25-OH-Vitamin D Levels in the Nephrotic Syndrome

H SCHMIDT-GAYK, E RITZ, W SCHMITT, Chr GRAWUNDER, G MASCHIO*, W SCHULTZ†
Ludolf-Krehl-Klinik, Heidelberg, GFR, *Division of Nephrology, Verona, Italy, and †4 Medizinische Klinik, Nurnberg, GFR

Summary

25-hydroxy Vitamin D (25-OH-D) levels were measured in 32 patients with the nephrotic syndrome without renal insufficiency (urinary protein > 3.5 g/24 h; GFR > 80 ml/min/1.73 m²). 25-OH-D levels in the serum were 20.1 ± 10.9 nM (normal range 25–150). Serum iPTH was in the upper normal range (25.9 ± 13.8 pM; normal range 2–30) as was urinary cAMP (4.1 ± 1.8 μM/g creatinine; normal range 2.1–4.7).

The study documents an acquired deficiency of circulating total 25-OH-D in patients with the nephrotic syndrome in the presence of a normal GFR, normal dietary intake and normal exposure to sunlight. Increased renal loss of the low molecular weight binding protein is suggested as the cause of low circulating 25-OH-D levels. The serum concentration of free unbound 25-OH-D is unknown. Although overt osteomalacia is absent, stimulated PTH secretion (secondary hyperparathyroidism) is compatible with borderline vitamin D deficiency in these patients.

Introduction

In patients with the nephrotic syndrome (NS), intestinal malabsorption of Ca (Emerson & Beckmann, 1945; Jones et al, 1967) and hypocalciuria (Boyd et al, 1926; Lichtwitz et al, 1960) are well known. In addition, Bonucci found disturbed primary mineralisation of lamellar bone in patients with the NS in the presence of a normal GFR (personal communication).

We speculated that in the NS, vitamin D depletion secondary to renal loss of
protein bound 25-hydroxy-vitamin D (25-OH-D) might occur. The present investigation was designed to test this hypothesis.

PATIENTS AND METHODS

Thirty-two patients with NS without renal insufficiency (urinary protein excretion > 3.5 g/24 h; GFR > 80 ml Ccr/min/1.73 m²) were studied. The age of the patients was 39.5 ± 14.2 years (range 14–74 y); 24 males; 8 females.

None of the patients received steroid therapy. All patients were on a high protein diet and had normal sun exposure as ascertained by questioning.

Serum chemistry was measured with the SMA 12 Technicon Autoanalyzer. Urinary protein was measured by the Biuret Method. iPTH was measured by RIA using a carboxyterminal antibody (A VI 2) with the procedure of Arnaud et al (1971); urinary cAMP was measured by the method after Tovey et al (1974).


RESULTS

Serum and urinary chemistry of the patients is given in the table on the following page. Serum 25-OH-D levels in patients with the NS, as compared with seasonally

![Graph showing 25-OH-D nmol/l over time]

Figure 1. Markedly lower 25-OH-D levels in patients with the nephrotic syndrome. The area enclosed by the two lines represents the normal range. The one patient in the upper normal range (month of February) underwent a remission under steroid therapy (daily protein excretion at the time of the study 4.5 g/24 h)
<table>
<thead>
<tr>
<th></th>
<th>250Hvit.D (nM)*</th>
<th>Serum protein (g/100 ml)</th>
<th>Serum alb. (g/100 ml)</th>
<th>Serum creat. (mg/100 ml)</th>
<th>Serum Ca (mEq/l)</th>
<th>iPTH (pM)†</th>
<th>Urin. prot. (g/24 h)</th>
<th>Urin. cAMP (µM/g creat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nephrotic syndrome (n = 32)</td>
<td>20.1 ± 10.9</td>
<td>5.2 ± 0.99</td>
<td>2.26 ± 0.85</td>
<td>1.1 ± 0.23</td>
<td>4.09 ± 0.28</td>
<td>25.9 ± 13.8</td>
<td>9.83 ± 7.11</td>
<td>4.1 ± 1.8</td>
</tr>
<tr>
<td>controls (normal range)</td>
<td>25–150</td>
<td>6.5–8.0</td>
<td>3.5–4.6</td>
<td>0.7–1.4</td>
<td>4.5–5.1</td>
<td>2–30</td>
<td>0.15</td>
<td>2.1–4.7</td>
</tr>
</tbody>
</table>

* Normal range depends on season, see Figure 1
† Purely C-terminal antibody (A VI 2)
Figure 2. Binding of $^3$H-25-OH-D to urinary proteins. Serial urinary dilutions (abscissa) were incubated with 1 picomole $^3$H-25-OH-D (specific activity 10.7 Ci/mmol). The bound fraction (separation on charcoal) is given on the ordinate.

Adjusted values in normal individuals with normal sun exposure, are shown in Figure 1. The lowering of serum 25-OH-D levels was accompanied by the appearance in the urine of 25-OH-D binding activity as shown in Figure 2.

DISCUSSION

The normal range of 25-OH-D levels found in our laboratories agrees well with that reported by other groups in Europe (Bec et al, 1971; McLaughlin et al, 1974). There was a clear diminution of serum 25-OH-D levels in patients with NS which reverted rapidly to normal after cessation of macroproteinuria. The relative decrease in serum 25-OH-D in patients with the NS was greatest during
summer months (Figure 1) and occurred in the presence of a normal GFR, normal dietary intake and normal exposure to sunlight. The assay for 25-OH-D, using rat serum as the binding protein, fails to distinguish between 25-OH-vitamin D₂ and 25-OH-vitamin D₃; therefore, both actinic (vitamin D₃) and nutritional (vitamin D₂ + vitamin D₃) vitamin D supply are reflected by the measured values.

Since concomitantly 25-OH-D binding activity appeared in the urine (Figure 2) we suggest renal loss of the low molecular weight binding protein (Edelstein et al, 1973) as the cause of low circulating 25-OH-D levels.

It is unknown whether the decrease of total 25-OH-D is accompanied by a decrease of free (i.e. non-protein bound) 25-OH-D and a concomitant failure of vitamin D action on target organs. However, the latter is suggested, by the findings of hypocalciuria, reduced intestinal absorption of calcium and defective mineralisation (demonstrable by electron microscopy).

Borderline vitamin D deficiency, albeit in the absence of overt clinical osteomalacia, might also account for the slight but definite secondary hyperparathyroidism suggested by the serum PTH and urinary cAMP measurements.

Acknowledgments

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Open Discussion

BONE (Liverpool) Did you measure the urinary excretion or the loss in the urine of 25-hydroxy D itself in addition to the binding protein?

RITZ Yes, we did. As a matter of fact in patients with a nephrotic syndrome without vitamin D supplementation the excretion of 25 was below the limit of detectability with our method. This is hardly surprising since serum levels are rather low; when you administer vitamin D in a dose of 50,000 international units per day there is clearly a loss of 25-hydroxy vitamin D in the urine.

MALLICK (Manchester) We documented, about ten years ago, the hypercalciuria of the nephrotic syndrome and this is extreme. There is often very little calcium in the urine of such patients even with a normal GFR. In patients with minimal change nephropathy, the change can be very dramatic with treatment; the excretion of calcium is very soon normal. Do you think from your results, you have completely explained this phenomenon, because there is only a relative drop in the hydroxy D levels in your cases?

RITZ I think clinical data cannot answer unequivocally the question of whether there is borderline vitamin D deficiency or not. This has to be done with animal experiments and this is currently being done in collaboration with Dr Brunner. I think there are two different ways to assess the vitamin D status of these animals; first measuring intestinal absorption of calcium and the calcium-binding protein, which is currently carried out by Dr Brunner with our animals, and second, measuring the response to exogenous bovine PTH, but I do not have the data.

MALLICK (Manchester) Yes I understand your point, but the hypocalciuria is so extreme, so dramatic and acute and reverses so quickly, do you think that your findings could really explain such dramatic change?

RITZ I hesitate to speculate in the absence of data, but certainly there are many other mechanisms that might contribute or be responsible for hypocalciuria in the nephrotic syndromes, such as changes of fractional reabsorption in the proximal tubules, etc.

COBURN (Los Angeles) Both Haddad of St Louis and Goodman of New York described at the Atlantic City Meetings characterisation of the vitamin D and 25-OH-D binding plasma protein; it is a globulin of substantial molecular weight. Also, it is present in large excess, being normally only 3–5% saturated. It would be surprising if urinary losses of this in nephrosis could account for significant depression of vitamin D binding protein. One might suggest altered synthesis rather than urinary losses. Was any correlation found with selectivity of proteinuria and the degree of suppression of plasma 25-OH-D levels?

RITZ These observations on low serum levels, and increased binding activity in the urine apply both to patients with minimal change glomerulonephritis and the patients with proliferative glomerulonephritis. That is to say both in the presence of a selective and non-selective proteinuria.

PARSONS (London) Did you correlate the serum cholesterol, which is raised in
many of these patients, with your vitamin D levels? There is a suggestion that in some varieties of the nephrotic syndrome the hepatic dysfunction resulting in the high plasma cholesterol may also overlap and produce alterations in vitamin D turnover.

RITZ No, we have not studied this specifically; the idea did not occur to us. Thank you for the suggestion.