Measurement of 1,25(OH)₂D₃ in serum has been laborious and time consuming. In most assays a preparative chromatographic procedure is used, followed by HPLC, before radioimmunoassay. Hollis (3) reported a method requiring a single preparative chromatographic procedure (C₁₈OH column). We measured 1,25(OH)₂D₃ in serum from normal adults and adult patients with functioning kidney grafts, using a commercial kit (Nichols Institute, cat. no. 40-2201) based on this procedure. The manufacturer’s instructions were rigorously followed.

Subjects studied were as follows: Group 1) 20 healthy volunteers (10 men, 10 women) without renal, skeletal, or hepatic diseases and with a normal biochemical profile. Group 2) 34 renal-transplant subjects, ages 19–59 years (mean 41), 20 men and 14 women. The follow-up interval after transplantation ranged from one to 60 months (mean, 12). For post-transplant immunosuppression, prednisone combined with cyclosporine was used. None had taken vitamin D compounds since the renal transplantation.

Serum creatinine values ranged from 76 to 418 µmol/L (mean 159, SD 73, normal range 50–120). Serum calcium ranged from 2.2 to 2.9 mmol/L (mean 2.5, SD 0.2, normal range 2.2–2.6). Serum phosphate ranged from 0.6 to 1.6 mmol/L (mean 1.1, SD 0.2, normal range 0.8–1.3). Serum alkaline phosphatase ranged from 1.3 to 23.6 µkat/L (mean 1.8, SD 4.2, normal range 0.7–1.8). Values for intact parathyrin in serum ranged from 1.9 to 38 pmol/L (mean 10, SD 8.8, normal range 1.1–5). Only 16 patients (47%) had intact parathyrin values within the normal range (hyperfunction of the parathyroid gland may persist indefinitely after a successful kidney transplant (4)).

Extraction efficiency varied between samples; mean recovery was 62.7% (range 47–74%). Figure 1 shows individual serum 1,25(OH)₂D₃ concentrations. The mean circulating 1,25(OH)₂D₃ for the patients was not significantly different from that in normal subjects (36.5 vs 41), but the range was considerably wider in the transplanted patients, in agreement with a previous report (2).

We found a positive correlation between serum 1,25(OH)₂D₃ and both months after renal transplantation ($r = 0.45, P <0.01, n = 34$) and alkaline phosphatase ($r = 0.47, P <0.01, n = 34$); a negative correlation between serum 1,25(OH)₂D₃ and serum creatinine ($r = -0.48, P <0.01, n = 34$); and no correlation between serum 1,25(OH)₂D₃ and parathyrin, calcium, or phosphate in serum.

Evidently this procedure is easier to perform and less time consuming than are elaborate HPLC methods, and it appears to be sufficiently accurate for evaluating circulating 1,25(OH)₂D₃ in normal subjects and in patients with altered mineral metabolism.

References


Monoclonal Gammopathies as a Cause of High Serum Laminin Concentrations, Juergen Kropf, Manfred Lammers, and Axel M. Greßner (Dept. of Clin. Chem. and Central Lab., Klinikum der Phillipps Universität, Baldingerstrasse, D-3550 Marburg, F.R.G.)

Concentrations of laminin, a main constituent of basement membranes, in serum are significantly increased in various diseases: chronic active liver disease (1), rheumatoid arthritis (2), hyperthyroidism (3), diabetes (4), and tumors (5). We now report increased concentrations of laminin in sera of patients with monoclonal gammopathies.

Laminin was determined in serum by a radioimmunoassay, kindly provided by Dr. Strecker (Hoechst AG/Behringwerke AG, Frankfurt, F.R.G.), the analytical criteria of which have been reported (2). Monoclonal gammopathies were identified by immunofixation electrophoresis. Immunoglobulins G, A, and M were measured and immunoglobulin light-chain composition was determined by using automated fixed-time immunonephelometry (Behring Nephelometer System; Behringwerke AG, Marburg, F.R.G.). For statistical evaluation, we used the Mann–Whitney test for comparison of means and the Spearman rank correlation coefficient.

From the patients with monoclonal gammopathies, those with serum creatinine concentrations above the upper reference limit (106 µmol/L) were excluded, because patients with renal insufficiency may show increased concentrations of laminin in serum (4). The remaining 54 patients with monoclonal gammopathies showed significantly increased concentrations (1.51 ± 0.42 kU/L, $P <0.001$) of laminin in serum as compared with a reference population of healthy blood donors (1.06 ± 0.15 kU/L, $n = 146$). This was most pronounced for IgG gammopathies ($n = 30, 19$ of them type kappa) followed by IgA gammopathies ($n = 16, nine$ type kappa), whereas the increase for IgM gammopathies ($n = 8, seven$ type kappa) was not statistically significant (Figure 1). Assuming an upper reference limit of 1.45 kU/L (1), the fractions of patients with increased laminin concentrations were 0.72 for IgG, 0.31 for IgA, and 0.25 for IgM gammopathies, respectively. There was no difference between laminin concentrations of type kappa and lambda gammopathies.

In the group of IgG gammopathies, a significant positive
correlation between the concentrations of IgG and laminin was observed ($r = 0.61, P < 0.001$).

Increased laminin concentrations in monoclonal gammopathies might be directly caused by the proliferating plasma cells or by delayed elimination from the circulation—maybe owing to association with the immunoglobulins—but our results provide no conclusive pathophysiological explanation. A possible but hypothetical explanation would be that an unspecific and relatively weak influence on connective-tissue metabolism accompanies different forms of diseases, whereas stronger and more specific effects are operational in diseases with direct involvement of connective tissue, e.g., liver cirrhosis.

**References**


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**Simpler Measurement of Caffeine and Paraxanthine in Saliva, N. R. Badcock (Dept. of Chem. Pathol., The Adelaide Children's Hospital, 72 King William Road, North Adelaide 5006, South Australia)**

Caffeine concentrations in serum and saliva correlate well (1). Various analytical approaches have been described for measuring caffeine and paraxanthine in serum, but no rapid, accurate, and inexpensive method, adaptable to automation, has yet been reported for saliva. The following cost-effective, quantitative micro-scale assay was developed for a study examining the association between caffeine and symptomatology, involving several thousand saliva samples.

Vortex-mix patients' saliva or caffeine/paraxanthine-supplemented saliva and filter the suspension through a sintered-glass funnel (Pyrex Size 0, 161- to 250-µm pore size). Vortex-mix 100 µL of the filtrate with an equal volume of methanol containing 7-(2-hydroxyethyl)theophylline, 11 µmol/L, as the internal standard, in a 0.5-mL polypropylene microfuge tube. Centrifuge (2000 × g, 2 min) and transfer an aliquot of supernate to an auto-sampler vial for liquid-chromatographic analysis on a reversed-phase Ultrasphere ODS 50 × 4.6 mm (i.d.) analytical column, average particle size 5-µm, with a 45 × 4.6 mm (i.d.) guard column containing the same material (pre-column and analytical column; Beckman Instruments, Berkeley, CA 94710). Elute the injected 100-µL samples isocratically with sodium acetate buffer (10 mmol/L, adjusted to pH 5.5/methanol/tetrahydrofuran (90/10, v/v) at a constant flow rate of 2.0 mL/min. Monitor caffeine and paraxanthine at their maximum absorbances, 273 nm and 269 nm, respectively, with a Model 165 variable-wavelength, dual-channel ultraviolet detector (Beckman Instruments). Under the above conditions, the retention time for paraxanthine is 3.5 min, for the internal standard 4.1 min, and for caffeine 5.6 min. Use peak-height ratios to prepare the standard curve and calculate concentrations. After each day's run of approximately 100 assays, wash the column with methanol/dimethyl sulfoxide (90/10, by vol), then store it overnight in methanol.

The detection limit, defined as twice the height of the noise level, is 0.5 µmol/L for both analytes. Concentration and peak height are linearly related throughout the concentration range measured, 0.5–150 µmol/L. For concentrations of 5 µmol/L in saliva, the within-day and day-to-day CVs were 2.9% and 4.1% for caffeine and 3.7% and 4.2% for paraxanthine, respectively. Analytical recovery of caffeine and paraxanthine added to saliva to give concentrations of 1, 10, and 50 µmol/L was calculated by comparison with methanol solutions of caffeine, paraxanthine, and internal standard of equivalent concentrations; absolute recoveries ranged from 95% to 102%. Other xanthine derivatives, including theophylline, did not interfere.

Filtration was used in preference to centrifugation because, in the initial work-up of the method, I found that concentrations of caffeine and paraxanthine were diminished when saliva was centrifuged, presumably through their binding to co-precipitated protein. Injection of untreated saliva, on the other hand, caused column blockages, making it unsatisfactory for automation. I precipitated protein with methanol, because denaturation with acetonitrile or trichloroacetic acid produced considerably distorted and broad xanthine peaks.

**Reference**


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**A Kinetic Method for Determination of Serum Ceruloplasmin, H. Mukerjee (Dept. of Pathology, Bellevue Hospital, First Avenue & 27th Street, New York, NY 10016)**

Ceruloplasmin, a good marker of Wilson's disease, might also be useful for monitoring fetal development in high-risk