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Original Article

Incomplete Renal Tubular Acidosis in 'Primary' Osteoporosis

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Abstract. Chronic metabolic acidosis may increase alkali mobilization from bone and thus promote the development of osteoporosis. While it is undisputed that overt metabolic acidosis is associated with metabolic bone disease, renal acidification in patients with idiopathic osteoporosis has not been studied systematically. The purpose of this study was to investigate the prevalence of renal acidification defects in patients with 'primary' osteoporosis. Thirty-two women (including 10 premenopausal women) and 16 men who were referred to our department for investigation of osteoporosis were enrolled in this study. Patients with obvious or possible secondary osteoporosis were excluded. None of the patients had overt metabolic acidosis. In random urine samples 12 of the 48 patients had pH levels below 5.5 and were therefore considered to have normal renal acidification. The remaining 36 patients underwent further testing by a short-course oral ammonium chloride load. In this test nine of these 36 patients (7 men and 2 premenopausal women) failed to lower urinary pH below 5.5 despite the induction of systemic metabolic acidosis. In these patients, therefore, the diagnosis of incomplete distal renal tubular acidosis was made (RTA I). Patients with incomplete RTA I had significantly lower spontaneous plasma pH (7.38 ± 0.0081 vs 7.41 ± 0.004, mean ± SEM, $p = 0.002$), a lower serum bicarbonate concentration (21.9 ± 0.49 mmol/l vs 23.1 ± 0.24 mmol/l, $p = 0.034$), a lower base excess (-2.33 ± 0.42 mmol/l vs -0.55 ± 0.21 mmol/l, $p = 0.001$) and lower *Z*-scores in bone densitometry (-2.18 ± 0.27 vs -1.40 ± 0.15, $p = 0.028$) than patients with normal renal acidification. In conclusion, a high prevalence of incomplete RTA I (in 44% of the male patients, 20% of the premenopausal female patients and 6% of all female patients) was found in patients with osteoporosis who, without testing, would have been diagnosed as having 'primary' osteoporosis. The mild metabolic acidosis observed in these patients may have contributed to loss of bone mass by a compensatory mobilization of alkali and calcium from bone. Because of possible therapeutic consequences (e.g., administration of alkali salts and high doses of vitamin D) we propose that measurements of urinary pH and, if necessary, ammonium chloride testing should be included in the diagnostic investigation especially of male and of premenopausal female patients with osteoporosis. Since referral bias, although unlikely, cannot be excluded in our study, the prevalence of RTA I in unselected patients with osteoporosis needs to be determined at primary screening institutions.

Keywords: Ammonium chloride test; Blood gases; Distal tubular acidosis; Osteoporosis

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Introduction

Primary osteoporosis is a common disease of largely unknown pathogenesis. Acid–base balance has a profound influence on bone calcium homeostasis [1]. In Western industrial societies an average 70-kg adult ingests a diet that generates appoximately 70 mequiv of acid per day. Physiologically, this amount of acid is excreted by the kidneys, 40 mequiv/day as ammonium ions and 30 mequiv/day as titratable acid. In the steady state the renal acid excretion equals the endogenous acid production and thus acid–base balance is neutral. A

positive body acid balance may be induced either by a significant rise in endogenous acid production which exceeds the renal acid excretion capacity, or by defects of renal acid excretion [2]. In response to a positive acid balance serum HCO_3^- concentration and pH decrease and activate homeostatic mechanisms for compensation. The renal excretion of NH_4^+ may more than double, whereas the excretion of titratable acid shows a minor increase. Despite these homeostatic mechanisms the acid balance may remain positive, triggering a release of alkali from the bone. Prolonged alkali release from the bone leads to increased bone resorption and a reduction of total bone substance in rats [3,4]. Subjects with reduced renal acid excretory reserve, such as occurs in incomplete distal tubular acidosis, may be at extra risk for osteoporosis even when ingesting a usual mixed Western diet. In this study we searched for renal tubular acidification defects in patients with primary osteoporosis.

Materials and Methods

Study Population

Forty-eight patients with osteoporosis – 32 women (mean age ± SEM: 58.8 ± 13.7 years, range 24–84 years) and 16 men (45.8 ± 13.8 years, range 19–71 years) – were referred for further investigation to our hospital. Osteoporosis was diagnosed according to WHO criteria, namely a reduced bone density on dual-energy X-ray absorptiometry (DXA) with a *T*-score of below −2.5, or radiologic evidence of vertebral fractures, respectively [5]. Bone density at the lumbar spine (L2–4) and/or the neck of the femur was assessed using DXA on a Sophos XLA machine. Clinical characteristics of the patients are given in Table 1. Patients with secondary causes of osteoporosis were excluded from the study by the following investigations: personal history, blood chemistry, including serum creatinine, serum calcium, phosphate, alkaline phosphatase, electrophoresis, immunoelectrophoresis, triiodothyronine, thyroxine, thyroid stimulating hormone, intact parathyroid hormone, Bence Jones proteinuria and urinalysis. Chronic pyelonephritis and analgesic nephropathy were excluded by personal history, a quantative sediment, and ultrasound studies of the kidneys. Particularly it must be emphasized that 11 additional patients in whom an incomplete RTA I was found had to be excluded from the study because a possible cause for RTA I was found, namely analgesic nephropathy (n = 3) autoimmunthyroiditis (n = 3), hypercalciuria with nephrolithiasis (n = 1), MGUS (n = 1), nephrocalcinosis (n = 1). All patients enrolled in this study had normal results of the above-mentioned investigations. Since urinary tract infections may raise urinary pH and therefore interfere with ammonium chloride testing, current urinary tract infection was excluded by a normal urinalysis. None of the patients received any diuretics either chronically or immediately before the test. The study was approved by the ethics committee of the hospital and all patients gave their informed consent.

Experimental Protocol

The patients in this study were all referred to our hospital for further investigation of established osteoporosis. They were all seen by the hospital dietician for dietary history and for dietary advice. None of the patients was a vegetarian and all consumed a diet typical for Western industrial societies [1]. Also, during admission all

Table 1. Clinical characteristics of the patients

	With acidification defect	Without acidification defect
Number	9	39
Gender	2 females (premenopausal), 7 males	30 females (8 pre- and 22 postmenopausal), 9 males
Age (years)	45±13.6	56±14.7
Body mass index (kg/m^2)	22.29±0.89	23.89±0.60
Blood pH (7.350 - 7.450)[a]	7.38±0.0081**	7.41±0.0038
Plasma bicarbonate (mmol/l) (21.00–26.00)	21.88±0.49*	23.1±0.24
Base excess (mmol/l) (−3.00 to +3.00)	−2.3±0.42***	−0.55±0.21
Plasma phosphate (mmol/l) (2.5–4.2)	3.28±0.24	3.24±0.10
Capillary pCO_2 (mmHg) (35.0–45.0)	38.5±1.18	37.8±0.49
Alkaline phosphate (IU/l) (60–170)	99.8±10.23	118.1±5.33
Plasma calcium (mmol/l) (2.00–2.60)	2.35±0.033	2.39±0.014
24 h urinary calcium (mmol/day) (2.5–10)	3.9±0.80	5.61±0.49
Index of phosphate excretion (−0.45 to +0.45)	−0.25±0.13	0.01±0.071
iPTH (pg/ml) (10–65)	23.36±3.12	33.26±2.73
Pyridinoline crosslinks (nmol/mmol creatinine) (F 5–65; M 3–51)	11.26±3.50 (n=4)	9.04±3.86 (n=17)
Osteocalcin (ng/ml) (F 2.4–10; M 3.4–11.7)	10.9±2.37 (n=4)	13.39±2.37 (n=18)

Results are the mean ± SEM.
Sample size was as stated at the top of the column unless otherwise indicated in parentheses.
BMI, body mass index [body weight (kg)/body height (m)^2]; ipTH, intact parathyroid hormone.
*p<0.05 between groups; **p<0.01 between groups; ***p<0.001 between groups.
[a] Values in parentheses are the normal range.

patients consumed the usual hospital diet containing 30% animal protein, 40% carbohydrate and 30% fat for at least 2 days before the study.

Blood gas analysis (pH, base excess, HCO_3, pCO_2) was performed from capillary blood using an AVL 995 Hb blood gas analyzer (AVL List GmbH, Graz, Austria) in all patients. Renal acidification was investigated by an established protocol [6]. In each patient at least five random urine samples were collected over 24 h. Urinary pH was measured by a potassium chloride electrode (Metrohm pH-Meter E512, Herisau, Switzerland). A urinary pH below 5.5 in any of the urine specimens was considered to exclude renal tubular acidosis and further ammonium chloride testing was withheld. In patients who failed spontaneously to lower their urinary pH below 5.5, an oral ammonium chloride loading test was performed. After an overnight fast, 0.1 g NH_4Cl per kilogram of body weight was given by mouth in coated gelatin capsules with 500 ml of tap water. Blood and urinary pH were determined before, and 2, 4 and 6 h after ammonium chloride ingestion [7]. A fall in serum bicarbonate concentration >3 mmol/l and a capillary pH <7.35 were taken as criteria for an adequate acidemia following ammonium chloride ingestion. Failure to lower urinary pH <5.5 despite the presence of systemic metabolic acidosis is consistent with some form of renal distal tubular acidosis [8].

Statistical Analysis

Data are given as the mean ± SEM. The *t*-test of untransformed data was used for group comparison. A two-tailed *p* value of <0.05 was considered to be significant. For obvious biologic reasons, in the case of blood gas variables the null hypothesis of lower bicarbonate, lower pH and lower pCO_2 in patients with incomplete renal tubular acidosis type I (RTA I) was tested. The prevalence of incomplete RTA I in women and men was compared by chi-squared test. Statistical analysis was performed with the SPSS statistical package (SPSS for Windows, v7.0, 1995, Chicago).

Results

Twelve (25%) of 48 patients showed a normal urinary pH profile (pH below 5.5 in one or more of the urine samples). In 36 (75%) patients urinary pH was >5.5 in all five random urine samples. In these patients a short-course oral ammonium chloride loading test was performed. Nine of these 36 patients (i.e., 19% of all patients) failed to lower urinary pH <5.5 following the oral ammonium chloride load. Seven pathologic ammonium chloride tests were obtained in men and two in female patients (44% of all male patients and 6% of all female patients; $\chi^2 = 7.54$, d.f. = 1, $p<0.05$). Both female patients with incomplete RTA were premenopausal (aged 37 and 39 years, respectively), so that 2 of 10 premenopausal women (20%) were diagnosed of having incomplete RTA I. Clinical characteristics of patients with and without incomplete RTA I are given in Table 1. As can be seen, pCO2, serum calcium, serum phosphate, serum alkaline phosphatase, 24 h urinary calcium excretion and index of phosphate excretion did not differ significantly between the groups (Table 1).

The individual results of basal blood gas analysis in patients with and without RTA I are shown in Figs 1–3. In patients with incomplete RTA I a lower basal plasma pH (7.38 ± 0.0081 vs 7.41 ± 0.0038, $p = 0.002$), lower base excess (BE) (−2.33 ± 0.42 mmol/l vs −0.55 ± 0.21 mmol/l, $p = 0.001$), lower serum bicarbonate levels (21.9 ± 0.49 mmol/l vs 23.1 ± 0.24 mmol/l, $p = 0.034$) and lower *Z*-scores in bone densitometry (−2.18 ± 0.27

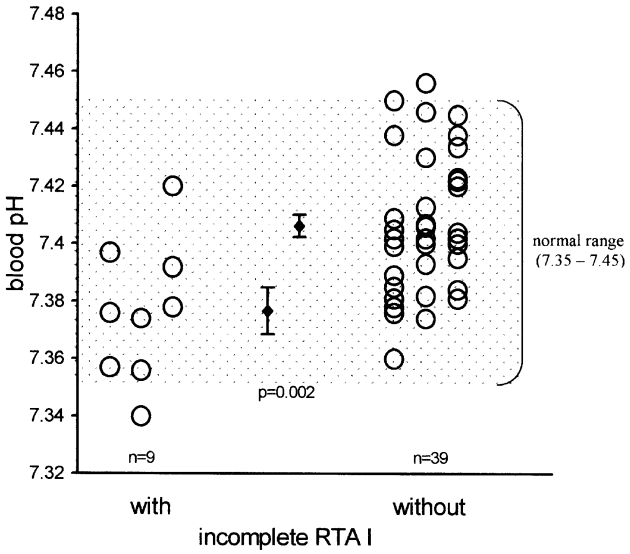

Fig. 1. Individual values of blood pH, and means ± SEM, of patients with and without incomplete RTA I.

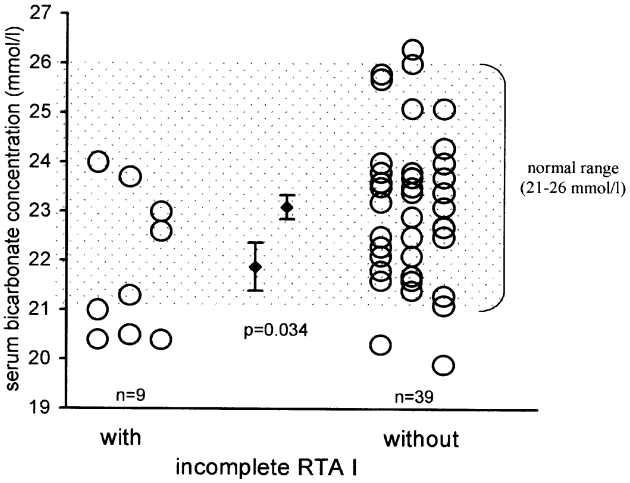

Fig. 2. Individual values of serum bicarbonate, and means ± SEM, of patients with and without incomplete RTA I.

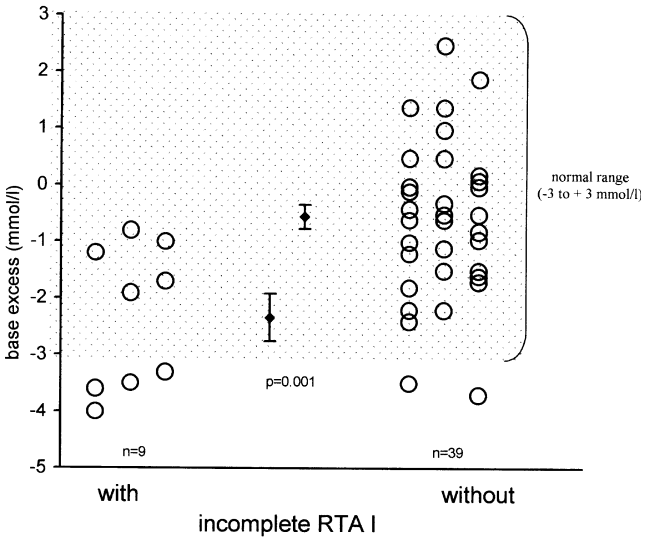

Fig. 3. Individual values of base excess, and means ± SEM, of patients with and without incomplete RTA I.

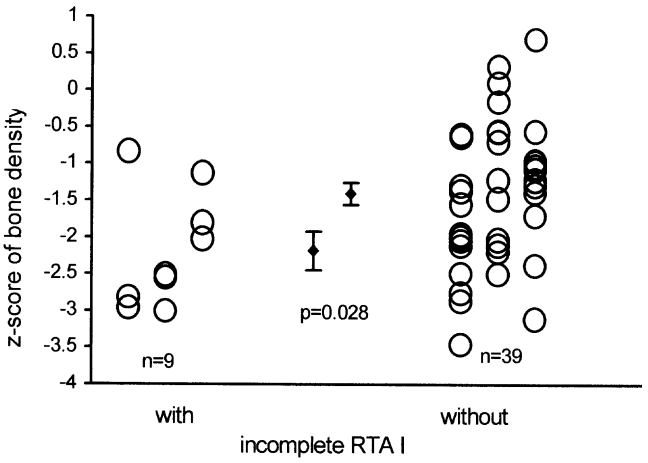

Fig. 4. Individual values of *Z*-scores, and means ± SEM, of patients with and without incomplete RTA I.

vs −1.40 ± 0.15, $p = 0.028$) were observed. Figure 4 shows the *Z*-scores of osteodensitometry in patients with and without RTA I for comparison. As can be seen, subjects with RTA I have a statistically significant lower *Z*-score than patients without RTA.

Discussion

The important findings of the present study are (a) that a renal acidification defect was found in 19% (44% of male patients, 6% of female patients and 20% of premenopausal female patients) of the patients with 'primary' osteoporosis in whom secondary causes of osteoporosis were excluded and (b) that in patients with acidification defect spontaneous blood pH and bicarbonate values were lower than in patients without acidification defect. There were 16 male, 10 premenopausal and 22 postmenopausal female patients in our study; both female patients with incomplete RTA were premenopausal, and aged 37 and 39 years, respectively.

This acidification defect was accompanied by significantly reduced spontaneous blood pH, plasma bicarbonate and base excess as compared with the group with normal renal acidification, which re-emphasizes the clinical significance of the acidification defect. Since none of the patients with RTA I had overt metabolic acidosis, the diagnosis of incomplete RTA I was made. Serum calcium, phosphate, potassium, 24 h calcium excretion and the index of phosphate excretion were normal, making associated renal tubular defects unlikely. Since all subjects were on the same acidogenic diet, we consider it unlikely that substantial differences in calciuria exist between the two groups.

Acid–base balance plays an important role in calcium homeostasis and bone metabolism. Renal tubular acidosis type I is an inherited or acquired disease in which the kidney fails to lower urinary pH below 5.5 despite the presence of systemic acidosis. Acquired forms of RTA I occur either sporadically or in association with a number of renal or extrarenal diseases such as interstitial nephritis, systemic lupus erythematosus, Sjögren syndrome or autoimmune thyroiditis [8]. None of these diseases was evident in our patients, so that a sporadic form of incomplete RTA I appears more likely. RTA I may be caused by impaired distal tubular H^+ secretion or an excessive backdiffusion of H^+ ions from the tubular lumen [8]. Typically, incomplete RTA I does not cause overt systemic acidosis. Although no overt metabolic acidosis was observed in the patients with RTA I, the mild derangement of acid–base balance might still have contributed to loss of bone calcium in these subjects. The high animal protein content of the diet of industrialized countries generates approximately 70 mequiv acid per day, and additionally many grain products, cheese and even a high salt intake may be acidogenic [9]. Any impairment of renal acid excretion may result in a positive acid balance. A chronically positive acid balance may trigger the loss of alkali and calcium from bone and may lead to osteoporosis [10]. Metabolic acidosis may promote bone alkali mobilization by physicochemical effects.

The fact that neither calciuria, nor pyridinoline and pyridinoline crosslinks were statistically different between the groups with and without incomplete RTA is problematic. However, it could be argued that these subjects never reached their maximal adult potential of bone development because of a lifelong abnormality in the renal handling of acid and that their current state of osteoporosis was primarily the result of this lifelong defect. Furthermore we can not exclude some mild form of osteomalacia induced by the acidosis which osteodensitometry would fail to detect. It would have been necessary to perform bone biopsies to differentiate mild osteomalacia from osteoporosis. It is interesting that the comparison of the bone density of subjects with and without RTA I shows significantly lower *Z*-scores in subjects with RTA I, which could be an indication of

more severely affected bone substance. None of the postmenopausal women with osteoporosis had defective renal acidification. In contrast, 2 of 10 premenopausal women had incomplete RTA I. We consider it possible that, if RTA I is present, the higher degree of loss of bone substance makes the disease already apparent before menopause. Moreover, a negative calcium balance may be induced by an acidosis-mediated renal loss of calcium. It has been shown that the short-term administration of potassium bicarbonate to women with osteoporosis leads to a positive calcium balance and to improved parameters of bone metabolism [11].

Since osteoporosis associated with overt metabolic acidosis may respond to alkali, [11,12] the administration of alkali salts may be a therapeutic concept also for osteoporotic patients with incomplete RTA I. It must be mentioned that calcium supplements, especially in the form of carbonate and citrate, also provide an alkali load. At least in older studies a response of bone mass to high doses of vitamin D has been shown [13]. In these older studies measurements of vitamin D metabolites were not available. Clearly, measurements of all vitamin D metabolites and prospective randomized studies will be necessary to test whether patients with incomplete RTA I will profit from such a therapeutic approach.

In conclusion, the present study shows a high prevalence of incomplete RTA I, especially in male patients with osteoporosis and in premenopausal women. It must be reemphasized that 11 additional patients with RTA I were excluded from the study because secondary causes of RTA I had been found. Some of the underlying diseases are difficult to detect and are very likely to be overlooked in the usual diagnostic work-up of patients with osteoporosis. Had these patients been included in the present study, the percentage of patients with RTA I would have been even higher. We consider it possible but unlikely that selection bias was responsible for the high prevalence of RTA I observed in the present study, since the only selection criterion for referral to our center was a low bone density. We would have liked to perform a case–control study including patients with high and low bone densities, but unfortunately we were able to obtain informed consent for the unpleasant ammonium chloride test only in patients with osteoporosis (in whom the test may have therapeutic implications) and not in patients with normal bone density. It should be considered whether the measurement of urinary citrate could serve as a surrogate for ammonium chloride loading [14]. We also plan to evaluate whether the administration of furosemide could replace the ammonium chloride test [15]. We propose a case–control study aimed at studying renal acidification in patients with and without osteoporosis. Such a study should be performed in unselected patients with high and low bone densities at an institution screening for osteoporosis.

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