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# Serum and Urinary Hydroxyproline in Patients with Severe Chronic Renal Failure

Hydroksyprolina w surowicy krwi i w moczu pacjentów z ciężką niewydolnością nerek

Гидроксипролин в сыворотке крови и в моче больных тяжелой почечной недостаточностью

Disturbances of calcium and phosphate metabolism is a common consequence of chronic renal failure. The primary pathogenic mechanisms may include retention of inorganic phosphate, impaired synthesis of the active vitamine D metabolite and diminished intestinal calcium absorption (1). Increased concentration of calcitonin in the blood is also discussed (3). These abnormalities lead to decreased serum calcium concentration which results in increased parathyroid hormone secretion and secondary hyperparathyroidism development (10). Although in chronic renal failure a skeletal resistance to PTH may be also observed (9), finally in most of cases these disturbances become a cause of the renal osteodystrophy (2).

The bone osteodystrophy in chronic renal failure may have different histopathological forms, however, one should suppose that besides mineral disturbance some significant changes in the organic matrix of the bone may also occur. Since the main constituent of this matrix is the bone collagen, the purpose of this study was to evaluate the relationship between renal failure, calcium and phosphate metabolism disturbances and the collagen catabolism determined through serum hydroxyproline concentration and urinary hydroxyproline excretion.

## MATERIAL AND METHODS

Patients: 15 healthy adults (8 males, 7 females) aged 19—68 served as a control. They did not have any mineral metabolism disturbances and their creatinine clearance was above 80 ml/min/1.73 $m^2$. The group of patients with chronic renal failure consisted of 23 persons (15 males, 8 females) aged 38—75 years. The duration of disease was 6.2 ±1.8 years and the mean creatinine clearance was 22 ±12 ml/min/1.73 $m^2$. All investigated persons were on the normal hospital diet with about 1 g of protein per kg of the body weight daily, from which obvious sources of gelatin were excluded. Blood samples were drawn in the morning after overnight fast. The 24-hours urine was collected with a few drops of toluene as preservative.

Analytical methods: Calcium, phosphate and creatinine were determined using standardized laboratory procedures. The serum level of calcium was corrected for individual variation in protein concentration, to a serum protein level of 70 g/l. The endogenous creatinine clearance was calculated from 24-hours urine creatinine and serum creatinine concentrations and corrected for the body surface. Alkaline phosphatase activity was measured by Bassey, Lowry and Brock method using Smith Kline Instr. reagents, according to the manufacturer instruction.

The total, protein-bound and free or peptide hydroxyproline in the serum, as well as the non-protein-bound hydroxyproline in urine were determined after acid hydrolysis by modified Prockop and Udenfriend method (13). In the cases of proteinuria, the protein was precipitated by solid sulphosalicylic acid (50 mg/ml) and discarded prior to hydrolysis.

## RESULTS

The mean serum creatinine, phosphate, calcium and protein-bound and free or peptide hydroxyproline concentration as well as alkaline phosphatase activity in control group and patients with severe chronic renal failure are presented in Table 1. As could be expected, the much higher creatinine concentration and the alkaline phosphatase activity in patients than in the control group, reflects both the severity of renal insufficiency and its osteodystrophic complications. However, unexpectedly, the phosphate concentration did not differ significantly whereas calcium in the patients sera was even higher. The both forms of serum hydroxyproline were considerably elevated in the patients with chronic renal failure.

Table 2 compares the mean values of creatinine, phosphate, calcium and non-protein-bound hydroxyproline excretion in urine of control and the patients groups. In comparison with the control group, in patients with severe renal failure a significant increase of calcium and decrease of phosphate and hydroxyproline urinary excretion are observed.

The phosphate, calcium and non-protein-bound hydroxyproline clearances, corrected to a standard body surface in both investigated

groups are presented in Table 3. It is worth notifying that in the patients group the most significant was about fourfold decrease of creatinine clearance and about threefold that of hydroxyproline clearance, in comparison with the control group. Decrease of the phosphate clearance has been also observed.

The relationship between serum concentration and urine excretion of three investigated biochemical parameters are presented in Figs. 1—3. No significant correlation was found between the phosphate concentration and excretion in both the control and patients groups. In the healthy

Table 1. Concentration of creatinine, phosphate, calcium and hydroxyproline and alkaline phosphatase activity in sera of control group and patients with renal failure (mean ±SD)

| | Control group $n=15$ | Patients group $n=23$ | Significance |
|---|---|---|---|
| Creatinine (μmol/l) | 90.20 ±14.10 | 565.70 ±442.00 | $p<0.001$ |
| Phosphate (mmol/l) | 1.38 ±0.11 | 1.36 ±0.48 | ns |
| Calcium (mmol/l) | 2.44 ±0.15 | 2.80 ±0.54 | $p<0.01$ |
| Protein-bound hydroxyproline (μmol/l) | 7.29 ±0.97 | 8.94 ±1.41 | $p<0.001$ |
| Free and peptide hydroxyproline (μmol/l) | 1.79 ±0.40 | 2.78 ±0.81 | $p<0.001$ |
| Alkaline phosphatase (U/l) | 55.40 ±13.50 | 112.80 ±43.20 | $p<0.001$ |

Table 2. Excretion of creatinine, phosphate, calcium and hydroxyproline in urine of the control group and patients with chronic renal failure (mean ±SD)

| | Control group $n=15$ | Patients group $n=23$ | Significance |
|---|---|---|---|
| Creatinine (mmol/24 h) | 12.01 ±2.34 | 12.74 ±4.46 | ns |
| Phosphate (mmol/24 h) | 28.09 ±4.56 | 19.08 ±5.49 | $p<0.001$ |
| Calcium (mmol/24 h) | 5.16 ±0.87 | 6.54 ±1.30 | $p<0.001$ |
| Free and peptide hydroxyproline (μmol/24 h) | 284.40 ±101.40 | 146.40 ±51.10 | $p<0.001$ |

Table 3. Clearance of creatinine, phosphate, calcium and non-protein-bound hydroxyproline in the control group and patients with chronic renal failure (mean SD)

| | Control group $n=15$ | Patients group $n=23$ | Significance |
|---|---|---|---|
| Creatinine ml/min/1.73 m² | 93.10 ±16.40 | 22.00 ±11.80 | $p<0.001$ |
| Phosphate ml/min/1.73 m² | 14.50 ±2.70 | 10.50 ±3.80 | $p<0.005$ |
| Calcium ml/min/1.73 m² | 1.51 ±0.21 | 1.68 ±0.43 | ns |
| Hydroxyproline ml/min/1.73 m² | 11.10 ±2.80 | 4.00 ±1.70 | $p<0.001$ |

subjects a statistically significant correlation between calcium concentration and calcium urinary excretion was observed ($r=0.608$, $p<0.01$). Such dependency has not been found in patients group. The similar results were observed concerning free and peptide hydroxyproline. In the control group the correlation coefficient was 0.721 ($p<0.005$), whereas in the patients group no correlation was found ($r=0.009$).

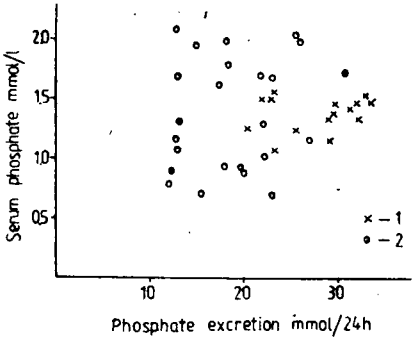


Fig. 1. Relationship between serum phosphate level and urinary phosphate excretion in: 1 — healthy persons, 2 — patients with chronic renal failure

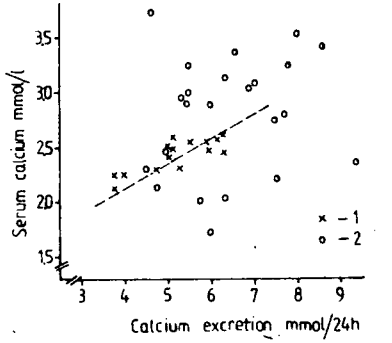


Fig. 2. Relationship between serum calcium concentration and urinary calcium excretion in: 1 — healthy persons, 2 — patients with chronic renal failure

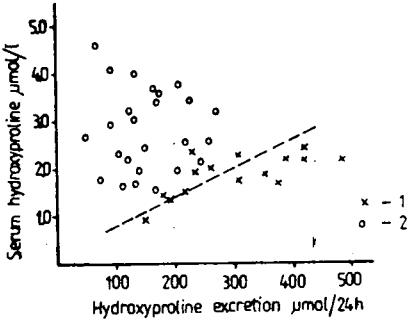


Fig. 3. Relationship between serum free and peptide hydroxyproline level and non-protein-bound hydroxyproline excretion in urine of: 1 — healthy persons, 2 — patients with chronic renal failure

## DISCUSSION

It is well accepted that the decreased level of serum calcium in chronic renal failure is the cause of secondary hyperparathyroidism and further osteodystrophy (7). Although in our patients both the clinical symptoms and biochemical findings indicate the secondary bone resorption the mean calcium concentration was even higher than in the control group. Only 5 of 23 patients had significantly decreased calcium level.

Taking into account severity and duration of the disease it seems that in the most cases, the cause of normo- or hypercalcemia may be fixed, autonomic hyperparathyroidism independent of the serum calcium level developed as an effect of the earlier mineral metabolism disturbances. The same observation in severe renal failure and in uremic patients has been done by Manitius and Rudowski (8) and others (4).

The increased parathyroid hormone secretion in secondary hyperparathyroidism leads to elevated resorption of both mineral and organic phase of the bone (5). Because the main component of the organic bone matrix is collagen, the increased serum non-protein-bound hydroxyproline in the patients group seems to be the result of this resorption. In comparison with the healthy group the mean free and peptide-bound hydroxyproline concentration was over 50% higher. It is worth emphasizing that the protein-bound hydroxyproline in the patients serum was also higher than in the control group. This is in agreement with our previous observation concerning the patients undergoing the peritoneal dialysis (6). Since the protein-bound hydroxyproline seems to be a constituent of such proteins as C1q subcomponent and IgM (14), it is possible that its increased concentration reflects some immunological disturbances in the course of chronic renal insufficiency.

Urinary hydroxyproline excretion is considered as index of the collagen metabolism (11). Our results clearly show that in normal conditions there is a significant correlation between non-protein-bound serum hydroxyproline and urinary hydroxyproline excretion. In patients with severe chronic renal failure not only the lack of this correlation but also a significant decrease of urinary hydroxyproline were observed. This last finding might be due to the considerable reduction of glomerular filtration in the examined patients.

There is a question arising whether the diminished hydroxyproline excretion may be the cause of increased nonprotein-bound hydroxyproline in serum of our patients. Nevertheless it is well known that only about 10% of the hydroxyproline derived from the collagen catabolism is eliminated through the kidney (12). Thus the high concentration of free and peptide serum hydroxyproline in chronic renal failure seems to be mainly the consequence of elevated bone collagen catabolism.

It also seems that increased serum non-protein-bound hydroxyproline is besides alkaline phosphatase activity a good index of osteodystrophic disturbances, whereas the urinary hydroxyproline excretion is of little diagnostic importance in patients with renal insufficiency.

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## STRESZCZENIE

W surowicy krwi 23 pacjentów z ciężką niewydolnością nerek oznaczano stężenie wapnia, fosforanów, hydroksyproliny białkowej oraz wolnej i peptydowej, a także aktywność fosfatazy zasadowej. Oznaczano również wydalanie wapnia, fosforanów i hydroksyproliny z moczem. W porównaniu z grupą kontrolną stwierdzono istotny wzrost stężenia wapnia, hydroksyproliny białkowej i nie związanej oraz aktywności fosfatazy alkalicznej. Wydalanie wapnia i hydroksyproliny nie związanej z moczem, podobnie jak nerkowy klirans hydroksyproliny, było istotnie obniżone. Omówiono związki obserwowanych zmian z osteodystrofią nerkowopochodną oraz ich ewentualne znaczenie diagnostyczne.

## РЕЗЮМЕ

В сыворотке крови 23 пациентов с тяжелой недостаточностью почек определено концентрацию кальция, фосфатов, протеинового, свободного и пептидного гидроксипролина, а также активность щелочной фосфатазы. Определено также выделение с мочей кальция, фосфатов и гидроксипролина. По сравнению с контрольной группой замечено существенное повышение концентрации кальция, протеинового и свободного гидроксипролина и активность щелочной фосфатазы. Выделение кальция и свободного гидроксипролина с мочей, а также почечный клиранс гидроксипролина были значительно понижены. Авторами обсуждается связь замеченных изменений с остеодистрофией почечного происхождения и их диагностическое значение.