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Determination of the Calcium Binding Capacity to Discriminate Between Urines of Calcium Stone Formers and Healthy Persons

H. Füredi-Milhofer[1], K. Kiss[2], F. Kahana[3] and S. Sarig[3]

[1] "Rudjer Boskovic" Institute, Zagreb, Croatia, Yugoslavia, [2] Akzo, Chemical Company, Dobbs Ferry, New York, USA, [3] Casali Institute of Applied Chemistry, The Hebrew University of Jerusalem, 91904, Jerusalem, Israel

Summary. Calcium binding by undiluted fasting urine has been tested as a means of demonstrating the capacity of urine from control subjects and calcium stone patients to hold spontaneous precipitation of stone forming compounds. Preliminary data are promising with respect to the possibility that controls and patients can be separated.

The causes of kidney stone disease are multiple complex events but the outcome is simply the inability of the patients urine to prevent stone formation. Thus in determining the risk of stone formation one needs to take into account the overall inhibitory potential of urine.

Some tests which were proposed to this effect measure the growth rate of calcium phosphate seed crystals in diluted urine (1) or the amount of seed crystals needed to produce a certain amount of precipitate (2). Another method is based on the measurement of the growth rate of calcium oxalate seed crystals incorporated in a gel matrix to which artificial or native urine has been added (3). Some of the above tests are tedious to perform or use expensive equipment. Another test to discriminate between normals and calcium oxalate stone formers (DI method) has been devised in the Casali Institute laboratory (4, 5). It is based on the determination of a discrimination index (DI) which is related to the initial rate of precipitation of calcium oxalate from a solution which contains the urine under investigation. The DI test has been recommended as a diagnostic tool and has also been successfully used in follow-up studies of the efficacy of therapeutic treatment (6). The main limitation of this test is that it is carried out in diluted (10%) urine. In the present work a new method to discriminate between urines of stone formers and healthy individuals, based on the determination of the calcium binding capacity of whole urine, has been proposed. For this purpose a new selective membrane electrode which measures calcium in whole urine has been introduced and tested.

Experimental

All experiments were performed at constant, physiological temperature (37 ± 0.5 °C).

The capacity of whole urine to "bind" calcium was assessed in first morning urine, which was free of infection (pH < 6). To that effect the urine was titrated with a calcium chloride solution, using a new PVC-matrix calcium selective electrode. The membrane of the selectrode was prepared in the laboratory and renewed when indicated by the decline of the calibration line (7).

Generally a linear relationship between total added calcium and the Ca^{2+} concentration was obtained up to a point where precipitation of calcium salts commenced. The slope and intercept of the titration line were determined by linear regression of values obtained by at least five consecutive measurements and the slope was used as a criterion for the calcium binding capacity (CBC) of the respective urine. For comparison the previously described DI test (4) was performed in diluted (10%) urine using an Orion calcium selective electrode.

Results and Discussion

Apart from acting as crystallisation inhibitors, urinary macromolecules also bind calcium ions, thus in effect lowering the supersaturation of urine with respect to calcium salts. The "calcium binding capacity" of urine, CBC, is reflected in the slope of the titration line, i.e. the slope should be inversely proportional to CBC while the intercept should coincide with the initially measured concentration of free calcium ions, c $(Ca^{2+})_i$.

The linear response of the new calcium selectrode is demonstrated in Fig. 1 in which lines obtained by titration of a urine donated by a diagnosed kidney stone patient (line 1) and a urine specimen obtained from a healthy donor (line 2) are compared. As expected the slope of line 1 (0.87) is appreciably higher than that of line 2 (0.32) indicating a lower CBC of the patient's urine. The intercepts of both titration lines coincided within 10% with the respective value for c $(Ca^{2+})_i$. The corresponding DI values (4) were 1.0 for patient and 0.08 for the control, respectively.

For statistical evaluation 26 uninfected urines of patients with diagnosed kidney stone and 13 urines of donors with no known history of kidney stone disease and at present on no medication (healthy donors) have been analysed by the new method and their DI has also been determined. All the patient's urines had DI > 0.9 while the healthy controls had DI < 0.6. A two sample t-test with unequal variances gave mean values of the slopes of the titration lines as 0.31 for healthy and 0.64 for patients, respectively with a p-value < 0.001. Individual 99% confidence intervals were (0.21, 040) for the controls and (0.54, 0.73) for the patients respectively. Apparently good separation between the urines of patients and healthy controls has been achieved.

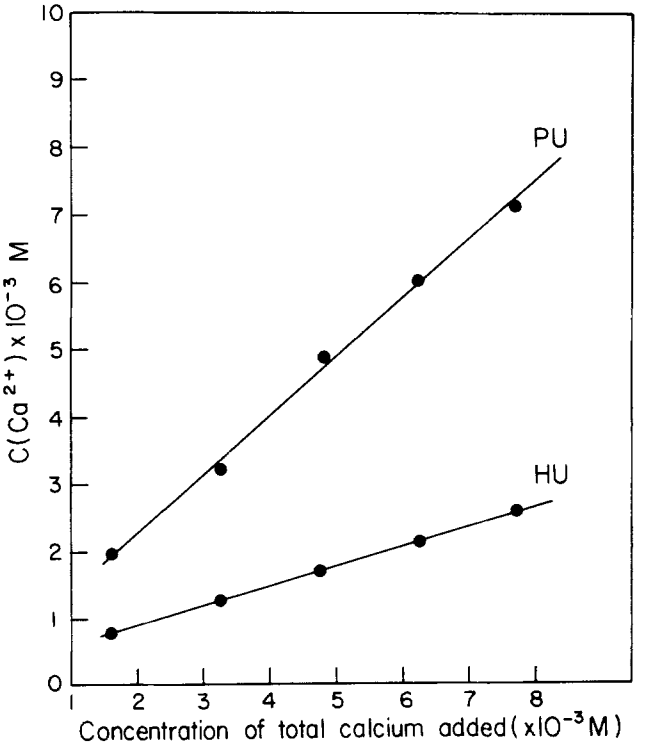

Fig. 1: Titration lines obtained from the urine of a diagnosed kidney stone patient (PU) and from urine donated by a healthy person (HU). Titrated with 0.1 M $CaCl_2$ solution, temperature 37 ± 0.5 °C.

The above results indicate that the slope of the line obtained by titration of a donor's first morning urine may be a reliable criterion for discrimination of calcium stone-formers from healthy people. The tests show good correlation with the clinical situation and with the previously developed D.I. test (4). Because of its simplicity the new method seems to be suitable for routine examinations in clinical laboratories. Pilot experiments to ascertain its value for routine tests are being considered.

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Prof. H. Füredi-Milhofer,
"Rudjer Boskovic" Institute,
Zagreb, Croatia, Yugoslavia

Importance of the Mineral Metabolic Study and Calculi Analysis in Urolithiasis Patients

L. Rodriguez Vela, C. Rioja Sanz, J. M. Ibarz Navarro, J. Lazaro Castillo, T. Abadia Bayona and L. A. Rioja Sanz

Dept. Urology, "Miguel Servet" Hospital, Zaragoza, Spain

We have performed a metabolic mineral study in 1600 urolithiasis patients. We did a complete blood, 24 hours and 2 hours urine analysis. Indeed with the microscope stereoscopic lens and infrared spectrophotometry we study the gravels and calculi eliminated by these patients. 1436 patients completed properly the protocol.

Overall, 647 patients (45.1 %) showed changes in calcium-oxalate metabolism. In 582 (40.1 %) uric acid metabolism changes were detected. 251 (17.5 %) had alkalinuria and 4 cystinuria. The mineral metabolism was normal in 173 (12.1 %). The chemical composition of the calculi were: Calcium oxalate and phosphate + calcium oxalate (68.2 %), uric acid 12.6 %, struvite 14.1 %, calcium phosphate 5.1 % and cystine 0.3 %. From the 974 patients (67.8 %) with calcium-oxalate calculi, 686 (70.4 %) had metabolic anomalies. Upon then the most frequent were: hypercalciuria (29.7 %), hyperuricosuria and/or hyperuricemia (18.8 %), hyperoxaluria (11.4 %), hypercalcemia with secundary hypercalciuria (2.7 %). In this group, 288 (29.6 %) no changes were detected.

From the 182 patients (12.7 %) with uric acid calculi: 15.4 %