

# DISTAL RENAL TUBULAR ACIDOSIS SCREENING BY URINARY ACIDIFICATION TESTING IN MEXICAN CHILDREN

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## ABSTRACT

**Background:** Primary distal renal tubular acidosis is a clinical disorder characterized by hyperchloremic metabolic acidosis, hypercalciuria, hypocitraturia, urinary acidification impairment, hypokalemia, metabolic bone disease, and nephrocalcinosis. Urinary acidification ability may be evaluated by an acidification test or maximum urinary pCO<sub>2</sub> assessment with alkaline urine. The maximum urinary pCO<sub>2</sub> test using acetazolamide and sodium bicarbonate is an easy test to confirm the lack of urine acidification in distal renal tubular acidosis in children. **Objective:** To determine the urinary acidification ability using the maximum urinary pCO<sub>2</sub> assessment in a group of children with a distal renal tubular acidosis diagnosis. **Material and methods:** Thirty children were evaluated (13 males and 17 females); 23 children had been diagnosed with distal renal tubular acidosis by other physicians and were under alkali treatment with potassium and sodium citrates (21) and bicarbonate (2), and five children were not under alkali treatment. Two children had been diagnosed with primary distal renal tubular acidosis by our medical group. The maximum urinary pCO<sub>2</sub> was determined by the oral intake of acetazolamide and sodium bicarbonate. **Results:** Two cases with primary distal renal tubular acidosis were found, and they had a history of dehydration episodes during infancy and showed hyperchloremic metabolic acidosis with hypokalemia. They also exhibited urine acidification impairment with furosemide and reduced urinary pCO<sub>2</sub> (< 60 mmHg), and the urine-blood pCO<sub>2</sub> gradient was reduced in both cases (< 30 mmHg). One of them developed bilateral sensorineural deafness, while the other showed severe hypocitraturia. One case of proximal or type 2 renal tubular acidosis with hyperaminoaciduria was identified. Twenty-eight children displayed normal urinary acidification and did not show signs of distal renal tubular acidosis. **Conclusions:** The urinary acidification test with furosemide and urinary pCO<sub>2</sub> assessment are reliable tests to identify the renal excretion of hydrogen ions (H<sup>+</sup>) and allow confirmation of the lack of urine acidification in distal renal tubular acidosis. (REV INVES CLIN. 2015;67:191-8)

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## INTRODUCTION

In 1936, Butler, Wilson and Farbes published similar clinical and biochemical characteristics in four infants with ages ranging from two weeks to 11 months. The patients had a clinical pattern that had not been described before, characterized by: (i) persistent dehydration in the absence of vomiting and excessive diarrhea regardless of adequate food, fluid, and salt intake; (ii) persistent hyperpnea associated with sustained elevated serum chlorine and low bicarbonate levels; and (iii) calcium salt deposits in and adjacent to some kidney tubules<sup>1</sup>. This clinical entity was initially called *nephrocalcinosis infantum*<sup>2-4</sup> and is currently known as distal or type 1 renal tubular acidosis (RTA). In addition to hyperchloremic metabolic acidosis, distal RTA (dRTA) is associated with hypercalciuria, hypocitraturia, defects in urinary acidification capacity, metabolic bone disease and, frequently, hypokalemia<sup>5</sup>. In recent years, molecular biology techniques have identified the genetic bases of a defect in the urinary excretion of ammonium<sup>5-7</sup>. Distal RTA is characterized by the loss of the ability to acidify urine. This condition is caused by a defect in acid excretion (mainly ammonium) by the collecting tubule<sup>7</sup>.

The urinary acidification test only confirms defects such as a urine pH decrease to  $< 5.35$ , or the inability to achieve a maximum urine  $p\text{CO}_2 > 70\text{-}60$  mmHg with a urine-blood  $p\text{CO}_2$  gradient  $< 20\text{-}30$  mmHg. This defect may be confirmed by performing a test of acidification with ammonium chloride or by determining the maximum urine  $p\text{CO}_2$  in alkaline urine. However, it is not necessary to perform these tests to reach a diagnosis because the main clinical manifestations of dRTA can be identified using a detailed clinical history. Complementary tests may determine the biochemical changes that characterize this disease. The assessment of urinary acidification is performed in patients with borderline bicarbonate levels or with an uncertain diagnosis<sup>7</sup>.

While this clinical entity is well defined, there has been an increase in the number of children diagnosed and treated for dRTA in our country in the last few years. Recently, this over-diagnosis has been reported<sup>8</sup>, and there are doubts regarding its veracity. In this paper, we have gathered a group of patients diagnosed with and treated for dRTA, and another group consisting of untreated children with the same clinical features in whom this disorder has been suspected. All the

patients were tested for acidification to confirm or exclude the diagnosis. To our knowledge, no test for urinary acidification has been used in our country to confirm the diagnosis of distal RTA in children.

## PATIENTS AND METHODS

A total of 30 children (13 males and 17 females) with a mean age of  $4.5 \pm 2.5$  years (range, 1.25-12.0) were evaluated at the Department of Pediatric Nephrology, Hospital General Centro Médico Nacional "La Raza" (Instituto Mexicano del Seguro Social, IMSS). Most of them ( $n = 23$ , 76.7%) had been previously diagnosed with dRTA and were treated with sodium citrate and potassium ( $n = 21$ ) or sodium bicarbonate ( $n = 2$ ). A smaller subgroup consisted of children in whom the diagnosis was suspected and who came to the hospital for diagnosis confirmation ( $n = 5$ , 16.6%). Two patients diagnosed with primary dRTA previously identified at our hospital ( $n = 2$ , 6.66%) were also included<sup>9</sup>. Citrate treatments were suspended for all treated patients for at least two days before the laboratory and acidification tests.

Data such as age and weight and height percentiles, and symptoms or clinical signs were collected from the medical records. All participants signed an informed consent. The Hospital's code of ethical behavior was followed. The levels of sodium, potassium, and chloride were determined in all the children. A test to assess the acid-base balance in venous blood, without using a tourniquet, was also performed. When possible, the values of the protein/creatinine ( $n = 24$ ), calcium/creatinine ( $n = 24$ ) and citrate/creatinine ( $n = 5$ ) ratios were determined using the first urine of the day. All the patients underwent renal ultrasonography. The maximum urine  $p\text{CO}_2$  ( $\text{UpCO}_2$ ) was measured using acetazolamide and sodium bicarbonate ( $\text{NaHCO}_3$ ) stimulation, and the urine-blood  $p\text{CO}_2$  gradient difference was determined. Twenty-seven children underwent an acidification test with furosemide stimulation. Measurements of the levels of calcium, citrate, and creatinine were performed by standard techniques in an autoanalyzer (Roche Modular Analytics SWA<sup>®</sup>). Ultrasonography was performed using an Orion<sup>®</sup> (Philips) scanner.

Metabolic acidosis was defined by a blood  $\text{pH} < 7.32$ . To define the lower limit of normal bicarbonate ( $\text{HCO}_3^-$ ) in the blood, the following values corresponding to  $-2$  SD

(standard deviation) published in the medical literature, which vary by age, were used: 18.6 mEq/l between one and 3.5 years, 19.9 mEq/l between 3.5 and 5.4 years, and 20.7 mEq/l between 5.5 and 12 years old<sup>10</sup>. Hyperchloremia was defined by values  $> 107$  mmol/l, and hypokalemia was defined by values  $< 3.5$  mmol/l. The diagnosis of hypercalciuria was established when the calcium/creatinine ratio was  $> 0.47$  mg/mg in children aged between 1-2 years<sup>11</sup>,  $> 0.28$  mg/mg in those aged 2-4 years<sup>(12)</sup> and  $> 0.20$  mg/mg in those older than four years<sup>12,13</sup>. Based on the Stapleton and Kroovand criteria, the diagnosis of hypocitraturia was made when the value of the citrate/creatinine ratio was  $< 400$  mg/g<sup>14</sup> in the first morning urine.

A urinary acidification defect was diagnosed when the urinary pH was  $> 5.35$  in the furosemide stimulus test, the urine  $p\text{CO}_2$  was  $< 60$ -70 mmHg in the acetazolamide bicarbonate stimulation test, and the urine-blood  $p\text{CO}_2$  gradient was  $< 20$ -30 mmHg<sup>15,16</sup>.

### Determination of the maximum urine $p\text{CO}_2$ in alkaline urine

Patients emptied their bladder at the beginning of the test. They were administered 2 mEq/kg of  $\text{NaHCO}_3$  orally together with 500 mg/1.73 m<sup>2</sup> of acetazolamide<sup>15,16</sup>. At 60 and 90 minutes, urine samples were collected in a sterile plastic container. The urine was aspirated with a syringe under sealed anaerobic conditions, and the urine pH (UpH) and Up $\text{CO}_2$  were measured immediately after collection in a GEM Premier 3000 gasometer (Werfen Group Instrumentation Lab). Furthermore, the sodium ( $\text{UNa}^+$ ) and potassium ( $\text{UK}^+$ ) contents were determined in each urine sample. To calculate the content of urinary  $\text{HCO}_3^-$  ( $\text{UHCO}_3^-$ ), the following formula was used:  $\text{UHCO}_3^- = 0.03 \times \text{UpCO}_2 \times \text{antilog}(\text{UpH} - \text{urine pK})$ <sup>17</sup>. Urine pK was calculated using the following formula:  $6.33 - 0.5 (\text{UNa}^+ [\text{mol/l}] + \text{UK}^+ [\text{mol/l}])$ <sup>1/2</sup>. The maximum  $\text{UHCO}_3^-$  and Up $\text{CO}_2$  values for the two samples were recorded. In 19 cases, these values corresponded to the collection at 60 minutes; in the other 11 cases, the values corresponded to the collection at 90 minutes. For the test to be considered normal, the Up $\text{CO}_2$  had to exceed 60-70 mmHg<sup>18,19</sup>. To be considered valid, the  $\text{UHCO}_3^-$  concentration had to be  $> 80$  mEq/l<sup>20,21</sup>. No child had clinical complications during the test.

### Acidification test with furosemide

A furosemide dose of 1 mg/kg was administered orally. Four urine samples were collected at hourly intervals. The minimum urinary pH (UpH) was measured using a Digital PH-200 Meter HM (HM Digital Inc.). The lowest value was recorded as UpH. For the test to be considered normal, the UpH value had to be  $< 5.35$ <sup>21</sup>.

### Statistical analysis

Quantitative variables were expressed as medians and interquartile ranges because of the small number of patients. The Mann-Whitney U test was used to compare the means of the quantitative variables. To study the correlation between variables, the Pearson correlation coefficient (normal distribution) was used. Values lower than 0.05 were considered statistically significant. These analyses were performed using SPSS statistical software (SPSS V 17.0, SPSS Inc., USA).

## RESULTS

The clinical signs that led to the supplementary examinations in children were as follows: height lower than or equal to the 3rd percentile ( $n = 18$ ), height between the 3rd and 25th percentile and a weight lower than the 10th percentile ( $n = 4$ ), arrest in the weight curve ( $n = 4$ ), episodes of dehydration and metabolic acidosis ( $n = 2$ ), repetitive vomiting ( $n = 1$ ), and adrenal hyperplasia ( $n = 1$ ). The two patients who had experienced episodes of dehydration with no other apparent renal cause had hyperchloremic and hypokalemic metabolic acidosis; they also exhibited urine acidification impairment with furosemide and reduced maximum urine  $p\text{CO}_2$ , the urine-blood  $p\text{CO}_2$  gradient was  $< 20$  mmHg, and they had nephrocalcinosis. One of them was diagnosed with sensorineural deafness. In one of the patients, citraturia was very low ( $< 0.2$  mg/day). These were the only two patients with dRTA type 1. A third patient had hyperchloremic and hypokalemic metabolic acidosis, but furosemide testing,  $p\text{CO}_2$  values, and renal ultrasonography revealed normal findings. This patient showed high fractional excretion of  $\text{HCO}_3^-$  (14%). Therefore, type 2 or proximal RTA was diagnosed with complex proximal tubulopathy because the symptoms included generalized hyperaminoaciduria (Table 1). A fourth patient showed no metabolic acidosis. However, acidification

**Table 1.** Patients with hyperchloremic metabolic acidosis and/or an acidification capacity defect using the maximum pCO<sub>2</sub> assessment

Patient/sex	pH/HCO <sub>3</sub> <sup>-</sup> (mEq/l)	K <sup>+</sup> /Cl <sup>-</sup> (mEq/l)	UCa/Cr	UpHmin <sup>+</sup> /UpCO <sub>2</sub> mmHg <sup>#</sup>	pCO <sub>2</sub> urine- plasma mmHg <sup>§</sup>	Renal ultrasound
1. Female*	7.20/7.0	1.6/117.0	0.27	6.8/49.0	8	Nephrocalcinosis
2. Male*	7.34/14.6	3/110	-	6.26/53.0	17	Nephrocalcinosis
3. Male	7.28/13.6	3.3/114.0	0.2	4.78/101.0	72	Normal
4. Female	7.39/25.4	4.11/102.7	0.66	6.17/65.0	23	Bilateral lithiasis

\*Female 1 and Male 2 had type 1 RTA. Male 3 had proximal tubular acidosis. Female 4 was diagnosed with idiopathic hypercalciuria.

<sup>#</sup>Furosemide testing. Normally, the minimum urine pH is < 5.35.

<sup>#</sup>Acetazolamide testing + sodium bicarbonate. Normally, the maximum urine pCO<sub>2</sub> should be > 60-70 mmHg.

<sup>§</sup>Difference in the urinary-blood pCO<sub>2</sub> gradient > 30 mmHg.

**Table 2.** Values of biochemical data and test results in children with normal maximum urinary pCO<sub>2</sub>

Boys	Age	Venous gas			Serum electrolytes		Acidification test		pCO <sub>2</sub> urine- plasma
		pH	pCO <sub>2</sub>	HCO <sub>3</sub> <sup>-</sup>	Potassium	Chloride	Furosemide UpH	Maximum UpCO <sub>2</sub>	
1	2 years 7 months	7.42	30.0	21.0	4.5	108	5.0	115	85.0
2	6 years 11 months	7.28	29.0	13.6	3.3	114	4.78	101	72.0
3	1 year 6 months	7.33	25.0	18.3	3.86	107	5.0	103	78.0
4	3 years 10 months	7.35	31.0	18.6	4.65	103	4.58	81	50.0
5	3 years 3 months	7.33	38.0	20.0	5.2	104	5.0	72	34.0
6	5 years 3 months	7.27	48.0	22.0	4.84	101	5.0	115	67.0
7	4 years 6 months	7.32	41.0	21.1	4.0	104		92	51.0
8	3 years 8 months	7.38	35.0	20.7	4.5	103		80	45.0
9	6 years 3 months	7.34	34.0	18.3	4.42	106	5.27	94	60.0
10	6 years 1 month	7.37	42.7	24.4	4.6	109	4.43	100	57.3
11	5 years 5 months	7.39	34.0	20.6	4.41	111	5.0	102	68.0

with furosemide was negative, and the maximum urine pCO<sub>2</sub> was slightly borderline (65 mmHg). This patient had been diagnosed with bilateral renal calculi associated with idiopathic hypercalciuria from six months of age (Table 1). The remainder of the children (n = 26, 11 males and 15 females) had normal maximum urinary pCO<sub>2</sub> values (86.5 ± 24.7 mmHg; range, 71-115) with a blood-urine pCO<sub>2</sub> gradient > 30 mmHg. These results exclude a diagnosis of dRTA (Tables 2 and 3). All the acidification tests were validated by normal bicarbonaturia (140.3 ± 53.5 mEq/l; range: 81.2-195.6). In plasma, all these patients had sodium levels between 136 and 143 mEq/l (139 ± 4 mEq/l) and potassium levels > 3.5 mEq/l (4.5 ± 0.47 mEq/l; range, 3.7-5.3). Furthermore, all but one patient had chloremia < 110 mEq/l (105 ± 3 mEq/l; range, 101-111). Hypercalciuria was observed in one child. Citraturia was normal when it was determined (n=4) was determined. Renal ultrasound scans were normal

in 24 of these 26 children. Nephrocalcinosis of unknown cause was found in two of them.

When the 26 children were divided into two subgroups according to whether they had received prior treatment with alkali (n = 19) or not (n = 7), no statistically significant differences were found in any of the parameters studied. For the acid-base balance, the blood pH and HCO<sub>3</sub><sup>-</sup> values were 7.38 ± 0.06 (range, 7.27-7.46) and 20.4 ± 2.7 mEq/l (range, 16.9-24), respectively (n = 26). When children with low HCO<sub>3</sub><sup>-</sup> blood levels (n = 10) were compared with those with normal levels (n = 16), statistically significant differences were only found in the chloremia values (Table 4). Finally, when children with positive acidification with furosemide stimulation (n = 14) were compared with those who did not show adequate acidification (n = 9), no statistically significant differences were found in any of the parameters studied. In the entire sample

**Table 3.** Values of biochemical data and test results in girls with normal maximum urinary pCO<sub>2</sub>

Girls	Age	Venous gas		Serum electrolytes			Acidification test		pCO <sub>2</sub> urine-plasma
		pH	pCO <sub>2</sub>	HCO <sub>3</sub>	Potassium	Chloride	Furosemide UpH	Maximum UpCO <sub>2</sub>	
12	3 years 6 months	7.38	36.0	20.5	4.8	103	6.0	81	45.0
13	7 years 10 months	7.36	38.0	21.5	4.47	109	6.28	115	77.0
14	2 years 8 months	7.41	32.0	20.3	4.68	105	5.0	115	83.0
15	1 years 5 months	7.43	34.0	23.20	4.5	104	6.33	72	38.0
16	1 years 3 months	7.41	30.0	18.4	5.3	104	6.16	80	50.0
17	8 years 8 months	7.4	29.3	19.1	4.2	107	5.5	77	47.7
18	8 years 8 months	7.38	32.8	18.5	3.8	106	5.5	84	51.2
19	2 years 11 months	7.36	33.0	18.6	4.67	105	5.0	83	50.0
20	12 years	7.42	25.0	17.5	4.3	106	5.0	107	82.0
21	3 years 7 months	7.4	33.0	20.4	4.8	104	5.15	80	47.0
22	4 years 5 months	7.43	30.0	19.9	4.6	103	4.85	91	61.0
23	1 years 8 months	7.38	27.0	16.9	4.81	106	6.0	115	88.0
24	2 years 6 months	7.43	33.0	23.9	3.7	105	5.0	89	56.0
25	2 years 8 months	7.39	35.0	21.2	4.3	105		71	36.0
26	4 years 7 months	7.46	28.0	19.8	4.25	108	5.5	75	47.0

(n = 30), the maximum UpCO<sub>2</sub> correlated with both the UHCO<sub>3</sub><sup>-</sup> concentration in the test acetazolamide and NaHCO<sub>3</sub> (r = 0.4; p = 0.03) (Figure 1) and minimum UpH obtained in the furosemide test (r = -0.5; p = 0.008).

## DISCUSSION

The determination of urine pH and net acid excretion in cases of acidemia is a basic step to assert the integrity of distal acidification mechanisms<sup>7</sup>. Throughout history, various tests have helped in the assessment of acidification capability. These tests include the overload ammonium chloride test, or other substances rich in H<sup>+</sup> ions (methionine, arginine, hydrochloride); fludrocortisone and furosemide test<sup>22-27</sup>, as well as UpCO<sub>2</sub> test, have been used to confirm the diagnosis of RTA<sup>28-32</sup>.

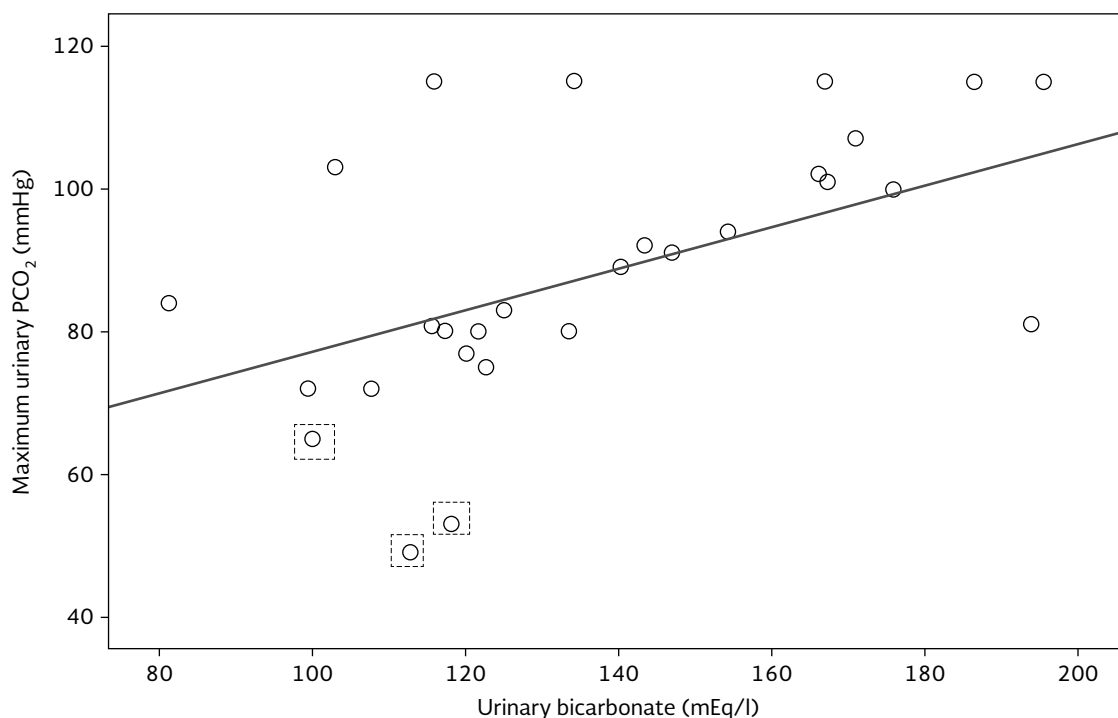
In the present study, in the group that was subjected to the pCO<sub>2</sub> test, we found that only three children had not shown properly increased UpCO<sub>2</sub> (10%). Two of them also had hyperchloremic metabolic acidosis, nephrocalcinosis, and dehydration episodes suggestive of polyuria. These symptoms are classic for dRTA. There were no records of dRTA in parents. It is possible that the children with sensorineural hearing loss might be carrying mutations in the gene *ATP6V1B1*

that encodes the subunit B1 of the H<sup>+</sup>-ATPase<sup>33</sup>. The other child is likely to be carrying mutations in the gene *ATP6V0A4* encoding the α4 subunit of the H<sup>+</sup>-ATPase that causes an autosomal recessive form of dRTA with late deafness<sup>34</sup>. The third patient showed better elevation of UpCO<sub>2</sub> than the first two, but presented an abnormal value (urinary pCO<sub>2</sub> of 65 mmHg) that was borderline, and the patient did not have hyperchloremic metabolic acidosis. This child was diagnosed with kidney stones at a young age and with idiopathic hypercalciuria. The urinary acidification defect observed particularly in patients with repeated nephrolithiasis<sup>35,36</sup> has been known for many years.

Another boy had hyperchloremic metabolic acidosis with normal urine acidification tests. Distal RTA, with which he had been diagnosed since childhood, was ruled out. Instead, significant proximal tubular acidosis with bicarbonate loss associated with hyperaminoaciduria within a proximal tubulopathy of unknown etiology was diagnosed. Therefore, because the distal portions of the nephron were unaffected, the acidification tests were normal.

The remaining 26 children showed no signs or biochemical parameters of dRTA. They had normal UpCO<sub>2</sub> and pCO<sub>2</sub> urine-blood gradients > 30 mmHg. Therefore, it was confirmed that dRTA was misdiagnosed. None had hypokalemia, and only one showed chlorine

Figure 1. Direct correlation between bicarbonaturia and maximum urinary  $p\text{CO}_2$  in the test combining acetazolamide and  $\text{NaHCO}_3$  stimulation. The points surrounded by squares correspond to the three children with the altered test.



plasma levels in the upper limit of the normal range. Some patients had levels of bicarbonaturia that were apparently reduced. However, compared with those patients with normal  $\text{HCO}_3^-$  values, there were no differences between the two subgroups, except, as expected, in the chloremia levels required to maintain plasma electroneutrality. The explanation for this paradox is that the apparently small values of bicarbonaturia should be normal in our local setting. Physiologists from the beginning of the last century knew that the alkalinity of the blood decreases with altitude<sup>37,38</sup>. Normal acid-base values reported in the international literature correspond to those determined at sea level, where the barometric pressure is 760 mmHg. It is widely known that barometric pressure and altitude determine  $p\text{CO}_2$ ,  $p\text{O}_2$  and  $\text{HCO}_3^-$  values. Mexico City is located approximately 2,240 meters above sea level, and the barometric pressure varies between 584 and 590 mmHg. Therefore, the partial pressure of inhaled breathing gases is much lower than that at sea level<sup>39-41</sup>. According to the Henderson-Hasselbalch equation, to maintain a normal pH with lower  $p\text{CO}_2$ ,  $\text{HCO}_3^-$  levels must also decrease. Therefore, there was no difference between patients in the two subgroups shown in table 4 because they are normal from the

viewpoint of acid-base balance. It is noteworthy that the normal  $\text{HCO}_3^-$  levels corresponding to  $-2$  SD (arterial blood) in the children of Mexico City are  $18.66 \pm 1.37$  mEq/l between 2-5 years and  $20.30 \pm 1.30$  mEq/l between 6-16 years<sup>39,40,42</sup>.

Another finding of our study was discussing is the inability of some children without dRTA to acidify urine after receiving furosemide. Puschett and Goldberg noted in 1968 that the urine was acid a few hours after furosemide administration<sup>43</sup>. A few years later, the effectiveness of furosemide administration was demonstrated for the diagnosis of cases of dRTA<sup>44</sup>. Furosemide inhibits the action of the Na-K-2Cl (NKCC2) co-transporter located in the ascending thick limb of the loop of Henle. This phenomenon increases the concentrations of the three ions in the distal portions of the nephron. Once  $\text{Na}^+$  and  $\text{K}^+$  are reabsorbed, the tubular lumen becomes electronegative because the concentration of  $\text{Cl}^-$  is higher. As observed for the  $\text{HCO}_3^-$  and  $p\text{CO}_2$  tests, this phenomenon stimulates the secretion of  $\text{H}^+$  ions. However, it was observed in a study conducted in children diagnosed with idiopathic hypercalciuria that 27.3% of them did not acidify urine with furosemide despite having normal

**Table 4.** Values of the parameters studied in children with a normal maximum urinary pCO<sub>2</sub>. Children were subdivided into two subgroups according to the levels of bicarbonaturia

	Patients with HCO <sub>3</sub> <sup>-</sup> levels "apparently" reduced (n = 10)	Patients with normal HCO <sub>3</sub> <sup>-</sup> levels (n = 16)	P value*
Age (years)	5.0 (7.04)	3.58 (1.86)	NS
Weight percentile	0.01 (0.10)	0.0 (0.02)	NS
Height percentile	0.02 (0.33)	0.01 (0.02)	NS
Blood pH	7.38 (0.06)	7.38 (0.06)	NS
Blood CO <sub>3</sub> H <sup>-</sup> (mEq/l)	18.45 (0.85)	21.1 (2.35)	-
Plasma Na <sup>+</sup> (mEq/l)	140.0 (3.50)	139.0 (3.00)	NS
Plasma K <sup>+</sup> (mEq/l)	4.35 (0.59)	4.55 (0.43)	NS
Plasma Cl <sup>-</sup> (mEq/l)	106.0 (1.50)	104.0 (2.00)	< 0.01
Protein/Cr in urine (mg/mg)	0.23 (0.15)	0.19 (0.06)	NS
Calcium/Cr in urine (mg/mg)	0.10 (0.08)	0.09 (0.12)	NS
Minimum UpH with furosemide	5.38 (0.62)	5.0 (0.96)	NS
Maximum urinary pCO <sub>2</sub> in alkaline urine (mmHg)	89.0 (24.75)	85 (35)	NS
Urinary UCO <sub>3</sub> H <sup>-</sup> in the urinary pCO <sub>2</sub> assessment (mEq/l)	138.56 (36.52)	140.28 (70.82)	NS
Plasma pCO <sub>2</sub> (mEq/l)	30.5 (5.50)	34.0 (5.25)	0.025
pCO <sub>2</sub> gradient (mmHg)	50.0 (18.00)	56.65 (29.75)	NS

Values are reported in medians (interquartile range).

\*The Mann Whitney U test was used to compare the groups.

UpCO<sub>2</sub><sup>43</sup>. The authors later demonstrated that the lack of acidification with furosemide was due to a partial resistance to the diuretic compound<sup>44-46</sup>. It seems that furosemide is less potent than NaHCO<sub>3</sub> in inducing the secretion of H<sup>+</sup> or that this effect is characteristic of some children predisposed to kidney stones<sup>47</sup>. Among the nine children in this study who did not show acidification with furosemide, one had nephrocalcinosis on renal ultrasonography, and two showed possibilities of future stone formation. One of them was a carrier of idiopathic hypercalciuria, and the father of the third had had lithiasis. A direct relationship was observed between bicarbonaturia and maximum UpCO<sub>2</sub> determined using the combined stimulus of acetazolamide and NaHCO<sub>3</sub> in the entire sample. This observation was not surprising because UpCO<sub>2</sub> depends on the concentration of UHCO<sub>3</sub><sup>-</sup>. Furthermore, an inverse relationship was noted between the minimum UpH obtained in the test with the furosemide stimulus and the maximum UpCO<sub>2</sub>. This observation occurred because these two variables measure the capacity of the kidney to properly remove ammonium.

Finally, it is important to remember that this study was conducted when most of the patients had been diagnosed with and treated for several years for dRTA.

Twenty-six of the 30 children were shown not to have this tubulopathy.

In summary, only two cases in our studied population were genuine cases of dRTA: one of them was referred to a hospital in southeastern Mexico, while the other case was found through social networks located in the center of the country. It is worth mentioning that the patients evaluated were misdiagnosed with dRTA and may be examples of the many children that come to us referred from other hospitals in Mexico City with that diagnosis. Therefore, our results confirm the impression of Muñoz-Arizpe, et al.<sup>8</sup> regarding the overdiagnosis of dRTA in Mexico. Thus, it is important for the Pediatric Nephrology Units to have the capacity to test for acidification using reliable methods, particularly the maximum urine pCO<sub>2</sub> and/or the furosemide with fludrocortisone tests. As indicated above, the test with furosemide alone causes some false positives, and it is only useful as a screening test in the case of acidic urine. Furthermore, given the altitude of Mexico City, the values obtained, particularly in venous gases and in infants, may induce diagnostic errors. Therefore, calciuria, citraturia and chlorine plasma level determination are very useful very useful in directing the diagnosis and may be confirmed by a reliable acidification test.

## DECLARATION OF INTEREST

The authors declare no conflicts of interest.

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