuminuria, and glomerulosclerosis) than wild-type counterparts in diabetic state [20] or under postrenal acute kidney injury [21], because of enhanced activation of the RAS in the kidney. In 5/6 nephrectomised rats given paricalcitol treatment attenuated tubulointerstitial and glomerular injury and decreased blood pressure and albuminuria by inhibiting the activation of the locally produced RAS in the remnant kidneys [22]. Doxercalciferol had an effect on modulating fat-induced renal injury by targeting

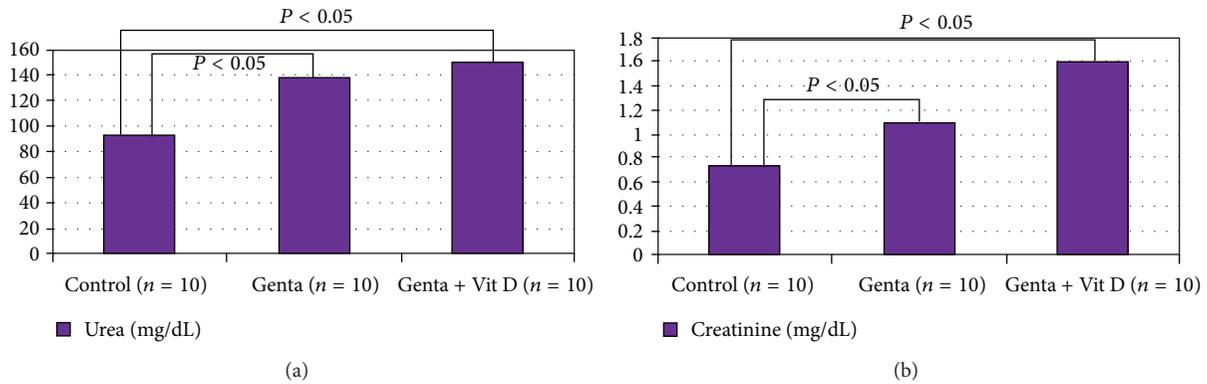


FIGURE 3: Renal function tests.

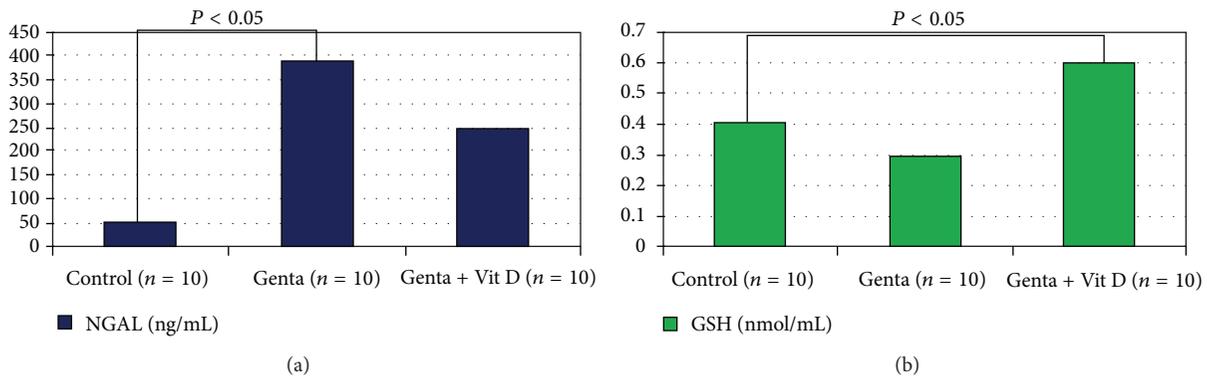


FIGURE 4: Urine neutrophil gelatinase-associated lipocalin and glutathione levels.

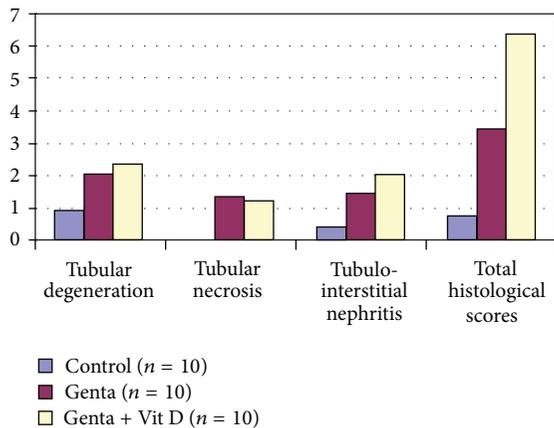


FIGURE 5: Renal histology. Pathological examination performed by semiquantitatively scored from 0 to 3.

the RAS and lipid metabolism [23]. Other studies proved that combination therapy with one RAS inhibitor (ACE inhibitor or ARB) and one vitamin D analog (paricalcitol or doxercalciferol) leads to additive or synergistic therapeutic effects in blocking renal injury in experimental rat models of type 1 and type 2 diabetes mellitus [24–27]. The renal protection of the combination therapy is the inhibition of the compensatory renin induction usually encountered in the use of both RAS inhibitors and the vitamin D analogues. Renin induction inhibition and accumulation of angiotensin II within the kidney leads to excellent therapeutic results

[28]. The combination strategy in these studies explains why vitamin D analogs are still effective in reducing albuminuria in CKD patients who are already receiving RAS inhibitors [29, 30]. In present study, we found that SBP is decreased in Genta + vitamin D group compared to Control group. This indicates that the RAS blocking effect of vitamin D was still strong enough even in the presence of acute kidney injury. Increased urine volume in this group also may be attributed to RAS blocking effect of vitamin D therapy.

Functionally, gentamicin-related nephrotoxicity is characterized by a decrease in glomerular filtration rate and high levels of serum creatinine and blood urea, indicating renal dysfunction [31–37]. In the present study, Genta-induced experimental AKI model is formed and proven by an increase of these renal function tests appropriately. Unfortunately, they were still higher in Genta + vitamin D group than in Control group.

In the literature, there are increasing multifactorial mechanisms suggested as the leading cause of gentamicin nephrotoxicity. Lysosomal apoptosis and phospholipidosis have been suggested to play a pivotal role in gentamicin-induced nephrotoxicity [38–40]. In the past, gentamicin was shown to increase reactive oxygen species (ROS) like superoxide anions, hydroxyl radicals and hydrogen peroxides, and reactive nitrogen species generation in the renal cortex that eventually lead to renal structural and functional deterioration [41–44]. Further, it is linked with marked increases in lipid peroxidation levels [45], nitrotyrosine formation [46] and protein oxidation [47]. In our study, we demonstrated

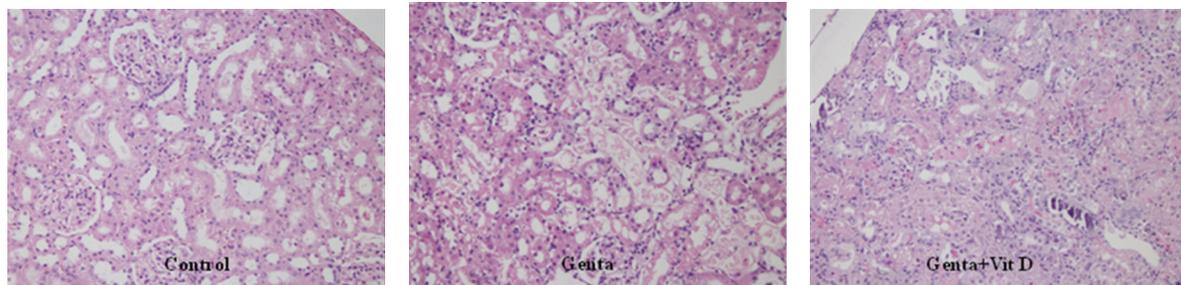


FIGURE 6: Renal pathology.

that in Genta group although it did not reach statistical significance a little GSH decrease occurred. On the other hand, Genta + Vit D group had a statistically significant GSH increase. In the literature, gentamicin has been also shown to cause changes in the composition of lipid membranes executed by free radicals mediated lipid peroxidation [48]. Furthermore, gentamicin-administered rat kidneys are more susceptible to ROS damage because of the induction of deficiency in antioxidant defense enzymes like superoxide dismutase and catalase [49, 50]. Here in our study, vitamin D might have some beneficial effect on gentamicin-induced AKI by increasing GSH levels and acting as an antioxidant mechanism, and also NGAL levels were not increased unlike to Genta group.

Structurally, gentamicin-related nephrotoxicity is associated with the edema of proximal tubular cells, glomerular hypertrophy, perivascular edema, inflammation, glomerular congestion, cellular desquamation, glomerular atrophy, tubular necrosis, and tubular fibrosis [40, 51–57]. Gentamicin causes macrophage infiltration and higher transforming growth factor- β which may lead to progression of TIN [40]. In the present study, TIN scores were significantly higher in Genta group, but surprisingly in Genta + Vit D group, histological scores were even higher than Genta group. Tubular histological parameters all were increased in Genta group indicating experimental AKI model occurred but unfortunately all these parameters were not decreased in Genta + Vit D group.

Acute kidney injury as a result of gentamicin-induced tubular necrosis stimulates inflammatory events by recruiting intercellular adhesion molecule-1 and monocyte chemoattractant protein-1 at the site of injury that enhance the migration of monocytes and macrophages to the site of tissue damage, ultimately leading to renal pathogenesis [58, 59]. In present study we demonstrated that GGT levels in Genta + Vit D group and KIM-1 levels in both Genta and Genta + Vit D groups were increased indicating that renal tubular damage occurred in Genta groups and also even using Vit D did not prevent progression of injury.

5. Conclusion

In the past, vitamin D was shown as an effective drug on podocytes preventing proteinuria, regulate bone remodeling, regulate cell cycles, and the renin-angiotensin system [60]. The present study indicates that the progression of

gentamicin-induced AKI was not stopped by vitamin D treatment shown by histological findings although it probably has some beneficial effects on the RAS system via blood pressure lowering and increase of urine volume and a promising effect on antioxidant system. As a result given the various overlapping pathways involved in AKI pathogenesis, intended therapies may need to use vitamin D in addition to other therapeutical approaches to target diverse pathways in order to achieve success.

Disclosure

The results presented in this paper have not been published previously in whole or part, presented in abstract form. All the authors have contributed substantially to the research, preparation and production of the paper and approve of its submission to the Journal.

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Research Article

Significant Independent Predictors of Vitamin D Deficiency in Inpatients and Outpatients of a Nephrology Unit

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Aims. Kidney disease was found to be a major risk factor for vitamin D deficiency in a population study of patients hospitalized. The aims of the study were to describe the prevalence of vitamin D deficiency inpatients and outpatients in a nephrology department during fall and to evaluate effect of assessing serum 25-hydroxyvitamin D (25(OH)D) levels and previous supplementation of cholecalciferol on vitamin D status. **Methods.** We studied 280 subjects in total, between October and January. The subjects were recruited from the following two groups: (a) inpatients and (b) outpatients in nephrology unit. We examined previous documentary evidence of vitamin D supplementation of the patients. **Results.** The prevalence of vitamin D deficiency among these 280 patients was 62.1% (174 patients). Fifty-three patients (18.9%) had severe vitamin D deficiency, 121 patients (43.2%) moderate vitamin D deficiency, and 66 patients (23.6%) vitamin D insufficiency. In logistic regression analysis female gender, not having vitamin D supplementation history, low serum albumin, and low blood urea nitrogen levels were significant independent predictors of vitamin D deficiency while no association of vitamin D deficiency with diabetes mellitus, serum creatinine, eGFR, and being hospitalized was found. **Conclusion.** Vitamin D deficiency, seems to be an important problem in both inpatients and outpatients of nephrology. Monitoring serum 25(OH)D concentrations regularly and replacement of vitamin D are important. Women in Turkey are at more risk of deficiency and may therefore need to consume higher doses of vitamin D.

1. Introduction

Vitamin D deficiency is reemerging as a major public health problem throughout the world. It is acknowledged that the prevalence of vitamin D deficiency and its associated morbidities are higher than previously thought worldwide. In addition to metabolic bone disease, recent studies reported that vitamin D deficiency could increase the risk of certain cancers, heart disease, and autoimmune diseases including rheumatoid arthritis and multiple sclerosis in adults and diabetes mellitus [1–8].

Chronic vitamin D deficiency may have serious adverse consequences in patients with kidney disease [9–13]. To test the hypothesis that low serum 25-hydroxyvitamin D (25(OH)D) levels are a risk factor for kidney disease progression, Melamed et al. analyzed data from 13,328 participants

in the National Health and Nutrition Examination Survey (NHANES) III Follow-Up Study, in which 25(OH)D levels were measured from 1988 through 1994, and then participants were followed for up to 12 years. The incidence of end-stage renal disease was 2.6 times greater in people whose serum 25(OH)D was less than 15 ng/mL than in those with higher levels [14].

However, it is still not common practice among nephrologists to monitor and correct vitamin D deficiency of patients with kidney disease, because it is widely believed that the capacity of the 1 α -hydroxylase to synthesize 1,25(OH)₂D₃ decreases progressively because of decreased renal mass and that any vitamin D deficiency associated with calcium-phosphate disturbances is better treated with activated vitamin D.

Although hypovitaminosis D has been reported frequently in patients with kidney disease, the prevalence of vitamin D deficiency among patients hospitalized in nephrology services is unknown.

The aims of the study were to describe and compare the prevalence of vitamin D deficiency inpatients and outpatients in the nephrology department during early winter and to evaluate effect of assessing serum 25(OH)D levels and previous supplementation of cholecalciferol on vitamin D status.

2. Material Methods

We undertook a retrospective audit of the serum 25(OH)D levels of inpatients and outpatients in the nephrology departments from October 15, 2010 to January 1, 2011, and previous serum 25(OH)D analysis, and previous vitamin D supplementation history of the patients. The study was approved by the Ethics Committee.

The following information was determined from the patients' medical records: age, gender, primary underlying renal diagnosis, blood urea nitrogen (BUN), serum creatinine, corrected total serum calcium, serum phosphorus, intact parathormone (iPTH), serum albumin levels, recent and preceding results of serum 25(OH)D analysis, and previous documentary evidence of vitamin D supplementation. Estimated GFR (eGFR), using the abbreviated Modification in Diet in Renal Disease (MDRD) equation, was determined [15].

Vitamin D deficiency was defined as 25(OH)D levels below 15 ng/mL (37.4 nM/L). For subanalysis the patients were divided into four diagnostic categories according to their serum 25(OH)D levels: severe vitamin D deficiency (serum 25(OH)D₃ level, less than 5 ng/mL, (12.5 nM/L)), moderate vitamin D deficiency (serum 25(OH)D₃ level, 5 to 15 ng/mL (12.5–37.4 nM/L)), vitamin D insufficiency (serum 25(OH)D₃ level, 15 to 30 ng/mL (12.5–37.4 nM/L)), and adequate vitamin D stores (serum 25(OH)D₃ level, more than 30 ng/mL (37.4 nM/L)) [16].

3. Statistical Analyses

Data are expressed as mean \pm SD. The significance of difference between continuous variables was tested using unpaired *t*-test or Mann-Whitney rank sum test and for categorical variables using χ^2 or Fisher exact test as appropriate. Correlations were tested using Spearman correlation coefficient. *P* values less than 0.05 were regarded as statistically significant. Logistic regression analysis, binary logistic (Hosmer-Lemeshow goodness of fit) by enter method, was studied.

4. Results

In the local biochemistry database we identified 25(OH)D requests in total 280 inpatients and outpatients in the Nephrology Department between October 15, 2010 and January 1, 2011.

Diagnosis of the patients were given in Table 1. A total of 49 (17.5%) patients were receiving dialysis (17 (34.7%) due to

TABLE 1: Diagnosis of the patients.

Diagnosis	Frequency (%)
Acute renal failure	13 (4.6)
Chronic kidney disease	230 (82.1)
Acute renal failure on chronic kidney disease	4 (1.4)
Hypernatremia	1 (0.4)
Hyponatremia	1 (0.4)
Glomerulonephritis	8 (2.9)
Renal transplantation	9 (3.2)
Urinary infection	2 (0.7)
Hypertension	12 (4.3)
Total	280 (100.0)

acute renal failure, and all these were inpatients. Sixty-three patients (22.5%) were diabetic. Demographic and clinical characteristics of inpatients and outpatients are shown at Table 2.

The prevalence of vitamin D deficiency among these 280 patients was 62.1% (174 patients). Fifty-three patients (18.9%) had severe vitamin D deficiency, 121 patients (43.2%) moderate vitamin D deficiency, and 66 patients (23.6%) vitamin D insufficiency. Only 40 (14.3%) patients had adequate vitamin D stores. Whereas in patients with vitamin D deficiency, age, BUN, and iPTH were higher, serum albumin, calcium levels were lower. There was no significant difference according to serum phosphorus, creatinine, and eGFR ($P > 0,05$) (Table 3).

While in 72% of the inpatients (n : 90) serum 25(OH)D levels were less than 15 ng/mL (37.4 nM/L), in 54.2% of the outpatients (n : 86) serum 25(OH)D levels were less than 15 ng/mL (37.4 nM/L) ($P < 0,05$). While age, BUN, serum creatinine, and phosphorus levels of inpatients were higher, serum 25(OH)D, serum calcium, albumin levels, and eGFR of inpatients were lower than those of outpatients ($P < 0,05$). No significant difference was found according to serum iPTH levels between inpatients and outpatients.

Of 280 patients, 156 (55.7%) had at least one measurement of 25(OH)D levels during the previous followup in our nephrology unit. Of these 280 patients, 55.7% (156) were previously supplemented with cholecalciferol according to vitamin D supplementation protocol of our nephrology unit. The mean duration between recent and preceding measurements of serum 25(OH)D levels and initiation of vitamin D supplementation was 9.9 ± 3.9 months. The preceding mean serum 25(OH)D level was 12.4 ± 6.8 ng/mL (30.9 ± 16.7 nM/L).

Of 174 patients with vitamin D deficiency, 83 (47.7%) and 73 (68.9%) of 106 patients without vitamin D deficiency had vitamin D supplementation history. Of 125 inpatients, 57 (45.6%) and 99 (63.9%) of 155 outpatients had vitamin D supplementation history ($P < 0,05$). No difference was found between patients supplemented with vitamin D previously or not according to BUN, serum albumin, and phosphorus. While inpatients supplemented with vitamin D previously, age ($50,8 \pm 16,6$ versus $58,1 \pm 16,7$ years), hospitalization days

TABLE 2: Demographic and laboratory results of the inpatients and outpatients.

Variable	All patients (<i>n</i> = 280)	Inpatients (<i>n</i> = 125)	Outpatients (<i>n</i> = 155)	<i>P</i> value
Age (years)	54.06 ± 16.9 (18–91)	56.9 ± 18 (18–91)	51.8 ± 15.4 (21–84)	0.008
Hospitalization days	—	13.8 ± 11.2 (1–60)	—	—
Serum 25(OH)D levels (ng/mL) (nM/L)	16.5 ± 15.9 (0.4–110) (41.2 ± 39.7)	13.5 ± 15.9 (0.4–110) (33.7 ± 39.7)	18.9 ± 15.6 (1–82.8) (47.2 ± 38.9)	<0.001
Blood urea nitrogen (mg/dL)	54.4 ± 35.54 (7–185)	66.3 ± 37.3 (10–185)	43.1 ± 19.8 (7–102)	<0.001
Serum creatinine (mg/dL)	5.8 ± 3.7 (0.6–17.8)	6.3 ± 3.8 (0.7–17.8)	5.4 ± 3.8 (0.6–16.8)	0.021
Serum albumin (mg/dL)	3.4 ± 0.7 (0.5–4.8)	3.10 ± 0.7 (0.5–4.5)	3.6 ± 0.6 (1.7–4.8)	<0.001
Serum calcium (mg/dL)	9.0 ± 0.9 (6.0–12.7)	8.6 ± 0.9 (6.0–12.0)	9.2 ± 0.8 (7.3–12.7)	<0.001
Serum phosphorus (mg/dL)	4.7 ± 1.6 (1.9–11.9)	5.2 ± 1.8 (1.9–11.9)	4.3 ± 1.3 (1.9–10.1)	<0.001
Serum parathyroid hormone (pg/mL)	376.9 ± 425.9 (10.9–2500)	377.2 ± 362.3 (35.3–2258)	376.8 ± 472.7 (10.9–2500)	NS
eGFR (mL/min/1.73 m ²)	24.6 ± 31.8 (1.5–125)	16.4 ± 20.8 (1.5–125)	31.3 ± 37.2 (2.1–125)	0.001
Patients previously supplemented with cholecalciferol		57 (45.6%)	99 (63.9%)	0.003

The data were mean ± SD (min–max) or frequency (%).

TABLE 3: Demographic and laboratory results of the patients with and without vitamin D deficiency.

Variable	Patients with vitamin D deficiency (<i>n</i> = 174)	Patients without vitamin D deficiency (<i>n</i> = 106)	<i>P</i>
Age (years)	55.6 ± 18.1 (18–91)	51.5 ± 14.8 (18–79)	0.033
Hospitalization days	7.8 ± 11.7 (0–60)	3.3 ± 5.9 (0–28)	0.001
Blood urea nitrogen (mg/dL)	59.1 ± 33.8 (9–185)	44.6 ± 25.1 (7–185)	<0.001
Serum creatinine (mg/dL)	5.8 ± 3.8 (0.6–17.9)	5.7 ± 3.9 (0.6–14.7)	NS
Serum albumin (mg/dL)	3.2 ± 0.7 (0.5–4.8)	3.6 ± 0.5 (2.4–4.7)	<0.001
Serum calcium (mg/dL)	8.9 ± 0.9	9.2 ± 0.9 (7–12.7)	0.002
Serum phosphorus (mg/dL)	4.8 ± 1.7 (1.9–11.9)	4.5 ± 1.4 (1.9–8.8)	NS
Serum parathyroid hormone (pg/mL)	398.5 ± 408.3 (20.3–2500)	342.2 ± 452.7 (10.9–2500)	0.004
eGFR (mL/min/1.73 m ²)	23.4 ± 32.3 (1.5–125)	26.7 ± 31.0 (2.1–125)	NS
Previously treated with cholecalciferol	83 (47.7%)	73 (68.9%)	0.001

The data were mean ± SD (min–max) or frequency (%).

(4,7 ± 9,2 versus 7,9 ± 11,1 days), and eGFR (15,2 ± 21,1 versus 36,5 ± 38,4 mL/min/1.73 m²) were significantly lower, and serum 25(OH)D (20,2 ± 18,3 versus 11,9 ± 10,8 ng/mL, 50,4±45,7 versus 29,7±26,9 nM/L), creatinine (7,1±3,7 versus 4,3 ± 3,9 mg/dL), calcium (9,4 ± 3,4 versus 8,7 ± 1,1 mg/dL), and PTH (443,9 ± 468,2 versus 289,6 ± 346,5 pg/mL) levels were significantly higher (*P* < 0.05).

Serum 25(OH)D levels were statistically lower in females than males (15,2 ± 16,8 versus 18,2 ± 14,7 ng/mL; 45,4 ± 42 versus 37,9 ± 41,9 nM/L, resp.) (*P* < 0.05). The prevalence of vitamin D deficiency among these 153 women was 69.3% (106 patients) and 43.5% in men (68 patients) (*P* < 0,05).

Of 153 female patients with vitamin D deficiency, 91 (56.8%) and 65 (59.3%) of 127 male patients with vitamin D deficiency had vitamin D supplementation history (*P* > 0,05). The mean duration between recent and preceding measurements of 25(OH)D levels and initiation of vitamin D supplementation was 10.3 ± 3.6 months in female patients and 9.7 ± 4.1 months in male patients (*P* > 0.05). No difference was found between males and females according to

age, BUN, serum albumin, calcium, phosphorus, PTH, eGFR, and hospitalization days (*P* > 0,05). Serum creatinine was lower in female patients (5,3±3,3 versus 6,6±4,6 mg/dL, resp.) (*P* < 0,05).

In logistic regression analysis female gender, not having vitamin D supplementation history, low serum albumin, and low BUN levels were significant independent predictors of vitamin D deficiency, while we were unable to demonstrate any relationship with vitamin D deficiency and presence of diabetes mellitus, serum creatinine, eGFR, being on hemodialysis, and being hospitalized (Table 4).

5. Discussion

Malatya City, which gets lots of sunlight, is at a latitude of 38:21 north, longitude 38:19 east, and an altitude of 998 m [17]. Average rainfall per year is 384,4 mm. However, 62.1% of our patients had vitamin D deficiency, 23.6% vitamin D insufficiency, while 14.3% of the patients had adequate vitamin D stores.

TABLE 4: Logistic regression models for probability of vitamin D deficiency.

Variable	β	S.E.	P	Odds ratio (95.0% C.I)
Gender (1)	0.907	0.277	0.001	0.404 (0.2–0.6)
Age	–0.007	0.009	0.404	0.993 (0.9–1.0)
Albumin	0.892	0.253	<0.001	2.5 (1.5–4.0)
Diabetes (1)	0.319	0.352	0.365	1.376 (0.7–2.7)
Supplemented with vitamin D (1)	1.188	0.329	<0.001	0.305 (1.6–0.6)
eGFR	0.005	0.005	0.309	1.005 (0.9–1.0)
Hospitalization	–0.231	0.322	0.475	1.259 (0.6–2.3)
Put on hemodialysis	0.198	0.409	0.628	1.219 (0.5–2.7)
Constant	–4.488	1.137	<0.001	0.011

$n = 280$. Forward stepwise (likelihood ratio) method; entry criteria = $P < 0.05$.
 β : standard regression coefficient; 25(OH)D, 25-hydroxyvitamin D.

Vitamin D deficiency was reported to be high in the general medical inpatient population. The results of a study reported a 57% prevalence of vitamin D deficiency in 290 patients hospitalized in the general medical wards at Massachusetts General Hospital. Sixty-three percent of the patients studied in March and 49% of those studied in September had serum 25(OH)D concentrations of 15 ng/mL (37.4 nM/L) or less. These authors reported that anticonvulsant-drug therapy, renal dialysis, nephrotic syndrome, and winter season were significantly associated with hypovitaminosis D [18].

Daoudi et al. [19] from Belgium reported that more than 60% of 332 patients hospitalized presented with serum concentrations below the lower limit of normal (12 ng/mL, 29.9 nM/L), whereas less than 5% reached 30 ng/mL (74.9 nM/L), a level generally recommended to avoid vitamin D insufficiency. These authors reported that young patients are not at a lesser risk of vitamin D deficiency than the older patients and women showed a lesser deficiency than men.

We found a higher prevalence of vitamin D deficiency in inpatients (63.9%) than those of outpatients (45.6%) in the nephrology department during early winter season ($P < 0.05$). However, in logistic regression analysis being female gender, not having vitamin D replacement previously, low serum albumin, and low BUN levels were significant independent predictors of vitamin D deficiency, while we were unable to demonstrate any relationship with vitamin D deficiency and presence of diabetes mellitus, serum creatinine, eGFR, and being hospitalized.

In our department we follow a vitamin D supplementation protocol which is similar to that recommended by K/DOQI guidelines [16] except cholecalciferol usage. If serum level of 25(OH)D <5 ng/mL (12.5 nM/L), we prescribe 50000 IU of vitamin D₃ (cholecalciferol)/week orally/12 weeks and then monthly for six months. If serum level of 25(OH)D is between 5 and 15 ng/mL (12.5–37.4 nM/L), we prescribe 50000 IU of vitamin D₃ (cholecalciferol)/weekly orally/4 weeks and then monthly for six months.

Of all patients, 55.7% were supplemented with vitamin D previously during followup in our nephrology unit. Vitamin D supplementation history was more common in outpatients.

Vitamin D deficiency was less common in patients supplemented with cholecalciferol previously; even vitamin D deficiency was also found among patients supplemented with cholecalciferol during followup. However, we do not have any information on compliance with vitamin D supplements.

In a retrospective study of 88 patients with CKD stages 1–5 and baseline 25-hydroxyvitamin D level <30 ng/mL (<75 nM/L) performed by Qunibi et al. [20] treatment with ergocalciferol as recommended by K/DOQI guidelines managed to achieve in only 25% \geq or =30 ng/mL (75 nmol/L). These authors reported that current K/DOQI guidelines are inadequate for correcting vitamin D deficiency in CKD patients and recommended to monitor serum 25(OH)D levels regularly and to give appropriate vitamin D supplementation in order to achieve normal vitamin D status. It seems that our findings are consistent with those of Qunibi et al.

In our study during fall vitamin D deficiency was more common among women (69.3% versus 43.5%); even percentage of the women supplemented with cholecalciferol previously was similar to that of men. In another a cross-sectional study from Turkey Hatun et al. [21] demonstrated that vitamin D insufficiency (43.8%) and deficiency (21%) are common among Turkish adolescent girls at the end of the winter (in April). This finding is more striking in girls who wear concealing clothing, and they did not improve significantly during the summer, whereas the vitamin D status of girls in the other groups did.

In Turkey, the main source of vitamin D is cutaneous synthesis because there is no food fortification with vitamin D, and supplementation of vitamin D is not a routine practice. Veiling or staying indoors is common in our female patients, although we did not have any information about these in this study. It is possible that these women benefited less from the decreased sunlight through the fall months than men do, possibly by exposing their skin less to the sun.

Our study has several limitations. Firstly, this study suffers from all the limitations of retrospective observational studies that rely on existent databases, and the information was collected only at one point in time. Secondly, we have no information about compliance with vitamin D supplements. However, we had some information about common practice and results.

As a conclusion, vitamin D deficiency seems to be an important problem in both inpatients and outpatients of nephrology in Turkey. Women are at more risk of deficiency and may therefore need to consume higher doses of the vitamin D. Monitoring serum 25(OH)D levels regularly and replacement of vitamin D are important. A governmental mandate about the supplementation of foods with vitamin D is urgently needed in Turkey.

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