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ACIDOSE TUBULAR RENAL DISTAL EM CRIANÇAS E ADOLESCENTES

PAULA CRISTINA DE BARROS PEREIRA

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Faculdade de Medicina da Universidade Federal de Minas Gerais, como requisito parcial para obtenção do grau de Mestre.

Área de Concentração: Saúde da Criança e do Adolescente

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DECLARAÇÃO

A Comissão Examinadora abaixo assinada, composta pelos Professores Doutores: Ana Cristina Simões e Silva, Eduardo Araújo Oliveira, Paulo Cesar Koch Nogueira, José Maria Penido Silva, aprovou a defesa da dissertação intitulada **"ACIDOSE TUBULAR RENAL DISTAL EM CRIANÇAS E ADOLESCENTES"** apresentada pela mestranda **PAULA CRISTINA DE BARROS PEREIRA** para obtenção do título de Mestre em Saúde da Criança e do Adolescente, pelo Programa de Pós-Graduação em Ciências da Saúde – Área de Concentração em Saúde da Criança e do Adolescente da Faculdade de Medicina da Universidade Federal de Minas Gerais, realizada em 12 de março de 2009.

Profa. Ana Cristina Simões e Silva
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ATA DA DEFESA DE DISSERTAÇÃO DE MESTRADO de **PAULA CRISTINA DE BARROS PEREIRA**, nº de registro 2008652534. Às nove horas do dia **doze do mês março de dois mil e nove**, reuniu-se na Faculdade de Medicina da UFMG, a Comissão Examinadora de dissertação indicada pelo Colegiado do Programa, para julgar, em exame final, o trabalho final intitulado: **"ACIDOSE TUBULAR RENAL DISTAL EM CRIANÇAS E ADOLESCENTES"**, requisito final para a obtenção do Grau de Mestre em Saúde da Criança e do Adolescente, pelo Programa de Pós-Graduação em Ciências da Saúde – Área de concentração em Saúde da Criança e do Adolescente. Abrindo a sessão, a Presidente da Comissão, Profa. Ana Cristina Simões e Silva, após dar a conhecer aos presentes o teor das Normas Regulamentares do Trabalho final, passou a palavra à candidata para apresentação de seu trabalho. Seguiu-se a argüição pelos examinadores, com a respectiva defesa da candidata. Logo após, a Comissão se reuniu sem a presença da candidata e do público para julgamento e expedição do resultado final. Foram atribuídas as seguintes indicações:

Profa. Ana Cristina Simões e Silva/Orientadora	Instituição: UFMG	Indicação: Aprovada
Prof. Eduardo Araújo Oliveira/Co-orientador	Instituição: UFMG	Indicação: Aprovado
Prof. Paulo Cesar Koch Nogueira	Instituição: UNIFESP	Indicação: Aprovado
Prof. José Maria Penido Silva	Instituição: USP	Indicação: Aprovado

Pelas indicações a candidata foi considerada APROVADA.

O resultado final foi comunicado publicamente à candidata pela Presidente da Comissão. Nada mais havendo a tratar, a Presidente encerrou a reunião e lavrou a presente ATA, que será assinada por todos os membros participantes da Comissão Examinadora. Belo Horizonte, 12 de março de 2009.

Profa. Ana Cristina Simões e Silva/Orientadora _____

Prof. Eduardo Araújo Oliveira/Co-orientador _____

Prof. Paulo Cesar Koch Nogueira _____

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Aos meus pais...

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RESUMO

Acidose tubular renal distal ou ATR tipo 1 compreende um grupo heterogêneo de afecções resultantes da disfunção dos túbulos distais que podem levar a um déficit de crescimento, nefrocalcinose, raquitismo e insuficiência renal crônica. O objetivo deste estudo foi descrever o curso clínico de um grupo de pacientes portadores de ATR distal e analisar os possíveis fatores preditivos independentes para o ganho de peso e de estatura ao final do tratamento. Os pacientes foram acompanhados entre 1984 e 2008 de acordo com nosso protocolo de seguimento. O teste t pareado foi utilizado para comparar os resultados dos dados clínicos e laboratoriais à admissão e no último controle. Um modelo de regressão logística foi utilizado para identificar as variáveis independentes associadas ao ganho de pelo menos um desvio padrão (DP) no z-escore para peso e altura. Foram analisados 33 pacientes (15 do sexo masculino) portadores de ATR distal. A etiologia primária predominou (60,6%). A idade ao diagnóstico foi de 2,67±3,1 anos e o tempo de seguimento clínico de 10,8±6,1 anos. Baseado nas curvas de peso/idade e estatura/idade, 58,3% dos pacientes recuperaram completamente o crescimento após o tratamento. O nível inicial de bicarbonato foi considerado um fator preditivo independente para o ganho de estatura. Em relação ao ganho de peso, os pacientes do sexo masculino apresentaram pior evolução. Acidose metabólica, distúrbios hidroeletrolíticos, hipercalciúria e nefrocalcinose apresentaram melhora significativa durante o seguimento clínico ($p<0,05$). Nossos dados mostraram o grande impacto do tratamento no controle metabólico, além de indicar fatores preditivos para a retomada do crescimento nos pacientes portadores de ATR distal.

Palavras-chave: acidose tubular renal. infância. deficit do crescimento. nefrocalcinose. acidose metabólica.

ABSTRACT

Distal renal tubular acidosis (RTA) refers to a heterogeneous group of diseases that result from distal tubular dysfunction and can lead to growth retardation, nephrocalcinosis, bone disease and, rarely, chronic kidney disease. This study aimed to describe the clinical course of distal RTA series and to analyze somatic growth by identifying possibly predictive factors of growth improvement. Patients were followed-up from 1984 to 2008 according to our standard protocol. Paired t test was used for comparison between pre and post-treatment results. A logistic regression model was applied to identify variables that were independently associated with the gain of at least one standard deviation (SD) in Z-score for height and weight. A total of 33 distal RTA patients (15 males) were analyzed. Primary disease was the commonest form (60.6%). The mean age at the diagnosis was 2.7±3.1 years and the mean duration of follow-up was 10.8±6.1 years. Based on weight/age and stature/age curves, 58.3% of the patients completely recovered growth after treatment. Bicarbonate levels at admission were independent predictors of stature gain at last visit and the male sex negatively affected the final weight gain. Metabolic acidosis, electrolyte disturbances, hypercalciuria and nephrocalcinosis also improved during follow-up ($p<0.05$). Our data showed the great impact of treatment on metabolic control and further indicated predictive factors of growth catch-up.

Key words: renal acidification. growth failure. nephrocalcinosis. metabolic acidosis.

LISTA DE ABREVIATURAS E SIGLAS

AC = anidrase carbônica

AE1 = basolateral chloride-bicarbonate exchanger
trocador aniônico cloro-bicarbonato

ATR = acidose tubular renal

CAII = carbonic anhydrase type II

CAIV = carbonic anhydrase type IV

CA2 = carbonic anhydrase gene

CI = confidence interval

COEP = Comitê de Ética em Pesquisa

DP = desvio padrão

eAE1 = red cell anion exchanger AE1

GPA = glycophorin A

HAZ = height for age Z-sore

HS = hereditary spherocytosis

IC = intervalo de confiança

kAE1 = kidney anion exchanger AE1

NBC-1 = sodium dependent bicarbonate co-transporter
co-transportador de bicarbonato dependente de sódio

ND = nefron distal

NHE-3 **=** sodium/hydrogenio exchanger
trocador aniônico sódio/hidrogênio

OR = odds ratio

PHA1 = pseudohypoaldosteronism type 1

PHA2 = pseudohypoaldosteronism type 2

RBC = red blood cells

RTA = renal tubular acidosis

SAO = Southeast Asian ovalocytosis

SD = standard deviation

TC = túbulo coletor

TD = túbulo distal

UFMG = Universidade Federal de Minas Gerais

WAZ = weight for age Z-score

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1 INTRODUÇÃO

O termo Acidose Tubular Renal (ATR) engloba diversas afecções caracterizadas por acidose metabólica secundária a um defeito na reabsorção tubular renal de bicarbonato (HCO_3^-) e/ou na excreção urinária de íons hidrogênio (H^+), enquanto a função glomerular é nada ou minimamente afetada. Todas as formas de ATR apresentam acidose metabólica hiperclorêmica, com intervalo aniônico normal. São doenças crônicas com impacto significativo na qualidade de vida dos pacientes quando não tratadas adequadamente, podendo evoluir com déficit do crescimento, osteoporose, raquitismo, nefrolitíase e até perda da função renal. Podem ser primárias, decorrentes de defeitos genéticos nos mecanismos de transporte dos túbulos renais, ou secundárias a doenças sistêmicas, ou ao efeito adverso de medicamentos [1, 2].

Os rins são responsáveis pela reabsorção do HCO_3^- filtrado e excreção de H^+ numa quantidade igual àquela produzida pelo metabolismo diário de proteínas. A resposta normal a acidemia consiste na reabsorção do HCO_3^- filtrado e aumento da excreção de ácidos, principalmente através da maior excreção de íons amônio (NH_4^+) na urina. Assim, para cada H^+ excretado há regeneração de um íon HCO_3^- no plasma [3, 4].

As ATR são classificadas em quatro categorias: ATR distal ou tipo 1; ATR proximal ou tipo 2 e ATR hipercalêmica ou tipo 4. A ATR mista ou tipo 3 é caracterizada por uma desordem que apresenta características mistas dos tipos 1 e 2 [1].

A seguir, serão brevemente descritos os subtipos de ATR em relação às características clínicas, fisiopatologia, etiologia, genética, diagnóstico e tratamento.

1.1 Acidose Tubular Renal Distal - ATR Tipo 1

A ATR distal ou tipo 1 caracteriza-se por uma inabilidade dos túbulos distal e coletor em promover uma adequada acidificação urinária, resultando numa urina com pH elevado,

mesmo em presença de acidose metabólica [1]. Inicialmente, a função glomerular encontra-se normal ou perto do valor normal em todos os casos [4].

Em relação ao quadro clínico, observam-se déficit de crescimento, poliúria, hipercalciúria, nefrocalcinose e nefrolitíase. Ao diagnóstico grande parte dos pacientes apresenta-se com baixa estatura e baixo peso [5-10]. O retardo do crescimento aparece como queixa principal em quase todos os estudos realizados até o momento [5-10]. Dentre os sintomas clínicos, vômitos e poliúria encontram-se entre os mais prevalentes [5-8]. A nefrocalcinose, presente em cerca de 50% dos pacientes ao diagnóstico [5, 6, 10], pode progredir para insuficiência renal crônica. Na ATR distal diagnosticada precocemente a instituição do tratamento com álcalis pode promover a retomada da curva de crescimento, impedir ou retardar o desenvolvimento da nefrocalcinose e preservar a função renal.

1.1.1 Fisiopatologia

A ATR distal pode resultar dos seguintes defeitos nos túbulos distais: diminuição da atividade da H^+ATPase (ATR-1 secretora); aumento da permeabilidade da membrana luminal (ATR-1 por difusão retrógrada) ou diminuição da reabsorção distal de sódio (Na^+) (ATR-1 voltagem dependente) [11].

A diminuição, ou mesmo ausência, de atividade da H^+ATPase das células intercaladas dos túbulos distais e coletores, geralmente é secundária a um defeito genético. Também já foram detectadas mutações no trocador Cl^-/HCO_3^- (AE1) que, assim como a H^+ATPase, participa do processo de acidificação urinária, reabsorvendo HCO_3^-. Outra possibilidade para explicar este sub-tipo da ATR distal seria a existência de um defeito na H^+/K^+ATPase, localizada na membrana apical das células intercaladas. No entanto, alguns autores acreditam que esta bomba esteja mais relacionada à homeostase do potássio (K^+) que do H^+ [1].

O modelo de Stewart [12] é uma forma alternativa de entendimento dos distúrbios ácido-básicos, que se baseia nas leis de conservação de massa e carga. Levando-se em conta que o plasma é formado por íons totalmente dissociados (íons “fortes” tais como Na^+, K^+, Cl^-, lactato), ácidos “fracos” parcialmente dissociados (albumina e fosfato) e tampões voláteis (espécies carbonadas), Stewart elaborou uma equação polinomial que relaciona a concentração de H^+ com três variáveis independentes: a diferença entre os íons “fortes”, a

concentração total de ácidos fracos e a pressão parcial de gás carbônico (CO_2) [12]. Dessa forma, o modelo de Stewart avalia as acidoses metabólicas através de mudanças nas concentrações de íons considerados "fortes" aferidas pela seguinte operação matemática: $[Na^+]+[K^+]-[Cl^-]$. Segundo essa nova visão, acredita-se que a ATR seja devida primariamente a um defeito nas proteínas de transporte do íon H^+ que acarretam, secundariamente, em alterações no transporte do cloro (Cl^-). Tais alterações no transporte de Cl^- reduzem a diferença entre os íons "fortes", resultando em acidose metabólica. Este modelo considera que as alterações na excreção do bicarbonato são apenas um epifenômeno, decorrente das mudanças na excreção urinárias dos íons "fortes" [13]. Esta nova visão dos distúrbios ácido-básicos permite um melhor entendimento dos achados clínicos e laboratoriais em alguns tipos de ATR distal como as decorrentes de mutações do trocador AE1, assim como explica a piora da acidose nesses pacientes, induzida por uma carga excessiva de cloreto de sódio (NaCl) 0,45% [14].

O exemplo clássico de ATR distal, causada por aumento da permeabilidade da membrana luminal do néfron distal (ND), está associado ao uso de anfotericina B. Postulava-se, inicialmente, que esta droga formaria canais aquosos na membrana das células do túbulo distal (TD) e promoveria o retorno de íons H^+ para o interior da célula. Este processo foi chamado de difusão retrógrada. Recentemente, entretanto, esta teoria tem sido questionada. Alguns autores acreditam que o H_2CO_3 e/ou o HCO_3^-, e não o H^+, possam retornar para a luz tubular [11]. O relato de crianças com quadros de ATR distal associada a doenças auto-imunes sugere que o mecanismo de difusão retrógrada como causa ainda não foi totalmente esclarecido [15].

A reabsorção de Na^+ no túbulo coletor (TC) cria uma diferença de potencial lúmen-negativa, que é fundamental para a secreção de íons H^+ e K^+ [16]. Os fatores relacionados à diminuição da reabsorção de Na^+ ou de seu aporte ao TD, podem reduzir a capacidade secretora deste segmento do néfron, que é voltagem dependente [11]. Os fatores mais comumente relacionados a este tipo de ATR são a uropatia obstrutiva, a depleção volumétrica e o uso de diuréticos poupadores de K^+ [17]. Como a secreção de K^+ está igualmente comprometida, também pode evoluir com elevação dos níveis séricos deste cátion [11]. Recentemente, entretanto, a teoria de que a ATR distal hipercalêmica seja causada por defeito isolado de uma diferença de potencial transmembrana tem sido questionada. Alguns estudos têm demonstrado que os mecanismos envolvidos são bem mais complexos, envolvendo também defeitos no funcionamento da H^+ATPase, H^+/K^+ATPase e Na^+/K^+ATPase [17].

1.1.2 Etiologia e Genética

A ATR distal pode ser primária, devido a defeitos genéticos nos mecanismos de transporte, ou secundária a uma variedade de doenças [4]. Dentre as formas primárias podemos encontrar as seguintes variantes: autossômica dominante e autossômica recessiva com ou sem surdez. Na criança, o defeito é, na maioria das vezes, primário, como uma forma de herança autossômica dominante ou recessiva [1].

Em algumas famílias, a presença da doença em várias gerações sugere uma forma autossômica dominante. Apesar das manifestações clínicas não serem diferentes das observadas nas formas autossômicas recessivas ou nas esporádicas, estes pacientes podem ter seu diagnóstico mais tardio e evoluírem com sintomatologia mais branda [18]. Acredita-se que mutações no gene *SLC4A1*, que codifica o trocador Cl^-/HCO_3^- (AE1), localizado na membrana basolateral das células intercaladas tipo α, possam estar associadas a esta forma de ATR [2, 19]. Ressalta-se que mutações nesse trocador já foram descritas em crianças do nosso meio, portadoras de ATR distal e nefrocalcinose [20].

Pacientes com a forma autossômica recessiva geralmente apresentam manifestações clínicas mais acentuadas com importante déficit de crescimento e nefrocalcinose precoce, podendo evoluir para insuficiência renal [19]. Os achados clínicos dos pacientes autossômicos recessivos acompanhados de surdez neurosensorial são idênticos aos dos pacientes portadores de ATR distal esporádica ou autossômica recessiva com audição normal [21, 22]. A evolução da surdez é progressiva e não há melhora, mesmo após terapia com álcalis [12]. As mutações no gene *ATP6V1B1*, que codifica a subunidade B1 da H^+ATPase, localizada na membrana apical das células intercaladas tipo α, já foram detectadas em pacientes portadores de ATR distal autossômica recessiva associada à surdez neurosensorial [22]. Demonstrou-se que as células auditivas interdentais e as células do saco endolinfático são muito semelhantes às células intercaladas do tipo α, apresentando tanto a H^+ATPase como o trocador AE1. Assim, uma secreção normal de ácidos por estas células é fundamental para a manutenção de um pH reduzido na endolinfa e uma função auditiva normal [18].

A ATR distal, autossômica recessiva, com função auditiva normal é a forma primária mais comumente encontrada. Acredita-se que mutações no gene *ATP6V0A4*, que codifica a subunidade a4 da H^+ATPase, possam ser responsáveis pelo desenvolvimento desta forma de ATR [14]. Por outro lado, alguns autores acreditam que pacientes com esta mutação possam vir a desenvolver a surdez após a segunda década de vida [1].

Dentre as causas secundárias, que são mais comuns em pacientes adultos do que pediátricos, incluem-se [23-25]:

a) doenças auto-imunes: síndrome de Sjögren, hepatite crônica ativa, tireoidite, poliarterite nodosa, hiperparatireoidismo primário, rim esponjoso medular, doença de Wilson, artrite reumatóide e lúpus eritematoso sistêmico;
b) uso de medicamentos: anfotericina B, sulfametoxazol-trimetoprim, amilorida, lítio, analgésicos;
c) exposição ao tolueno (cheiradores de cola) e ao mercúrio;
d) doenças túbulo-intersticiais: uropatia obstrutiva, pielonefrite crônica, transplante renal;
e) doenças genéticas: Síndrome de Ehlers-Danlos.

1.1.3 Diagnóstico

O diagnóstico de ATR distal deve ser suspeitado em presença de acidose metabólica hiperclorêmica acompanhada de *anion gap* urinário positivo, ou seja, de uma concentração de Cl^- na urina inferior à soma das concentrações de sódio e potássio. Nessas circunstâncias, se a concentração plasmática de potássio é normal ou está reduzida, e o paciente é incapaz de reduzir o pH urinário para valores inferiores a 5,5, estabelece-se o diagnóstico de ATR distal. A excreção urinária de citrato geralmente está diminuída, devido a sua maior reabsorção proximal, estimulada pela acidose. A excreção urinária aumentada de cálcio (Ca^{2+}) associada a hipocitratúria e ao pH urinário persistentemente elevado, pode contribuir para o desenvolvimento de nefrolitíase e nefrocalcinose. Tais alterações são comuns na ATR distal não tratada, embora existam relatos de nefrocalcinose na ausência de hipercalciúria [26].

O raquitismo e a diminuição da massa óssea também podem ser encontrados, mas sua real incidência ainda é incerta. A acidose metabólica também pode, por si só, alterar o metabolismo da vitamina D, diminuindo sua produção renal, com conseqüente déficit na reabsorção intestinal de Ca^{2+} e doença óssea secundária [23].

A hipocalemia está presente em 30 a 50% dos casos. Pode manifestar-se como fraqueza muscular, às vezes com episódios agudos de paralisia flácida, que podem evoluir para tetraplegia em até 48h [27].

A apresentação clínica da ATR distal engloba, além do déficit de crescimento nas crianças, um quadro de anorexia, vômitos e poliúria [28]. Algumas condições podem

mimetizar a ATR distal com pH urinário maior que 5,5. Pacientes portadores de infecção urinária por bactérias urease-positivas podem apresentar pH urinário alcalino, porém, em geral, não apresentam acidose sistêmica. Além disso, o exame microbiológico e o sedimento urinário exibem alterações típicas. A fase inicial da ATR proximal, quando ainda há perda urinária de álcalis e hipovolemia, também pode confundir-se com a ATR distal. A depleção de K^+ e o aumento da excreção urinária de amônia (NH_3), que podem ocorrer na acidose metabólica por diarréia aguda, simulam, algumas vezes, o quadro laboratorial desta patologia [1].

É importante ressaltar ainda que existem formas incompletas de ATR distal que dificultam o diagnóstico, pois, muitas vezes, os pacientes apresentam-se com pH sangüíneo normal e pH urinário apenas levemente aumentado. Nesses casos, pode ser necessária a utilização de provas de acidificação urinária por meio da administração oral de cloreto de amônio (NH_4Cl), em pó ou em cápsula, na dose de 0,1 grama/Kg. Recomenda-se que seja coletada a urina a cada hora, nas próximas 8 horas; e a gasometria seja realizada no início do teste e a cada hora, nas 4 horas subseqüentes à administração do ácido. Se o pH urinário falha em cair abaixo de 5,5 durante a quarta hora após o NH_4Cl, é provável que a ATR distal esteja presente, desde que um pH sangüíneo inferior a 7,35 e um bicarbonato menor que 20 mEq/l sejam documentados.

1.1.4 Tratamento

O objetivo do tratamento consiste não só na correção das alterações bioquímicas, mas principalmente na retomada do crescimento e na prevenção da nefrocalcinose e da insuficiência renal. Os pacientes adequadamente tratados geralmente são assintomáticos e podem levar uma vida normal, a não ser que já tenha havido lesão renal ou óssea irreversíveis [1]. A normalização do pH sérico diminui a perda urinária de K^+ e previne a litíase e o desenvolvimento da nefrocalcinose [29]. A correção da acidose também reverte as alterações no metabolismo das células ósseas, aumentando, conseqüentemente, a densidade mineral do osso [30].

A base do tratamento constitui na administração de doses contínuas e adequadas de álcalis, sob a forma de HCO_3^- ou citrato. A quantidade ofertada deve ser suficiente para suprir as perdas urinárias de HCO_3^-, além da demanda diária gerada pela contínua produção de

ácidos pelo organismo, secundária ao catabolismo protéico. Em pacientes mais jovens, podem ser necessários de 4 a 14 mEq/Kg de bicarbonato de sódio, que deve ser oferecido em doses fracionadas [1, 28]. Crianças maiores e adultos, em geral, necessitam de doses menores. O citrato de potássio também pode ser utilizado em doses de 4 mEq/Kg/dia. A dose de álcalis é considerada adequada quando é suficiente para corrigir a maioria das anormalidades urinárias, inclusive a hipercalciúria. Deve-se evitar o uso de doses excessivas de álcalis, que podem elevar excessivamente o pH urinário, propiciando a precipitação do cálcio excretado em excesso. Recomenda-se, então, evitar que o pH urinário torne-se superior a 8,0. Idealmente, o pH urinário deve ser mantido entre 6,5 e 7,5 e a gasometria revelar equilíbrio ácido básico. A monitoração individual é fundamental para o ajuste das doses [31].

A correção da hipercalciúria é mandatória, mesmo em presença de uma excreção urinária adequada de citrato. O citrato pode melhorar a saturação urinária para o oxalato de cálcio, mas não reverte a tendência para a saturação renal do fosfato de cálcio no osso [31]. A monitoração do Ca^{2+} urinário, através da relação cálcio/creatinina em amostra de urina e/ou dos níveis de cálcio na urina de 24 horas é importante para a avaliação do tratamento [32]. O uso de diuréticos tiazídicos é uma opção terapêutica para controlar a hipercalciúria, quando a excreção urinária de cálcio persiste aumentada mesmo após correção do distúrbio ácido-básico [33].

Pacientes portadores de ATR distal primária vão requerer tratamento prolongado, possivelmente por toda a vida. Em geral o prognóstico é excelente, sobretudo para as crianças precoce e adequadamente tratadas. O uso adequado da terapia alcalina pode restabelecer o crescimento e prevenir a progressão para nefrocalcinose [1].

1.2 Acidose Tubular Renal Proximal - ATR Tipo 2

A ATR proximal ou do tipo 2 caracteriza-se por um defeito na reabsorção tubular proximal de HCO_3^-, determinando acidose metabólica hiperclorêmica. A hipercloremia se deve ao aumento da reabsorção do Cl^-, estimulada pela diminuição do volume extracelular [1]. Como esta porção do néfron é responsável pela reabsorção da maior parte do HCO_3^- filtrado, a acidose tende a ser mais acentuada e de difícil controle [26].

A ATR proximal isolada é uma forma rara de ATR [26]. Do ponto de vista clínico, os pacientes apresentam-se com vômitos, poliúria, polidipsia, desidratação, fraqueza muscular e,

principalmente, déficit de crescimento [34-36]. A ATR proximal também pode ocorrer como parte de um defeito generalizado do transporte no TP, caracterizando a Síndrome de Fanconi [11, 23, 34], que veremos mais adiante.

O papel principal dos túbulos proximais no processo de acidificação urinária consiste na secreção de H^+, através do trocador Na^+/H^+ (NHE-3), e no transporte de HCO_3^-, através do co-transportador Na^+-HCO_3^- (NBC-1) [37]. A enzima anidrase carbônica (AC) tem papel fundamental nesse mecanismo. As suas isoformas citossólica (II) e intraluminal (IV) são altamente estimuladas pela acidose metabólica crônica [1, 3, 18].

1.2.1 Etiologia e Genética

A ATR proximal pode ocorrer como uma desordem isolada, sem associação com outras doenças e/ou anomalias do TP [26]. Esta forma pode ser transitória ou persistente, esporádica ou adquirida. A natureza transitória sugere uma imaturidade da função dos trocadores NHE-3 [18] que, após o crescimento, se normaliza. Pode ser também decorrente à exposição a alguns tipos de medicamentos, como ifosfamida (análogo da ciclofosfamida) e a tetraciclina [25]. Já a ATR proximal permanente pode ser autossômica recessiva ou dominante. A forma recessiva está associada a anormalidades oculares (catarata, glaucoma) e é causada por uma mutação no gene *SLC4A4* que codifica o co-transportador NBC-1 (mesma família *SLC4* que codifica o AE1), localizado na membrana basolateral das células do TP [21]. Sobre a forma dominante pouco se sabe atualmente. Alguns estudos experimentais sugerem alterações genéticas em ratos [18]; porém, em humanos, nenhuma mutação foi ainda descrita.

1.2.2 Diagnóstico

O diagnóstico de ATR proximal ou tipo 2 deve sempre ser suspeitado em presença de acidose metabólica hiperclorêmica que se acompanha de normo ou hipopotassemia e de um *anion gap* urinário negativo, isto é, de uma concentração de Cl^- na urina superior à soma das concentrações de Na^+ e K^+.

Se o diagnóstico desta doença for realizado precocemente, observa-se um pH urinário alcalino devido às perdas excessivas de HCO_3^-. Com o passar do tempo, os níveis séricos de HCO_3^- caem de tal forma que o TD é capaz de reabsorver essa carga, ocorrendo acidificação urinária normal. Nesta fase, os níveis sangüíneos de HCO_3^- encontram-se na faixa de 15 a 18 mEq/L [26].

1.2.3 Tratamento

O tratamento tem como base a reposição de álcalis sob a forma de HCO_3^- ou citrato. A maioria das crianças requer doses de bicarbonato de sódio de 10 a 20 mEq/Kg/dia, para manter o pH sérico dentro dos limites da normalidade [1, 23].

As formas autossômicas dominante e recessiva geralmente são permanentes e vão requerer o uso do HCO_3^- por toda a vida. A maioria desses pacientes retomará seu crescimento, mas dificilmente atingirá uma estatura normal [36]. As formas esporádicas, em contraste, são transitórias e o tratamento com álcalis pode ser descontinuado após alguns anos, sem reaparecimento dos sintomas [35].

1.2.4 ATR proximal com Síndrome de Fanconi

Como já mencionado, caso haja acometimento isolado do transporte proximal de HCO_3^- tem-se a ATR proximal isolada, sem Síndrome de Fanconi associada. Se, por outro lado, todos os mecanismos de transporte do TP forem acometidos, produzindo deficiência na reabsorção de glicose, aminoácidos, fosfato (PO_4^{-3}) e também de HCO_3^-, tem-se a Síndrome de Toni-Debrè-Fanconi, mais comumente denominada Síndrome de Fanconi [3, 26, 35]. As manifestações clínicas dependem do grau de acometimento tubular e da etiologia da síndrome. Na criança, estão presentes atraso do crescimento e raquitismo resistente à vitamina D. No adulto, observa-se osteomalácia [34]. A poliúria está presente com freqüência, podendo ser causa de febre e desidratação [36].

A Síndrome de Fanconi pode ocorrer devido à ocorrência de erros inatos do metabolismo transmitidos geneticamente (cistinose, intolerância à frutose, galactosemia,

glicogenose, síndrome de Lowe, tirosinemia e doença de Wilson) e em algumas doenças adquiridas bem como na exposição acidental a toxinas, metais pesados (cádmio, chumbo e mercúrio), ou a certas drogas (tetraciclina, gentamicina, ácido valpróico, cisplatina e azatioprina). Mais comumente, a Síndrome de Fanconi é idiopática e sua ocorrência pode ser esporádica, sem qualquer evidência de transmissão genética. Mais raramente, há relatos de casos herdados como um traço dominante ou recessivo [36].

A fisiopatologia da Síndrome de Fanconi ainda não foi completamente elucidada. Acredita-se que o defeito possa estar relacionado à produção deficiente de energia com conseqüente alteração do funcionamento da Na^+/K^+ATPase. Outras possibilidades referem-se às anormalidades na permeabilidade da membrana apical e basolateral, ao fluxo bilateral ou retrógrado através das *tight-junctions*, ou às alterações patológicas numa organela celular específica [28, 36].

Os exames laboratoriais mostram acidose metabólica hiperclorêmica com intervalo aniônico normal, hipocalemia, hipofosfatemia e hipouricemia. A fração de excreção de PO_4^{-3} está elevada assim como a atividade da fosfatase alcalina. A glicosúria está presente com níveis séricos normais de glicose. Há aminoacidúria inespecífica. O pH urinário está normal na ausência de tratamento, podendo haver baixos níveis de amônia e acidez titulável. Se houver queda da função glomerular durante a evolução da doença, ocorre uma melhora paradoxal dos níveis séricos de eletrólitos e uma redução da aminoacidúria, glicosúria e fosfatúria [34].

Não há nenhum método diagnóstico específico para a Síndrome de Fanconi. Os achados laboratoriais citados associados à clínica de déficit de crescimento e de raquitismo resistente à vitamina D são bastante sugestivos.

A expressão clínica e bioquímica varia de paciente para paciente, de modo que não há um tratamento universal. Em pacientes com Fanconi secundário, o tratamento está voltado para a causa primária da doença. Naqueles com a síndrome primária, a terapia de reposição eletrolítica pode restabelecer o balanço mineral e de eletrólitos, prolongar a sobrevida e, em alguns casos, permitir uma vida normal. O raquitismo pode ser corrigido bem como as deformidades ósseas, porém um crescimento normal raramente é obtido [35, 38].

1.3 Acidose Tubular Renal Hipercalêmica - ATR Tipo 4

O espectro clínico da ATR hipercalêmica abrange pacientes com hipoaldosteronismo, tanto primário quanto associado à hiporreninemia em pacientes com doença renal crônica, e pacientes com pseudohipoaldosteronismo [18, 39]. São descritos também casos de causa indeterminada e caráter transitório que surgem nos primeiros anos de vida, desaparecendo até os três a cinco anos de idade [18].

1.3.1 Etiologia

A ATR hipercalêmica pode resultar de doenças da glândula adrenal, como Doença de Addison, hiperplasia congênita de supra-renal e hipoaldosteronismo primário, onde há déficit na produção de aldosterona. Nestes casos a função renal está normal, há freqüentemente perda urinária de sódio, a atividade de renina plasmática está elevada e a aldosterona urinária baixa.

O hipoaldosteronismo hiporreninêmico consiste numa forma de ATR hipercalêmica resultante de doenças renais associadas a dano intersticial e destruição do aparelho justa-glomerular, como lesões obstrutivas, pielonefrite, nefrite intersticial, diabetes mellitus e nefroesclerose, apesar de também poder ser percebido em casos de hipervolemia com inibição da ação das prostaglandinas. Neste caso, os níveis séricos de renina e conseqüentemente de aldosterona estão diminuídos e a função renal pode não estar preservada.

Raramente a ATR hipercalêmica pode ocorrer devido a uma não resposta do túbulo distal à aldosterona (pseudohipoaldosteronismo). Os níveis plasmáticos de renina e de aldosterona estarão elevados, a função renal estará preservada e a perda urinária de NaCl é a regra.

1.3.2 Fisiopatologia

A ATR hipercalêmica ou tipo 4 foi identificada em pacientes com hipercalemia de diversas causas [18, 39]. O defeito tubular é de caráter complexo, ocorrendo simultaneamente alterações na reabsorção de bicarbonato e na secreção de H^+, que se manifestam fundamentalmente por uma diminuição da excreção urinária de amônio [39]. Os mecanismos

de acidificação urinária estão intactos e o defeito de reabsorção de bicarbonato é menos acentuado do que na ATR proximal [18].

A alteração tubular situa-se nos segmentos do túbulo distal sensíveis à aldosterona [39]. A principal causa é a deficiência de aldosterona, embora também possa ocorrer em decorrência de refratariedade da célula tubular renal à ação da aldosterona (pseudohipoaldosteronismo) e a alterações tubulares primárias [39]. Quando a alteração tubular se deve ao mecanismo de diminuição da reabsorção de Na^+, pode ser afetada tanto a secreção de H^+ como de K^+, podendo levar à ATR hipercalêmica [40].

1.3.3 Diagnóstico

O diagnóstico é sugerido pela presença de acidose metabólica hiperclorêmica associada a *anion gap* urinário positivo e a uma elevação, ainda que discreta, da concentração plasmática de K^+ [18]. Para adequada avaliação desses pacientes, utiliza-se a administração aguda de furosemida na dose de 1 mg/ Kg. Pacientes com hipoaldosteronismo hiporreninêmico apresentarão uma urina com pH inferior a 5,5, acentuada hipoamoniuria e persistência de níveis plasmáticos reduzidos de renina e aldosterona. Pacientes com expansão do volume extracelular e inibição secundária do eixo renina-aldosterona exibirão, por outro lado, uma resposta que será indistinguível de controles sadios [18]. O diagnóstico do pseudohipoaldosteronismo é realizado em pacientes com quadro sugestivo de hiperplasia adrenal congênita [41], caracterizado por hiponatremia e hipercalemia, mas que se associa a níveis plasmáticos aumentados de renina e aldosterona bem como excreção urinária normal de 17-cetosteróides e pregnantriol [39].

1.3.4 Tratamento

O tratamento da ATR hipercalêmica deve ser voltado à causa primária do distúrbio. Nos casos de hipoaldosteronismo primário, a suplementação com fluorocortisona será a terapêutica de eleição [18, 39]. Caso haja contra-indicações para o seu uso, tais como hipertensão e edema que podem ser exacerbados com o uso de mineralocorticóides, está indicada dieta hipocalêmica e diurético de alça para o controle da hipercalemia. Em caso de

pseudohipoaldosteronismo, o tratamento consiste na suplementação oral de cloreto de sódio (3-5 gramas/ dia), após correção venosa do estado de hidratação [39].

1.4 Considerações

É importante ressaltar ainda que, apesar de não apresentarem incidência tão elevada em nosso meio como outras nefropatias, as acidoses tubulares assumem grande importância, não só pela dificuldade diagnóstica, mas também pelo grande impacto sobre o crescimento pôndero-estatural das crianças acometidas. Dessa forma, justifica-se claramente a necessidade de um maior entendimento da fisiopatologia, evolução, tratamento e prognóstico das acidoses tubulares na infância. Dentro dessa perspectiva, esta dissertação de Mestrado está inserida em uma linha de pesquisa que aborda as tubulopatias em pediatria. O presente estudo tem, então, por objetivo descrever o curso clínico de uma coorte de pacientes pediátricos com ATR distal, acompanhados de forma sistemática por longo período de tempo no intuito de identificar as variáveis que contribuem para a melhora dos parâmetros de crescimento. O foco principal do presente estudo em ATR distal se deveu a maior prevalência deste subtipo e pelo fato de que sua detecção precoce reveste-se de especial importância, uma vez que pode modificar completamente o futuro da criança acometida, não só em relação ao crescimento, mas também à prevenção de lesão renal, muitas vezes irreversível.

Finalmente, é necessário explicar que essa dissertação foi elaborada conforme o modelo aprovado pelo Programa de Pós-Graduação em Ciências da Saúde – Saúde da Criança e do Adolescente da Faculdade de Medicina da UFMG, que permite sua confecção sob a forma de artigos científicos já submetidos ou a serem submetidos para publicação em revistas médicas. Sendo assim, a apresentação do trabalho segue a seguinte estrutura:

a) Seção de Introdução (que acaba de ser apresentada);
b) Seção de Revisão da Literatura, apresentada sob a forma do artigo: *Molecular Pathophysiology of Renal Tubular Acidosis* (artigo publicado pelo periódico Current Genomics em janeiro de 2009);
c) Seção de Objetivos;
d) Seção de Pacientes e Métodos;

e) Seção de Resultados e Discussão, apresentada sob a forma do artigo original: *Clinical course of 33 children with distal renal tubular acidosis* (artigo submetido ao periódico Pediatric Nephrology);

f) Seção de Comentários Finais;

g) Anexos.

As referências bibliográficas estão dispostas ao final de cada artigo ou seção. As referências dos artigos seguem as normas do periódico específico para o qual o mesmo foi ou será submetido. As referências listadas ao final de cada seção estão dispostas em ordem de citação e seguem as normas de Vancouver.

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2 REVISÃO DA LITERATURA (REVIEW ARTICLE)

MOLECULAR PATHOPHYSIOLOGY OF RENAL TUBULAR ACIDOSIS

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ABSTRACT

Renal tubular acidosis (RTA) is characterized by metabolic acidosis due to renal impaired acid excretion. Hyperchloremic acidosis with normal anion gap and normal or minimally affected glomerular filtration rate defines this disorder. RTA can also present with hypokalemia, medullary nephrocalcinosis and nephrolitiasis, as well as growth retardation and rickets in children, or short stature and osteomalacia in adults. In the past decade, remarkable progress has been made in our understanding of the molecular pathogenesis of RTA and the fundamental molecular physiology of renal tubular transport processes. This review summarizes hereditary diseases caused by mutations in genes encoding transporter or channel proteins operating along the renal tubule. Review of the molecular basis of hereditary tubulopathies reveals various loss-of-function or gain-of-function mutations in genes encoding cotransporter, exchanger, or channel proteins, which are located in the luminal, basolateral, or endosomal membranes of the tubular cell or in paracellular tight junctions. These gene mutations result in a variety of functional defects in transporter/channel proteins, including decreased activity, impaired gating, defective trafficking, impaired endocytosis and degradation, or defective assembly of channel subunits. Further molecular studies of inherited tubular transport disorders may shed more light on the molecular pathophysiology of these diseases and may significantly improve our understanding of the mechanisms underlying renal salt homeostasis, urinary mineral excretion, and blood pressure regulation in

health and disease. The identification of the molecular defects in inherited tubulopathies may provide a basis for future design of targeted therapeutic interventions and, possibly, strategies for gene therapy of these complex disorders.

Key words: renal tubular acidosis, acid-base homeostasis, molecular physiology, tubular transport, gene mutations

2.1 Introduction

The term Renal Tubular Acidosis (RTA) defines many disorders characterized by metabolic acidosis, secondary to defects in renal tubular reabsorption of bicarbonate (HCO_3^-) and/or in urinary excretion of hydrogen (H^+), while glomerular function is little or not affected [1, 2, 3, 4, 5, 6]. All forms of RTA present hyperchloremic metabolic acidosis, with normal anion gap and are chronic diseases with significant impact on the quality of life of affected patients when left untreated, possibly leading to growth failure, osteoporosis, rickets, nephrolithiasis and even renal insufficiency [1, 2, 3, 4, 5, 6].

Defects in proximal bicarbonate reclamation or distal acid secretion give rise to the respective clinical syndromes of proximal or distal RTA [1, 2, 3, 4, 5, 6]. These disorders can be primary, originating from genetic defects on tubular transport mechanisms [7], or secondary to systemic diseases and to adverse drug reactions [8, 9, 10, 11, 12]. The familial conditions exhibit distinct inheritance patterns. Distal RTA can be transmitted as either an autosomal dominant or an autosomal recessive trait, whereas isolated proximal RTA usually occurs as an autosomal recessive disease [6, 7, 13]. In the past few years, the molecular genetic strategies of positional cloning and candidate gene analysis have been combined to identify the genes responsible for these inherited conditions [6, 13]. This review will summarize the mechanisms of acid-base regulation by the kidney and the current understanding of the genetic causes of primary inherited RTA. It will, in addition, evaluate the ability of known functional and biochemical properties of these mutant proteins to explain the pathophysiology of associated renal acidification defects.

2.2 Brief overview of renal acid-base homeostasis

The kidney plays two major roles in acid-base homeostasis. First, the filtered bicarbonate load (approximately 4000 mmol/day) must be reabsorbed, mainly in the proximal tubule and beyond in the loop of Henle and distal nephron. This reclamation process in the proximal tubule minimally requires the following: hydrogen (H^+) secretion of an equivalent amount via the luminal Na^+/H^+ exchanger (NHE-3) and the vacuolar H^+-ATPase; luminal carbonic anhydrase type IV (CAIV) and cytosolic carbonic anhydrase type II (CAII); and basolateral bicarbonate exit through the electrogenic Na^+-dependent bicarbonate cotransporter (NBC-1) [2, 14, 15, 16, 17]. Second, the kidney must regenerate new bicarbonate (approximately 50 ± 100 mmol/ day) in the process of acid-secretion, mainly in the collecting ducts, to match the amount of newly produced acid load by systemic metabolism [18, 19]. In addition to sufficient buffer in the lumen, this process requires activities of several transport proteins of the acid secreting α-intercalated cells, including the luminal vacuolar H^+-ATPase, CA II, and the basolateral chloride-bicarbonate exchanger, AE1 [18, 20, 21].

2.2.1 Proximal tubular bicarbonate reabsorption

HCO_3^- is freely filtered at the glomerulus and approximately 80 to 90% of this is reabsorbed in the proximal tubule [6]. In the tubular lumen, HCO_3^- combines with H^+ in a reaction catalyzed by CA IV, which is bound to the luminal membrane of proximal tubular cells [2, 14, 15]. This reaction produces carbonic acid, which is promptly converted to CO_2 and H_2O. The resulting CO_2 rapidly diffuses into the tubular cells and is combined with water to produce intracellular H^+ and HCO_3^-. This intracellular reaction is catalyzed by CA II. HCO_3^- is then cotransported with Na^+ into blood (with a probable stoichiometry of 3 HCO_3^- to 1 Na^+) [6] via the NBC-1, located on the basolateral cell membrane. The intracellular H^+ produced by CA II is secreted into the tubular lumen predominantly via the NHE-3, situated on the luminal membrane [6, 15, 22]. This transport process is called facilitated diffusion and depends on the sodium concentration gradient generated by the action of a basolateral membrane Na^+-K^+-ATPase. It should be mentioned that there is minimal net acid excretion in the proximal tubule, since most of the H^+ secretion is coupled with HCO_3^- reabsorption [6, 13]. The small amount of remaining H^+ will be buffered by phosphate as titratable acid.

HCO_3^- reabsorption is influenced by luminal HCO_3^- concentration and pH, luminal flow rate, peritubular pCO_2, and angiotensin II [2, 6, 17].

Proximal tubular cells are capable of generating "extra" bicarbonate through the deamination of glutamine to glutamate, then forming α-ketoglutarate and eventually glucose. This metabolic process produces HCO_3^- and NH_4^+: the former reclaimed via the basolateral membrane and the latter secreted into the tubular lumen. This pathway can be upregulated in states of chronic acidosis [3, 6, 15].

The main mechanisms of proximal tubular bicarbonate reabsorption are displayed in Fig. (**2.1**).

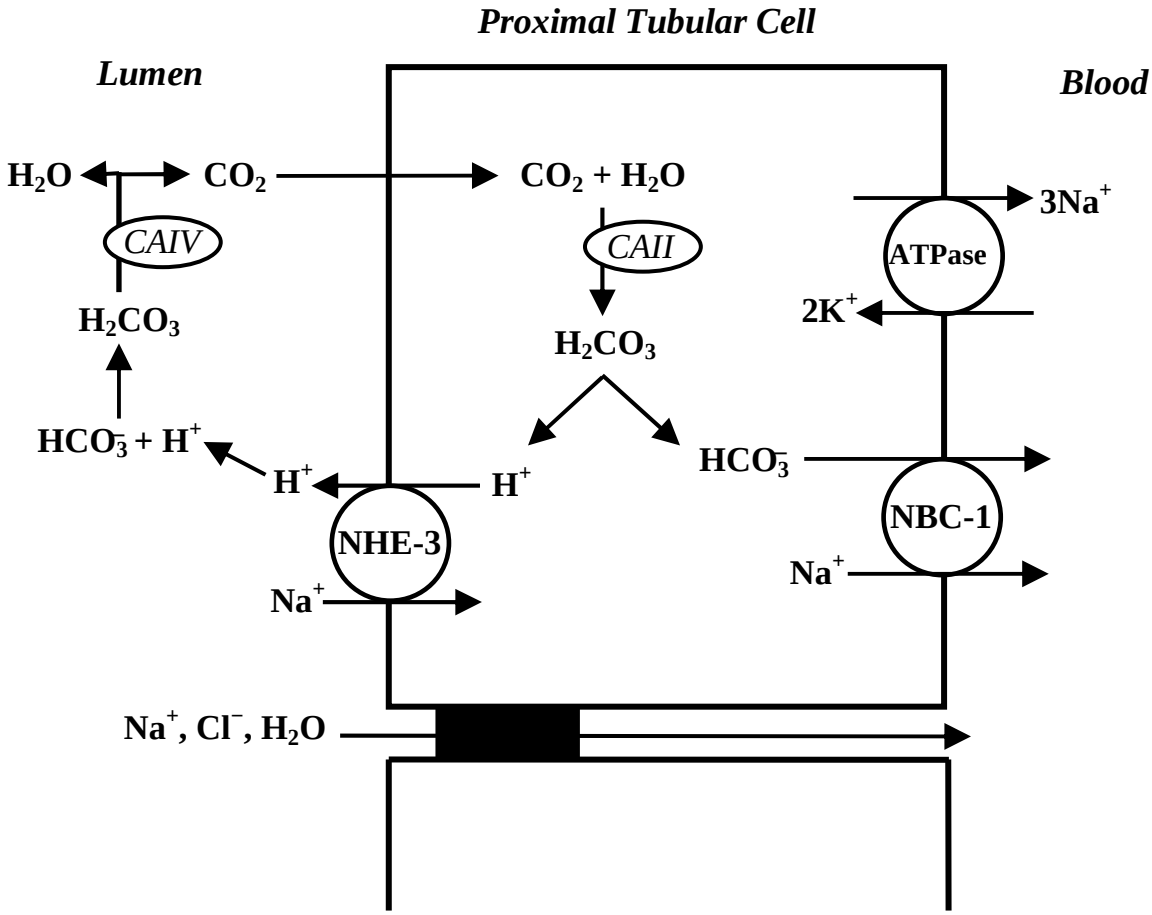

FIGURE 2.1 - *Schematic model of bicarbonate (HCO_3^-) proximal reabsorption. The intracellular carbonic acid (H_2CO_3) dissociates into H^+ and HCO_3^- in a reaction catalysed by a cytoplasmic carbonic anhydrase (CAII). At the luminal membrane, H^+ secretion is due to an especific $Na^+ - H^+$ exchanger (NHE-3), while, at the basolateral membrane, the 1 Na^+ - 3 HCO_3^- cotransporter (NBC-1) is responsible for HCO_3^- transport to the peritubular capilar. The secreted H^+ reacts with filtered HCO_3^- to form luminal H_2CO_3, which is dissociated into H_2O and CO_2 by the action of membrane-bound carbonic anhydrase (CAIV). The generated CO_2 diffuses back into the cell to complete the HCO_3^- reabsorption cycle.*

2.2.2 Distal tubular hydrogen secretion

One of the important roles of the collecting duct segment of the nephron is acid secretion, combined with reclamation of the approximately 10% of filtered HCO_3^- that is not reabsorbed by more proximal nephron segments [18]. The average omnivorous human diet in the `Western' world is rich in protein, and generates 1±1.5 mmol hydrogen/kg body weight each day [23]. Urinary acid excretion is therefore essential, and urine pH can drop as low as 4.5. The α-intercalated cell is the main responsible for hydrogen secretion into the urine. In humans at least, hydrogen pumps, called H^+-ATPases, mainly carry out hydrogen secretion [18, 19, 23]. H^+-ATPases are present at high density on the luminal membrane of α-intercalated cells [18]. Studies in nonhuman mammals show that these H^+-ATPases are also present within specialized intracellular tubulovesicles close to the membrane, allowing additional pumps to be recruited to the membrane quickly in to response to stimuli, such as systemic acidosis, for example [23]. These cells secrete H^+ into the lumen of the distal tubule and collecting duct not only via H+-ATPase but possibly also by an exchanger, H^+/K^+-ATPase [7, 10]. In addition, the normal function of the luminal H^+-ATPase in α-intercalated cells is coupled, in a poorly understood manner, to the electroneutral transport of HCO_3^- back across the basolateral surface into the interstitial fluid, and thence to blood. The transporter responsible for this activity in renal α-intercalated cells is the Cl^-/HCO_3^- exchanger AE1 [7, 20, 21]. The AE1 exchanger is homologous with the red cell anion exchanger known as ‘band 3’ (eAE1) [6, 24]. After the red cell, the kidney is the next richest source of this protein (kAE1) [24]. Proton secretion varies with systemic pH and it is also aldosterone-dependent and voltage-dependent [24].

Once secreted, net urinary elimination of H^+ depends on its buffering and excretion as titratable acid (mainly phosphate - $HPO_4^{2-} + H^+ \leftrightarrow H_2PO_4^-$), and excretion as NH_4^+ [24]. Notably, the production of NH_4^+ from glutamine by the proximal tubule, and its subsequent excretion in the urine, also generates ‘new’ bicarbonate, which is added to plasma [24]. Availability of phosphate as a buffer depends on its filtration, whereas NH_4^+ depends on normal function of the proximal tubule, as well as a complex process of secretion, reabsorption, and secretion again along the nephron [24]. The final secretory step for NH_4^+ excretion is ‘diffusion trapping’ in the collecting duct. Anything that interferes with H^+ secretion in the collecting duct will reduce diffusion trapping and cause a decrease in excretion of both H^+ and NH_4^+ [6, 24]. As previously mentioned, chronic metabolic acidosis

stimulates renal NH_4^+ synthesis and excretion [3, 6, 15]. Fig. (**2.2**) shows renal acidification process in α-intercalated cells of the distal nephron.

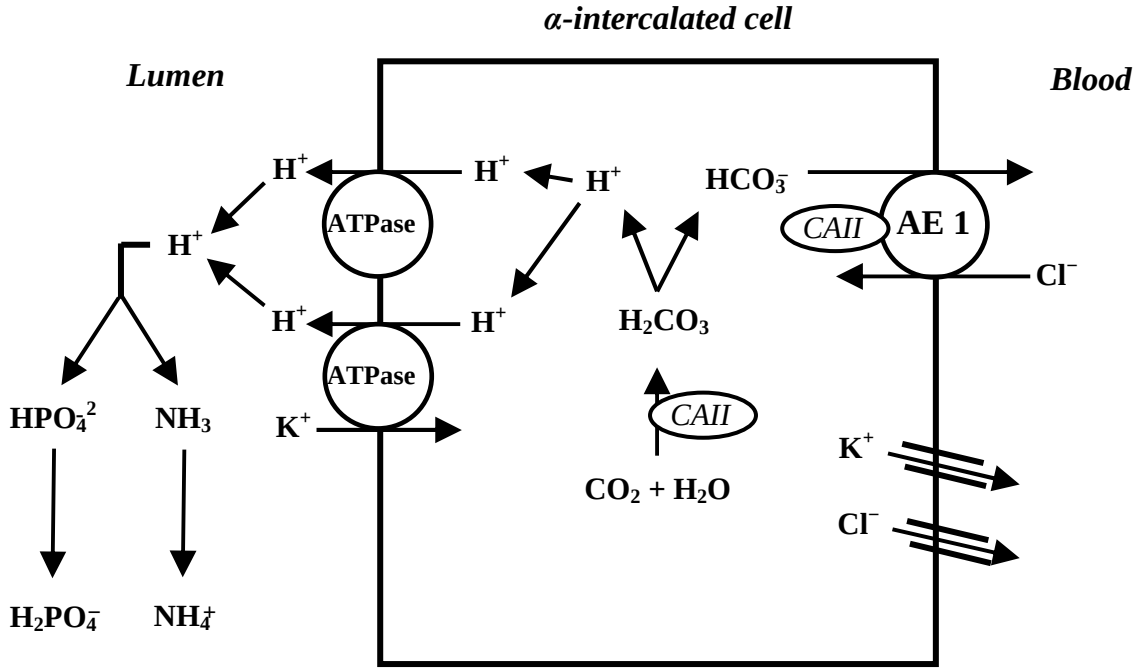

FIGURE 2.2 - *Schematic model of the α-intercalated cell and the H^+ secretion in cortical collecting tubule. The α-intercalated cell is responsible for H^+ secretion by a vacuolar H^+-ATPase (main pump) and also by a H^+-K^+-ATPase. The luminal ammonia (NH_3) buffers H^+ to form nondiffusible ammonium (NH_4^+) and divalent basic phosphate (HPO_4^-) is converted to the monovalent acid form ($H_2PO_4^-$) in H^+ presence. Intracellularly formed HCO_3^- leaves the cell via Cl^- - HCO_3^- exchange, facilitated by an anion exchanger (AE1). Cytoplasmic carbonic anhydrase II (CA II) is necessary to secret H^+.*

2.3 Classification and clinical features of renal tubular acidosis

Clinically, RTA is characterized by a normal anion gap, hyperchloremic metabolic acidosis, and associated failure to thrive secondary to growth failure as well as anorexia [13]. Polyuria and constipation can also be seen, although neither may be apparent in the neonatal period [13]. Hyperchloremic metabolic acidosis in pediatric practice is most often associated with diarrheal disease. Both diarrhea and RTA result in hypokalemia. For this reason, in a young infant with diarrhea and underlying RTA, the true diagnosis may be obscured. Thus, inordinately slow resolution of hyperchloremic metabolic acidosis following diarrheal disease should suggest the possibility of an underlying primary RTA [13].

Beyond the difficulties inherent in delineating RTA, RTA can be subcategorized into different disorders with distinctly diverse prognoses [13]. The diagnostic cataloguing of RTA is based on the underlying pathophysiology. The current model of how the nephron reabsorbs HCO_3^- and secretes H^+ has led to a clinical and functional classification of proximal (tubule) versus distal (tubule and collecting duct) forms of RTA [24]. Thus, the main types of RTA are proximal (or type 2) RTA and distal (or type 1) RTA. Type 3 RTA is a mixed type RTA that exhibits both impaired proximal HCO_3^- reabsorption and impaired distal acidification, and more disturbingly osteopetrosis, cerebral calcification and mental retardation [4]. Hyperkalemic (or type 4) RTA is a heterogeneous group of disorders that is characterized by low urine NH_4^+, which is probably caused by the hyperkalemia or by aldosterone deficiency or defective signaling [4].

In distal RTA, distal nephron net acid secretion is impaired. This leads to a high urine pH, even in the presence of systemic acidosis [2, 4]. However, there is often no metabolic acidosis and the blood bicarbonate concentration is normal, so-called ‘incomplete’ distal RTA, and a defect in renal acid excretion must be demonstrated by a failure to lower urine pH below 5.5 following an NH_4Cl load or a modified furosemide test [2, 6, 24]. Acquired distal RTA is often secondary to autoimmune diseases, such as Sjogren’s syndrome [6, 24]. Inherited distal RTA can be essentially of three types: autosomal dominant distal RTA (the commonest form) and autosomal recessive distal RTA with and without sensorineural deafness [24]. In the complete forms of both dominant and recessive distal RTA bone disease is common (rickets or osteomalacia), as well as nephrocalcinosis (often) complicated by renal stone disease. The occurrence of renal stones is attributed to the combination of hypercalciuria, low urinary citrate excretion (due to systemic and intracellular acidosis) and high urine pH, all favouring calcium phosphate stone formation. Hypokalaemia, another characteristic feature, is less troublesome than in the acquired autoimmune form of distal RTA, but it can become symptomatic, especially if a thiazide diuretic is prescribed to reduce hypercalciuria [24]. In recessive distal RTA, some patients suffer from sensorineural deafness, which can be late in onset [24].

Conceptually, the proximal tubule is charged with the task of reclaiming filtered HCO_3^- (~ 85% of the total) [13]. Failure of this process leads to reduction in systemic base, resulting in metabolic acidosis – proximal RTA [13]. Proximal RTA typically manifests as part of a generalized defect of proximal tubule function, namely the renal Fanconi’s syndrome (with glycosuria, low molecular weight proteinuria, urinary phosphate wasting, hypophosphataemia and hypouricaemia) [24]. Isolated proximal RTA occurs rarely and

usually presents as growth retardation in childhood. Like distal RTA, it can be divided into three types: autosomal recessive proximal RTA with ocular abnormalities, autosomal recessive proximal RTA with osteopetrosis and cerebral calcification, and autosomal dominant proximal RTA [24]. Autosomal recessive proximal RTA with ocular abnormalities is the commonest form of isolated and inherited proximal RTA, but even this is rare. Ocular abnormalities include band keratopathy, glaucoma and cataracts [24]. Short stature is usual; dental enamel defects, mental retardation, hypothyroidism, abnormal pancreatic function and basal ganglia calcification are also features [24, 25]. In inherited CA II deficiency, isolated proximal RTA presents with osteopetrosis (due to impaired osteoclast function), cerebral calcification and variable mental retardation [26]. Although this form of inherited RTA is clinically more proximal in type, it can also present with a mixed proximal and distal phenotype, which reflects the presence of CA II in cells all along the renal tubule.

Type 3 RTA can be caused by recessive mutation in the *CA2* gene on chromosome 8q22, which encodes CAII [4] or could involve direct interaction between CA II and the NBC1 [27] or Cl^-/ HCO_3^- exchanger, *SLC26A6* [4, 28].

The causes of type 4 RTA include various types of adrenal failure or pseudohypoaldosteronism type 1 (PHA1) due to defects in the mineralocorticoid receptor or the epithelial Na^+ channel, all characterized by salt loss and hypotension [4]. A similar picture may be seen in obstructive uropathy or drug induced interstitial nephritis [4]. Furthermore, a number of drugs may impair signalling in the renin–angiotensin-aldosterone system and cause hyperkalemia and metabolic acidosis (e.g. potassium sparing diuretics, trimethoprim, cyclo-oxygenase inhibitors, angiotensin converting enzyme inhibitors) [4]. Lately, much interest has been given to a group of rare autosomal dominant diseases characterized by hyperkalaemia and acidosis and age-related hypertension [4]. In spite of hypervolaemia, aldosterone is not low and the disorders have been collectively termed pseudohypoaldosteronism type 2 (PHA2) [4].

2.4 Inherited forms of distal renal tubular acidosis

Inherited forms of distal RTA have three variants: autosomal dominant and autosomal recessive with or without deafness. Dominant disease typically presents more mildly in adolescence or adulthood, and recessive variant occurs in infancy/early childhood, where

growth retardation is common [6]. In the table below we can see the chromosome mapping of distal RTA.

TABLE 2.1 - Chromosome mapping of the Inherited Distal Renal Tubular Acidosis

Inherited distal RTA	**Gene**	**Mapping**	**Protein encoded**
Autosomal dominant	*SLC4A1*	Chromosome 17q21-q22	AE 1 exchanger
Autosomal recessive *(with deafness)*	*ATP6V1B1*	Chromosome 2q13	B1 subunit of H^+-ATPase
Autosomal recessive *(with preserved hearing)*	*ATP6V0A4*	Chromosome 7q33-q34	a4 isoform subunit of H^+-ATPase.

2.4.1 Autosomal dominant distal RTA (distal RTA type 1a)

Distal RTA occurs with the greatest frequency as an isolated defect, often transmitted as an autosomal dominant trait [13]. In few reported families, the presence of the disorder in several generations suggests an autosomal dominant transmission. Although clinical findings are not different from those observed in autosomal recessive or sporadic cases, in these patients the disease may be diagnosed later (in adolescence or adulthood) [6] or manifest with milder symptomatology.

Autosomal dominant distal RTA has been found to be associated in several kindred with mutations in the *SLC4A1* gene encoding the Cl^-/HCO_3^- exchanger, AE1 [15].

The electroneutral anion exchanger (AE1)

The Cl^-/HCO_3^- anion exchanger, AE1, is a glycoprotein encoded by a gene (*SLC4A1*) present on chromosome 17 q21-22. *SCL4A1* gene is a member of the *SLC4* family comprising 10 genes of which 8 encode bicarbonate ion transporters [6, 24, 29]. AE1 is an integral membrane glycoprotein containing a long cytoplasmic N-terminus (~ 400 amino acids), 12–13 transmembrane domains (responsible for anion transport and dimerization), and a short

cytoplasmic C-terminus (~ 35 amino acids) [30, 31]. It is predominantly expressed in the erythrocytes (eAE1) and in the kidney (kAE1).

kAE1 is a truncated isoform of eAE1 with lacking of 65 amino acids at the N-terminus owing to the use of differential transcription and translation start sites [32]. This extra NH2-terminal sequence confers additional roles for eAE1, including facilitation of red cell metabolism and maintenance of erythrocyte structural stability via interaction with a glycolytic enzyme complex and cytoskeletal elements, respectively [6]. kAE1 mediates an electroneutral exchange of chloride for bicarbonate at the basolateral membrane of acid secreting α-intercalated cells of the distal nephron and collecting duct [32, 33]. This ion exchanger promotes the reabsorption of bicarbonate into the blood. Therefore, eAE1 defect results in morphological changes of red blood cells (RBC) while kAE1 abnormality leads to distal RTA [32].

The physiological role of kAE1 in the regulation of distal nephron acid secretion is well established. In the acidification process of the distal nephron, basolateral kAE1 mediates Na^+ independent, electroneutral Cl^-/HCO_3^- exchange, allowing HCO_3^- to exit the α-intercalated cells in concert with apical H^+ secretion via the vacuolar H^+-ATPase.

AE1 gene (SLC4A1) mutations

Because of the expression of AE1 in two different cells (RBC and α-intercalated distal tubular cells) with distinct functions, AE1 mutations show pleiotrophic effects resulting in two distinct and seemingly unrelated phenotypes: hereditary spherocytosis (or other forms of erythrocyte abnormalities) and distal RTA [31]. The largest group of mutations in human AE1 is associated with autosomal-dominant red cell dysmorphologies (hereditary spherocytosis – HS; and Southeast Asian ovalocytosis - SAO), where renal acid-base handling is normal [6]. AE1 mutations also result in distal RTA, because the defect in AE1 affects anion Cl^-/HCO_3^- exchanger at the basolateral membrane of the α-intercalated cells in the distal nephron [31].

SAO, a well-known erythrocyte disorder, is caused by a deletion of 27 bp in codons 400–408 in exon 11 (Ex11D27) of AE1 leading to a lack of 9 amino acids in the protein, which is inactive for anion transport.

How can be explained either the absence of red cell abnormalities in patients with distal RTA or the rarity of defects in distal urinary acidification in patients with hematological

disorders, when, in both circumstances, mutations in the same *SLC4A1* gene are present? [15]. One exception is the homozygous AE1 mutant V488M (Band 3 Coimbra; GTG → ATG), which presents with severe anemia and renal acidification defect [34, 35].

The majority of AE1 mutations apparently cause only erythroid abnormalities without renal phenotype. Most cause autosomal dominant forms of HS and are not encountered in homozygous form, suggesting embryonic lethality [7].

Dominant HS-associated AE1 mutations are generally not associated with distal RTA. Conversely, distal RTA-associated AE1 mutations are also not commonly accompanied with HS. Whereas HS missense mutations are distributed throughout AE1 cytoplasmic and transmembrane domains, distal RTA mutations are restricted to AE1's transmembrane domain. Although, the almost complete segregation between mutations associated with HS and with distal RTA is not fully understood [7].

Autosomal dominant distal RTA was first associated with exon 14 nucleotide substitutions encoding missense mutations in residue 589 (R589), in which the wild-type Arg is converted to His, Ser, or Cys [30, 36]. A single base change alters the identical AE1 residue, R589, in eight of the ten reported kindred with dominant distal RTA, supporting the importance of this residue in the normal acidification process. R589 lies at the intracellular border of the sixth transmembrane domain of the protein, adjacent to K590. These basic residues are conserved in all the known vertebrate anion exchanger isoforms and are thought to form part of the site of intracellular anion binding. Arginine at this position is conserved in all vertebrate AE proteins, indicating its functional importance [37].

Three different mutations at this position (R589C, R589H, and R589S) were found in autosomal dominant distal RTA and two *de novo* R589H mutations have also been reported [30, 32, 36]. A high prevalence of AE1 R589 mutations and the presence of at least two *de novo* mutations at this position suggest that codon 589 (CGC) is a "mutational hotspot" of AE1. The mechanism of recurrent mutations probably involves methylation and deamination altering cytosine (C) to thymine (T) in the CpG dinucleotides [37].

Another missense mutation alters serine to phenylalanine at position 613 [36] within the adjacent transmembrane loop, evidencing the importance of this region of the protein. A further complex mutation results in a C-terminally truncated AE1 protein lacking the last 11 amino acids [29].

AE1 in Autosomal Recessive Distal RTA

Recent gene studies have shown that some of the AE1 mutations are responsible for autosomal recessive distal RTA in several countries in Southeast Asia; these patients may be homozygous for the mutation or be compound heterozygotes of two different AE1 mutations, one of which is usually the SAO mutation [38, 39]. The evaluation of the AE1 G701D mutation has provided the first explanation for how any distal RTA-associated AE1 mutation might cause the disease [40].

Recessive distal RTA appears to result from the absence or a very marked deficiency of chloride-bicarbonate exchange activity in the basolateral membrane of the distal α-intercalated cell. In the case of the G701D mutation this occurs because the mutant protein is totally dependent on the presence of glycophorin A (GPA) for its movement to the cell surface. GPA is a glycosylated protein that is associated with band 3 and has a single span across the erythrocyte membrane [38]. Expression in *Xenopus* oocytes demonstrated that GPA completely rescues the cell surface movement of the G701D mutant band 3 to normal levels. This contrasts with normal band 3, which moves to the cell surface even in the absence of GPA, although GPA further enhances this movement. Red blood cells contain GPA but GPA is absent from the kidney, hence individuals homozygous for the G701D mutation have normal levels of band 3 in their red cells. It is proposed that, in homozygotes, the mutant G701D protein does not reach the basolateral membrane of the α-intercalated cell, but is turned over within the cell. In SAO/G701D compound heterozygotes, the SAO protein is presumed to reach the cell surface, but since it is inactive in anion transport, it acts as if it were a band 3 null allele [38].

2.4.2 Autosomal recessive distal RTA with deafness (distal RTA type 1b)

Recessive forms of distal RTA are related to mutations in the proton pump in α-intercalated cells. The gene involved (*ATP6V1B1*) is located on chromosome 2q13, and encodes the B1-subunit of H^+-ATPase expressed apically on α-intercalated cells and also in the cochlea and endolymphatic sac [4, 23].

In the human cochlea, the H^+-ATPase appears to be required to maintain normal endolymph pH [6] given that the very high potassium concentration (approximately 150 mmol/l) in this closed compartment is not normally accompanied by alkalinity of the

endolymph [23]. *ATP6V1B1* expression has also been observed in the male genital tract (with acidification requirement for sperm maturation) [29].

Clinical findings, other than deafness, are identical to those present in patients with sporadic or autosomal recessive distal RTA and normal hearing. There is great variation in the presentation of deafness, from birth to late childhood, it is progressive and does not respond alkali therapy [15]. The defects in B1 cause irreversible hair cell damage in human cochlea because of ambient electrolyte and pH abnormalities [29].

Screening for mutations in this gene revealed fifteen different mutations in kindred. The majority of these mutations are likely to disrupt the structure, or abrogate the production, of the normal B1 subunit protein [29].

The human vacuolar H^+-ATPase

The vacuolar-type proton ATPase (V- or H+-ATPase) is a multisubunit pump that is essential for normal acidification of intracellular vesicular structures. In each individual cell, H^+-ATPases may function in a variety of distinct but essential cellular processes. However, the mechanisms by which cells regulate the intracellular trafficking, final destination and activity of these proton pumps are unclear [41].

The H^+-ATPases are composed of two structural domains (membrane-bound V_0 and cytoplasmic or peripheral V_1) each formed of multiple subunits (a–e and A–H, respectively), which are responsible for ATP hydrolysis and proton transport, respectively [6, 23]. The mammalian H^+-ATPase is presumed to be similar to that of yeast (in which most of the structural studies have been performed) [23].

2.4.3 Autosomal recessive distal RTA with preserved hearing (distal RTA type 1c)

Individuals without hearing defects carry mutations at chromossome 7 q33-q34. The defective gene is *ATP6V0A4*, which encodes a kidney-specific a4 isoform subunit of H^+-ATPase. The involvement of the a4 subunit in distal RTA shows that it must be essential for proper proton pump function in the kidney [29], but its role is not totally clear.

Site-directed mutagenesis studies of the yeast ‘a’ subunit ortholog Vph1p (the ‘a’ subunit in proton pumps localized to the yeast vacuole) have yielded some potential functions

[42]. Some mutations showed that this subunit is important for the assembly of the proton pump, whereas other mutations had greater effects on ATPase activity and proton transport. These studies suggest that the ‘a’ subunit is important for both assembly and function of the pump. [29, 42].

2.5 Inherited forms of proximal renal tubular acidosis

Proximal RTA is caused by a reduction in bicarbonate reabsorption at the proximal tubules, resulting in low renal bicarbonate threshold. The most common proximal RTA in children is secondary to Fanconi Syndrome [2, 43]. Rarely, RTA might also be consequence of an inherited or sporadic primary renal disorder.

The acquired proximal RTA follows exposure to drugs or some toxins and the etiopathogenesis is still unknown [2]. Among drugs that cause Fanconi Syndrome are gentamicin, cisplatin, ifosfamide, and sodium valproate [6]. In addition, some hematologic and autoimmune conditions, such as myeloma and Sjogren syndrome respectively, might also course with proximal RTA.

The proximal RTA resulting from Fanconi Syndrome is frequently part of a systemic syndrome. Among systemic disorders that result in RTA, the inheritance pattern is usually autosomal recessive. Some of these disorders are cystinosis, tyrosinaemia, galactosaemia, Fanconi-Bickel syndrome and others (table 2.2) [44]. These syndromes are a heterogeneous group of disorders, which genes are mapped in many chromosome regions.

TABLE 2.2 - Chromosome mapping of the Inherited Fanconi syndromes

Inherited Fanconi Syndromes	**Gene**	**Mapping**
Autosomal recessive	*SLC4A4*	Chromosome 4q21
Dent´s syndrome	*CLCN5*	Chromosome Xp11.22
Cystinosis	*SLC3A1*	Chromosome 2p21
	SLC7A9	Chromosome 19p13.1
Tyrosinaemia type 1	*FAH* gene	Chromosome 15q23-q25
Galactosemia	*GALT* gene	Chromosome 9p13
Wilson´s disease	*ATP7B* gene	Chromosome 13q14.3-q21.1

The RTA non-related to Fanconi Syndrome is a rare disorder and might be sporadic, autosomal dominant or autosomal recessive. The autosomal recessive disorder is associated with ocular abnormalities, frequently coursing with mental retardation. Other clinical features are short stature, dental enamel defects, pancreatitis, and basal ganglia calcification [45]. Loss-of-function mutations in the gene that codifies the NBC-1, the *SLC4A4* gene, were first identified in two Japanese subjects with proximal RTA associated with cataracts, glaucoma and band keratopathy [46]. NBC-1 is formed by 1,035 amino acids; it contains ten transmembrane domains and two cytoplasmic termini, and it is present in kidney, brain, eye, pancreas, heart, prostate, epididymis, stomach, and intestine. In the kidney, NBC-1 is expressed mainly at the basolateral membrane of the proximal tubule. At least two genes encode the NBC proteins. Mutations were identified in the human NBC-1 gene (*SLC4A4*) mapped at chromosome 4p21 [47, 48].

Another interesting candidate gene for proximal RTA is the *TASK* gene. TASK2-potassium channel is a member of the tandem-pore domain potassium channel family and is located in pancreas, placenta, lung, small intestine, colon and kidney. TASK2 seems to be important to bicarbonate absorption in renal proximal tubules. Knockout mice for *TASK2* gene course with metabolic acidosis associated with low bicarbonate levels [49]. However, no mutation in these genes was yet identified in individuals with proximal RTA.

Other inherited form of proximal RTA is the one resulting from mutations in the gene *CA2* that encodes CAII. The carbonic anhydrases (CA) are member of a family of zinc metalloenzymes that catalyzes the hydration of CO_2. The human CA2 maps to the chromosome region 8q22. In the kidney, the majority of CA activity is attributable to CA II, which is localized in proximal tubular cells and in α-intercalated cells of the cortical and outer medullary collecting tubules [50]. Due to their localization, this RTA course with some proximal and distal components. In terms of clinical aspects, this RTA present osteopetrosis, cerebral calcification and different levels of mental retardation.

The autosomal dominant proximal RTA was originally described in a large Costa Rican family [51, 52], consisting of nine individuals presenting growth retardation and osteomalacia. No gene was found to be associated with this clinical presentation. Recently, another family with isolated proximal RTA inherited as an autosomal dominant disease was described [53]. The father and all four children had RTA with blood bicarbonate levels of 11-14 mEq/L and urine pH of 5.3-5.4 and all presented high bicarbonate fractional excretion. In terms of clinical aspects, they course only with short stature without other organ dysfunction. This family was investigated at the following genes: *CA II, CA IV, CA XIV, NCB1,* Na^+/H^+

exchanger (NHE-3), NHE-8, the regulatory proteins of *NHE3, NHRF1 and NHRF2* and the *Cl-HCO$^-_3$ exchanger, SLC26A6*. However, no mutation was found in any of the candidate genes studied. The study of these families might clarify other mechanisms involved in renal bicarbonate balance and a genome wide investigation of a pool of these families might result in interesting findings.

2.6 Inherited forms of renal tubular acidosis type 3

Type 3 RTA is a mixed type that exhibits both impaired proximal HCO_3^- reabsorption and distal acidification. The condition is due to an inherited deficiency of CAII caused by a recessive mutation in the *CA2* gene on chromosome 8q22, which encodes this widely expressed enzyme [4, 6]. The expression of CAII is affected in bone, kidney (in both proximal and distal nephron segments, explaining the mixed acidosis) and brain.

The mechanisms that underlie the clinical picture in type 3 RTA, apart from much slower conversion of carbonic acid to and from bicarbonate, apparently also involve direct interaction between CA II and the kidney NBC1 [27] or Cl^-/ HCO_3^- exchanger, *SLC26A6* (a plasma membrane Cl^-/ HCO_3^- exchanger with a suggested role in pancreatic HCO_3^- secretion) [4, 28]. Mutation of the identified CAII binding site reduced *SLC26A6* activity, demonstrating the importance of this interaction. [28].

Patients with this deficiency exhibit osteopetrosis and cerebral calcification, as well as a mixed RTA with proximal and distal components [29]. This association of osteopetrosis and RTA is known as Guibaud-Vainsel syndrome or marble brain disease. Osteopetrosis is a condition of increased bone density, but also augmented bone fragility, leading to increased fracture risk, plus intracerebral calcification, intellectual impairment, growth failure, and facial dysmorphism. Excess bone growth leads to conductive deafness and can also cause blindness through compression of the optic nerve [6].

There is a considerable degree of heterogeneity, both in the predominance of proximal or distal acidosis and in the osteopetrotic phenotype [6]. In different kindred, mild or severe mental retardation has also been described.

Different mutations in *CA2* gene have been described; for example, the common ‘Arabic’ mutation, consisting of loss of the splice donor site at the 5’ end of intron 2 [6, 29].

2.7 Inherited forms of hypercalemic renal tubular acidosis

Type 4 RTA is a heterogeneous group of disorders associated with hyperkalemia due to aldosterone deficiency or impairment in aldosterone molecular signaling.

Type 4 RTA might result from a PHA1. Some clinical aspects associated are hyponatremia, hyperkalemia, and elevated plasma aldosterone and plasma renin activity. The inheritance might be autosomal dominant or autosomal recessive [54]. The autosomal dominant is a frequent and mild kidney disorder without any other organ involvement [55]. This disorder seems to be associated to loss-of-function mutations in the mineralocorticoid receptor gene, the *MRL* gene. MRL-knockout mice develop symptoms of pseudohypoaldosteronism. In humans clinical presentation varies from non-symptomatic to important neonatal sodium loss. The recessive inheritance is associated to sodium transport defects in all aldosterone target tissues, not only kidney, but also colon, lungs, salivary and sweat glands. The recessive disorder is more severe and salt wasting is normally more pronounced. However, both types of inheritance might result in the same degree of natriuresis, hyperkalaemia and metabolic acidosis.

Other inherited cause of type 4 RTA includes hyperkalaemia associated with hypertension and low or normal levels of plasma aldosterone [57, 58]. This syndrome is called pseudohypoaldosteronism type 2 (PHA2), or Gordon's syndrome, which results in a renal aldosterone resistance inherited as an autosomal dominant pattern [6]. Mutations in the gene of two isoforms of WNK serine-threonine kinases, *WNK4* and *WNK1* genes, were identified in patients with PHA2 [59]. WNKs are serine kinase proteins lacking a lysine residue at the active site, being the WNK type 1 a regulatory protein from *WNK 4*. *WNK4* is found in the distal nephron and controls the sodium and chloride reuptake and inhibits potassium efflux [6].

2.8 Concluding remarks

Renal tubular acidosis (RTA) is characterized by metabolic acidosis due to renal impaired acid excretion. In this review, we summarized our current understanding of the hereditary diseases caused by mutations in genes encoding transporter or channel proteins operating along the renal tubule. Further molecular studies of inherited tubular transport

disorders may shed more light on the molecular pathophysiology of these diseases and may significantly improve our understanding of the mechanisms underlying renal salt homeostasis, urinary mineral excretion, and blood pressure regulation in health and disease. The identification of the molecular defects in inherited tubulopathies may provide a basis for future design of targeted therapeutic interventions and, possibly, strategies for gene therapy of these complex disorders.

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3 OBJETIVOS

3.1 Objetivo Geral

Descrever o quadro clínico, a abordagem diagnóstica, o tratamento e a evolução dos pacientes com ATR distal, acompanhados na Unidade de Nefrologia Pediátrica do Hospital das Clínicas da UFMG no período de novembro de 1984 a setembro de 2008.

3.2 Objetivos Específicos

a) descrever a apresentação clínica, etiologia, abordagem diagnóstica, achados laboratoriais evolução e tratamento dos casos de ATR distal;

b) comparar a evolução clínica e laboratorial dos pacientes com ATR distal por meio da análise dos dados obtidos ao diagnóstico e no último controle ambulatorial realizado dentro do período de estudo;

c) avaliar os parâmetros clínicos e laboratoriais que foram preditores independentes para o ganho de peso e de estatura nos pacientes com ATR distal.

4 PACIENTES E MÉTODOS

4.1 Pacientes

O grupo foi composto por crianças e adolescentes, sabidamente portadores de ATR distal, que foram atendidos e acompanhados pelo setor de Nefrologia Pediátrica do Hospital das Clínicas da UFMG no período de novembro de 1984 até setembro de 2008.

4.1.1 Critérios de inclusão

Foram incluídos as crianças e os adolescentes com diagnóstico confirmado de ATR distal, baseado em critérios clínicos e laboratoriais [1], que foram acompanhados pela Unidade de Nefrologia Pediátrica de novembro de 1984 a setembro de 2008.

4.1.2 Critérios de exclusão

Foram excluídos do estudo os pacientes cujo seguimento não foi suficiente para confirmar o diagnóstico de ATR distal, os pacientes que não seguiram o protocolo de abordagem de ATR da referida unidade e os casos cujos prontuários médicos apresentam dados insuficientes para análise.

4.1.3 Aspectos éticos

Esta linha de pesquisa já foi aprovada pelo Comitê de Ética em Pesquisa da UFMG (COEP), conforme o parecer número 144/02, em anexo (ANEXO A).

Ressalta-se ainda que somente os pesquisadores envolvidos no projeto tiveram acesso aos dados, ficando assim garantida a confidencialidade das informações obtidas.

Os resultados da pesquisa se destinam única e exclusivamente ao interesse científico, sendo mantido o anonimato do paciente participante da pesquisa. Os resultados obtidos não foram utilizados para outros fins senão os estritamente relacionados aos objetivos do projeto.

4.2 Métodos

4.2.1 Desenho do Estudo

Trata-se de um estudo de coorte retrospectivo, onde os dados foram coletados a partir da revisão dos prontuários médicos. Ressalta-se ainda que todos os pacientes incluídos no estudo foram submetidos ao mesmo protocolo de avaliação e seguimento na Unidade de Nefrologia Pediátrica do Hospital das Clínicas da UFMG. O início do período do estudo foi definido pela data do primeiro diagnóstico confirmado de ATR distal entre os pacientes selecionados.

4.2.2 Protocolo geral

Inicialmente, foi realizado o levantamento dos prontuários de todos os pacientes com acidose tubular renal, acompanhados pela Unidade de Nefrologia Pediátrica do HC-UFMG, a partir de uma listagem obtida por programa de computador da referida unidade. A seguir, foram selecionados os pacientes que preenchiam critério diagnóstico para ATR distal (vide item 4.2.3).

Foram coletados os dados de cada paciente, por meio de revisão pormenorizada das papeletas e preenchimento de fichas individuais, adaptáveis a banco de dados (ANEXO B). Foi elaborado um banco de dados no programa SPSS versão 15. As informações colhidas de cada paciente foram, então, lançadas neste banco para a realização das análises estatísticas.

4.2.3 Critérios para diagnóstico e classificação

Foram classificadas como portadoras de ATR distal aquelas crianças que apresentaram características clínicas sugestivas da doença, como déficit de crescimento, anorexia, vômitos e poliúria associados a um pH urinário maior que 5,5, em presença de um quadro de acidose metabólica hiperclorêmica (pH < 7,3) com intervalo aniônico normal [1-3]. A presença de nefrocalcinose ou nefrolitíase também foi considerada como alteração sugestiva.

Foram classificadas como ATR proximal ou tipo 2 as crianças com pH urinário inicialmente ácido e que passaram a apresentá-lo alcalino após reposição oral de bicarbonato [2-6]. A associação entre a clínica de déficit de crescimento e desenvolvimento e a presença de raquitismo resistente à vitamina D foi considerada bastante sugestiva [6-8].

Como não há nenhum método diagnóstico específico para a síndrome de Fanconi, foram considerados portadores desta síndrome pacientes com quadros de acidose metabólica hiperclorêmica com *anion gap* normal, hipofosfatemia, raquitismo, aminoacidúria e glicosúria em presença de séricos de glicose dentro dos limites da normalidade. Também foram consideradas a elevação da fração de excreção de fosfato e a elevação da atividade da fosfatase alcalina, assim como pH urinário inapropriadamente elevado após iniciado o tratamento com álcalis [1, 9, 10].

Outros exames complementares também foram realizados para avaliação e diagnóstico da doença de base subjacente à ATR: ultra-sonografia dos rins e vias urinárias para pesquisa e acompanhamento de nefrocalcinose; radiografias simples de punho e ossos longos para pesquisa e acompanhamento de raquitismo; exame de fundo de olho com lâmpada de fenda; *screening* metabólico em sangue e urina; dosagem de lactato e amônia para triagem de erro inato de metabolismo; as dosagens de cistina intraleucocitária e de oxalato urinário, além de avaliação de mutações genéticas, foram realizadas em casos específicos, de acordo com critérios clínicos para sua indicação.

A partir da classificação nos subtipos de ATR, foram selecionados para o presente estudo aqueles que preenchiam os critérios para o diagnóstico de ATR1.

4.2.4 Avaliação clínica

Todas as crianças e adolescentes selecionados para o estudo realizaram exame clínico completo, com ênfase na avaliação de déficit de crescimento e desenvolvimento, sendo determinada idade ao diagnóstico assim como peso e estatura neste momento e nas consultas subseqüentes. As consultas tiveram periodicidade entre 2 a 6 meses, podendo este período variar de acordo com as necessidades individuais de cada paciente. Os dados antropométricos foram verificados pela equipe de enfermagem do ambulatório de Nefrologia Pediátrica através do estadiômetro para aferição da altura e de balanças calibradas, para o peso. Estes dados foram colocados nos gráficos ou curvas de percentis de peso e estatura propostos pelo Ministério da Saúde. Foram considerados como portadores de déficit grave de crescimento, os pacientes que apresentaram percentil da curva peso/idade menor que 3 e déficit moderado para percentis entre 3 e 10. Valores acima do percentil 10 e inferiores ao percentil 90 foram considerados normais.

A análise dos percentis estatura/idade foi utilizada para avaliação final de retomada da curva de crescimento após o tratamento, assim como a reavaliação dos percentis peso/idade. Foi utilizado o escore z para avaliar e classificar os déficits pôndero-estaturais ao diagnóstico e no último controle dentro do período do estudo.

Também foram pesquisados outros sinais e sintomas como vômitos, anorexia, constipação, fraqueza muscular, poliúria, polidipsia, episódios de desidratação e febre de origem indeterminada.

4.2.5 Avaliação laboratorial e exames complementares

Os exames laboratoriais foram solicitados de acordo com o protocolo de seguimento da referida unidade (ao diagnóstico, trimestralmente nos primeiros anos de seguimento e a cada seis meses a seguir), respeitando-se também as necessidades clinicas individuais. Dentre os exames mais importantes ressaltam-se: gasometria venosa, uréia, creatinina e ácido úrico séricos, sódio, potássio, cloreto, cálcio, fosfato e magnésio séricos, fosfatase alcalina, urina rotina com pH de urina recém emitida e dosagem de eletrólitos (sódio, potássio, cloreto, cálcio, fosfato e magnésio urinários) e creatinina em urina de 24h. Todas as determinações

laboratoriais foram realizadas por metodologia convencional, no setor de bioquímica do laboratório central do HC-UFMG. A partir desses resultados, foram calculados o *clearance* de creatinina, as frações de excreção dos eletrólitos, o intervalo aniônico (*anion gap*) sérico e urinário.

Outros exames complementares também foram realizados para seguimento bem como para diagnóstico de possível doença de base subjacente à ATR1. Dentro dessa categoria incluíram-se como ultra-sonografia dos rins e vias urinárias para pesquisa e acompanhamento de nefrocalcinose e nefrolitíase realizada a cada 2 anos; radiografias simples de punho e ossos longos para pesquisa e acompanhamento de raquitismo; exame de fundo de olho com lâmpada de fenda; *screening* metabólico em sangue e urina; dosagem de lactato e amônia para triagem de erro inato de metabolismo; as dosagens de cistina intraleucocitária e de oxalato urinário, além de avaliação de mutações genéticas, que foram realizadas em casos específicos, de acordo com critérios clínicos para sua indicação.

4.2.6 Evolução e tratamento

A evolução clínica e laboratorial dos pacientes foi avaliada, sobretudo a partir da análise dos dados obtidos ao diagnóstico e no último controle incluído no período de estudo. O tratamento realizado, incluindo as fórmulas de suplementação de eletrólitos com a quantidade de cada componente expressa em relação ao peso do paciente, também foi avaliado ao diagnóstico e no último controle assim como a resposta terapêutica a cada medida de suplementação de acordo com critérios clínicos (retomada do crescimento, melhora da sintomatologia) e laboratoriais (melhora dos distúrbios metabólicos).

Para o tratamento da ATR distal foi utilizado protocolo pré-estabelecido que consistiu na administração de doses adequadas de álcalis (sob a forma de bicarbonato ou citrato), suplementação de eletrólitos e controle da hipercalciúria. A quantidade de base necessária por dia variou de 5 a 8 mEq/kg/dia nos pacientes mais jovens e de 2 a 4 mEq/kg/dia em crianças maiores e adolescentes. O citrato de potássio também foi utilizado em doses iniciais de 2 a 4 mEq/Kg/dia. Procurou-se manter o pH urinário entre 6,5 e 7,5 para evitar a precipitação do cálcio excretado em excesso. A monitoração do cálcio urinário, através da determinação da concentração de cálcio na urina de 24 horas e/ou da relação cálcio/creatinina em amostra de

urina foi também utilizada para a avaliação do tratamento [11, 12]. Hidroclorotiazida (1 a 2mg/kg/dia) foi iniciada nos casos onde a hipercalciúria (>4mg/kg/dia) persistia apesar do adequado controle do distúrbio ácido-básico [12]. A dose de álcalis foi considerada adequada quando corrigiu a maioria das anormalidades urinárias, inclusive a hipercalciúria. Tanto para escolha da medicação quanto para o ajuste das doses foi levado em consideração a resposta terapêutica e a monitoração individual de cada paciente.

4.2.7 Análise estatística

Para a realização da análise estatística utilizamos os programas SPSS versão 15.0 e Epi-Info versão 6.0. Os resultados foram apresentados como mediana ou média e desvio padrão (DP) para as variáveis contínuas ou porcentagens para as variáveis categóricas. Cada parâmetro foi avaliado quanto à normalidade de distribuição pelo teste de Kolmogornov–Smirnov.

Foram utilizadas técnicas de análise descritiva e exploratória dos dados com tabelas de freqüências e medidas de estatística descritiva. Os testes do qui-quadrado e t exato de Fisher foram usados para verificar a existência ou não de associação entre as variáveis categóricas ao diagnóstico e ao fim do período do estudo. O teste *t* pareado foi utilizado para analisar uma possível alteração da distribuição normal das variáveis continuas ao diagnóstico e a última consulta do período em estudo. Odds ratio (OR) e intervalo de confiança de 95% (IC 95%) foram utilizados para a comparação entre os grupos de risco. O nível de significância utilizado em todos os testes foi de 5% (α=0,05).

O método de regressão logística foi utilizado para modelar a relação entre uma variável resposta binária ou dependente (apenas dois valores possíveis; ganho ou não de pelo menos 1 DP no escore Z de peso (WAZ) e de estatura (HAZ) no último controle dentro do período do estudo) e uma ou mais variáveis explicativas ou independentes. O delta HAZ e o delta WAZ foram calculados com base na seguinte fórmula:

HAZ ou WAZ (final) – HAZ ou WAZ (ao diagnóstico)

Inicialmente, foi realizada a regressão logística simples para cada variável independente. A seguir, foram selecionadas as variáveis que apresentaram um valor de p no

teste de hipótese menor que 0,25. A partir deste momento, foi utilizada a técnica de *stepwise backward* para a seleção das variáveis significativas. Para o modelo final, apenas aquelas com nível de significância de 0,05 permaneceram. O teste U não paramétrico de Mann-Whitney foi utilizado para comparação das medianas do delta HAZ e do delta WAZ.

As variáveis testadas como fatores preditivos independentes estão listadas a seguir:

a) Sexo: feminino, masculino;
b) Idade ao diagnóstico: idade 1 (variável contínua) - idade em meses; idade 2 (variável categórica de acordo com a mediana das idades em meses) - menor ou igual a 15 meses ou maior que 15 meses; idade 3 (variável categórica de acordo com o terceiro quartil das idades em meses) - menor ou igual a 42 meses ou maior que 42 meses;
c) Peso ao diagnóstico e à última consulta do período do estudo: em quilogramas (kg);
d) Estatura ao diagnóstico e à última consulta do período do estudo: em centímetros (cm);
e) Percentis de peso e estatura ao diagnóstico e à última consulta do período estudado: $p \leq 3$ e $p > 3$;
f) Escores z do peso e estatura ao diagnóstico e à última consulta do período estudado;
g) Etiologia: primária, secundária (qual doença de base subjacente) ou forma transitória;
h) Sinais e sintomas (ao diagnóstico e ao último controle do período estudado): divididos inicialmente em presentes e ausentes. Quando presentes, foram listados os sinais e sintomas relacionados à apresentação clínica;
i) Exames laboratoriais (ao diagnóstico e ao último controle do período estudado): gasometria venosa, uréia e creatinina séricos, sódio, potássio, cloreto, cálcio, fosfato e magnésio séricos, urina rotina com pH de urina recém emitida, eletrólitos (sódio, potássio, cloreto, cálcio, fosfato e magnésio urinários) e creatinina em urina de 24h;
j) Parâmetros de função glomerular e tubular (ao diagnóstico e ao último controle do período estudado): foram calculados o *clearance* de creatinina, as frações de excreção dos eletrólitos, o intervalo aniônico (*anion gap*) sérico e urinário;
k) US dos rins e vias urinárias para avaliar a presença de nefrocalcinose (ao diagnóstico e ao último controle do período estudado);
l) RX de punho e ossos longos para avaliar a presença de raquitismo (ao diagnóstico e ao último controle do período estudado);
m) Tratamento (ao diagnóstico e ao último controle do período estudado): álcalis e fórmulas de suplementação de eletrólitos expressos em relação ao peso do paciente;

n) Tempo de seguimento clínico: em anos (variável contínua) e em faixas (variável categórica) - menos de cinco anos ou mais de cinco anos, inclusive.

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5 RESULTADOS E DISCUSSÃO (ORIGINAL ARTICLE)

CLINICAL COURSE OF 33 CHILDREN WITH DISTAL RENAL TUBULAR ACIDOSIS

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ABSTRACT

Distal renal tubular acidosis (RTA) refers to a heterogeneous group of diseases that result from distal tubular dysfunction and can lead to growth retardation, nephrocalcinosis, bone disease and, rarely, chronic kidney disease. This study aimed to describe the clinical course of distal RTA series and to analyze somatic growth by identifying possibly predictive factors of growth improvement. Patients were followed-up from 1984 to 2008 according to our standard protocol. Paired t test was used for comparison between pre and post-treatment results. A logistic regression model was applied to identify variables that were independently associated with the gain of at least one standard deviation (SD) in Z-score for height and weight. A total of 33 distal RTA patients (15 males) were analyzed. Primary disease was the commonest form (60.6%). Based on weight/age and stature/age curves, 58.3% of the patients completely recovered growth after treatment. Bicarbonate levels at admission were an independent predictor of stature gain at last visit and the male sex negatively affected the final weight gain. Metabolic acidosis, electrolyte disturbances, hypercalciuria and nephrocalcinosis also improved during follow-up ($p<0.05$). Our data showed the great impact of treatment on metabolic control and further indicated predictive factors of growth catch-up.

Key words: renal acidification, growth failure, nephrocalcinosis, metabolic acidosis

5.1 Introduction

Renal tubular acidosis (RTA), the main cause of tubular dysfunction in pediatric practice, represents a clinical syndrome in which either an inherited (primary) or acquired (secondary) defect in tubular transport mechanisms can lead to failure to maintain the metabolic homeostasis [1-4]. RTA can be basically divided into four categories: distal (type 1), proximal (type 2) with or without Fanconi's syndrome, combined distal and proximal defects (type 3), and hyperkalemic (type 4).

In the past decade, remarkable progress has been made in our understanding of the molecular pathogenesis of hereditary tubulopathies and the physiology of renal tubular transport processes [4-8]. Distal RTA is the commonest type of primary RTA in childhood [1-3], which is characterized by impaired urinary acidification leading to hyperchloremic acidosis with inappropriately alkaline urine [1, 9]. Affected children might course with nephrocalcinosis, nephrolitiasis, failure to thrive, growth retardation, bone disease and, more rarely, chronic renal disease [9, 10]. If detected early in life, therapeutic correction of the acidosis by continuous alkali administration may induce growth catch-up, arrest of nephrocalcinosis and preservation of renal function [2].

There have been a number of studies of distal RTA [1-3, 6, 7, 9-14] but few of them evaluated the outcome of treatment [9-14]. In addition, as expected the majority of the series comprised a few number of pediatric patients [10-12]. Moreover, none of these studies have systematically investigated baseline features associated with growth parameters after treatment. In this regard, the aim of this retrospective cohort study was to describe the clinical course of 33 pediatric patients with distal RTA followed by 1984 to 2008 and to analyze the somatic growth of the patients in order to identify possibly predictive factors of growth improvement.

5.2 Patients and Methods

In this retrospective cohort study, data from 33 patients with distal RTA consecutively admitted to our Pediatric Nephrology Unit from 1984 to 2008 were analyzed. Data were compiled from November 1984 to September 2008.

5.2.1 Inclusion criteria

Patients with a confirmed diagnosis of distal RTA based on clinical and laboratorial findings were included in our analysis [1-3, 15-17]. These findings consisted at least of history of polyuria or polydipsia and/or failure to thrive accompanied by normal anion gap, hyperchloremic metabolic acidosis with abnormally high urine pH (>5.5 in the presence of blood pH<7.30) and normal or near normal glomerular filtration rate [1-3].

5.2.2 Exclusion criteria

Patients whose follow up were not enough to confirm the diagnosis of distal RTA and those whose medical records had insufficient data for analysis were automatically excluded from the study.

5.2.3 Study protocol

Medical records of 33 patients diagnosed with distal RTA were reviewed from a database of all patients with RTA admitted to our Unit during study period. All distal RTA patients followed a systematic protocol, including: blood gas analysis and concomitant spot urine pH to confirm the acidification defect, complete metabolic evaluation (serum and 24-hour urine electrolytes and nitrogen waste levels measurements), images (renal ultrasonography, X-rays) and specific exams to define secondary cases of distal RTA (screening inborn metabolic diseases, autoimmune, hematological and endocrine disorders).

The visits were scheduled periodically at intervals of about two to six months, depending on the clinical and metabolic condition of each patient. A complete examination was performed on each occasion including clinical and laboratory evaluation. The data analyzed were obtained at diagnosis and at the time of the last visit.

The following variables were studied: gender, age at diagnosis, clinical presentation (polyuria, polydipsia, failure to thrive, metabolic acidosis, nephrocalcinosis and signs rickets were evaluated as present or absent), etiology of distal RTA, follow-up duration, height, weight, laboratorial data (blood gas analysis and serum levels of creatinine, urea, uric acid and electrolytes, urinary pH and 24-hour urine samples for electrolytes and creatinine measurements). Anthropometric data were collected in all visits. Weight was measured to the nearest 100 g using a baby scale for infants and a beam scale for older children. Length was checked using a measuring board of standard design for children younger than two years and height was measured using a wall-mounted scale for those aged two years or older. Glomerular filtration rate was estimated by the Schwartz formula [16]. Bone X-ray and ultrasound scan evaluated the presence of rickets and nephrocalcinosis, respectively. Rickets was defined radiologically as widened and irregular epiphyseal-metaphyseal junctions or evidence of bone softening in the long bones and nephrocalcinosis as diffuse calcification of the renal pyramids [18]. Image studies were periodically repeated to pursue the evolution of these complications in all positive cases.

Treatment was based on standard guidelines, which generally included alkali replacement therapy, electrolyte supplementation and control of hypercalciuria. Patients were treated with sodium-potassium citrate or bicarbonate. Basically, the amount of base given daily per kg of body weight to young infants was 5 to 8 mmol/kg per day (bicarbonate or citrate), 3 to 4 mmol/kg per day and 1 to 2 mmol/kg per day, to children and adolescents, respectively. Potassium citrate alone could also be used in a mean amount of 4 mmol/kg per day [2, 3, 14]. Supplements of potassium were given according to blood biochemistry findings. Patients with persistent hypercalciuria (>4 mg/kg/day) despite correction of acidosis were started on hydrochlorthiazide (1 to 2 mg/kg/day). Medication was chosen and doses was adjusted individually to achieve plasma bicarbonate in the range of 20-24 mEq/L and to successfully revert most of the urinary abnormalities, including hypercalciuria.

5.2.4 Statistical analysis

Data were analyzed with SPSS (release 15, SPSS Inc., Chicago, Illinois) and were presented as mean and standard deviation (SD) for continuous variables and as proportions for categorical variables. Growth parameters were expressed as Z-score. Weight-for-age (WAZ) and height-for-age (HAZ) Z-scores were used to assess weight and stature, respectively. These parameters were calculated with the public domain software EPI-INFO (version 6.0). The normality of the distribution was evaluated by the Kolmogornov–Smirnov test for each parameter. The delta HAZ or delta WAZ was calculated by the following equation: HAZ or WAZ at last visit – HAZ or WAZ at baseline. The Mann-Whitney or Kruskal-Wallis nonparametric test was used to compare medians of delta HAZ or delta WAZ. Paired t-test was used to analyze possible changes in normally distributed continuous variables from entry to follow-up.

To further analyze the association between baseline factors and growth improvement, the response variable was set as an increase of at least one SD in WAZ or HAZ at last visit. The following variables were assessed as independent predictors: gender; etiology (primary/secondary and transitory disease); nephrocalcinosis (present/absent); and bicarbonate levels (continuous variable). Age at diagnosis was assesses as a continuous variable and also as a categorical variable using two cut-off points (≤ 15 months and > 15 months, based on the median value; ≤ 42 months and > 42 months, based on the third quartile value). The analysis was conducted in two steps. In the first step, univariate analysis was performed by the chi-square test with Yates correction for comparison of proportions between growth parameters and clinical features and Mann Whitney U-test to compare growth parameters with continuous variables at admission. Then, a logistic regression model was applied to identify variables that were independently associated with the gain of at least one standard deviation (SD) in HAZ and WAZ. Only those variables that were found to present different proportions in univariate analysis ($p<0.25$) were included in the regression model. Next, using a backward elimination strategy, those variables that retained a significant independent association ($p<0.05$) were included in the final models.

5.2.5 Ethical aspects

The Ethics Committee of the Federal University of Minas Gerais approved the study. Informed consent was obtained from all included subjects and their parents.

5.3 Results

5.3.1 Clinical and laboratorial characteristics at baseline

a) *Clinical characteristics* - We analyzed data from 33 patients classified as distal RTA among 48 patients with RTA (68.8%). The clinical characteristics at baseline were displayed in Table 5.1. These 33 patients comprised 15 males and 18 females with mean age at diagnosis of 2.67±3.1 years (one month to 10 years). Primary disease was the commonest form of distal RTA (n=20, 60.6%) and in three patients it was associated to deaf. The transitory form of distal RTA was detected in six patients (18.2%) and distal RTA due to an underlying disease corresponded to seven patients (21.2%). The etiologies of secondary distal RTA were uropathies (n=3, 9.1%), primary hyperoxaluria type 2 (n=2, 6%) and autoimmune diseases (n=2, 6%). The main related symptoms were growth retardation (63.6%), polyuria (24.2%) and polydipsia (24.2%). The physical examination revealed growth impairment in 24 patients (72.7%), since all of them presented weight under 3rd percentile and 79.2% also exhibited height under this score. Females presented lower baseline values of WAZ (p=0.025) and HAZ (p=0.016) than males. The image evaluation showed nephrocalcinosis in 13 patients (39.4%) and rickets in 11 (33.3%).

TABLE 5.1 - Clinical characteristics of distal RTA patients at baseline.

Features	Female	Male	Total
	18	15	33
Age (*years*)*	2.95 (3.3)	2.33 (2.8)	2.67 (3.1)
Etiology (*n*)			
Primary	13	7	20
Secondary	2	5	7
Transitory	3	3	6
Family history (*n*)			
Present	9	3	12
Absent	9	12	21
Presentation (*n*)			
Growth retardation	12	9	21
Polyuria / Polydipsy	5	3	8
Acidosis	3	2	5
Nephrolitiasis	2	0	2
Persisting fever	2	0	2
Hematuria	2	0	2
Recurrent infections	0	1	1
Dehydration	0	1	1
Image evaluation findings (*n*)			
Nephrocalcinosis	7	6	13
Rickets	9	2	11
Follow-up duration (*years*)*	12.4 (6.7)	8.9 (4.8)	10.8 (6.1)

(*) Data are given as mean, with the standard deviation given in parenthesis

b) *Laboratorial characteristics* - Main laboratorial findings at diagnosis included metabolic acidosis with low bicarbonate levels (14.8 ± 3.9 mmol/L) and high urinary pH (7.20 ± 0.83) in all patients, hypopotassemia (< 3.5 mmol/L) in eight (24.2%), hypophosphatemia (< 3 mg/dL) in seven (21.2%) and hypercalciuria (>4 mg/kg/day) in 17 (51.5%). Estimated glomerular filtration rate (102.3 ± 49.9) were within the normal limits in all patients. Females presented lower initial bicarbonate (p=0.001), base excess (p=0.005), serum calcium (p=0.016) and potassium levels (p=0.009) than males. No more differences were detected in the comparison between genders.

5.3.2 Clinical and laboratorial characteristics at last visit

a) *Clinical findings* - The mean duration of follow-up was 10.8 years (1.4 to 26 years). Twenty-eight patients (84.8%) were followed for more than five years. Only one patient abandoned the treatment after 14 years of follow-up. Some symptoms at presentation such as dehydration, persistent fever and recurrent infections were not reported at last visit. Polyuria and polydipsia improved in six patients but remained unchanged in two. Among 13 patients with nephrocalcinosis at baseline, eight of them clearly improved, in one the alteration was no more detected and it remained stable in four. None of them worsened the nephrocalcinosis. Among 11 patients with rickets at diagnosis, six had their X-rays completely normalized and, in five, the improvement was evident. Only two patients developed chronic kidney disease due to the progression of their underlying disease (complex uropathies in both cases). These two cases did not exhibit hypercalciuria or nephrocalcinosis.

The treatment consisted of sodium bicarbonate alone in 18 children, potassium citrate and sodium bicarbonate in five patients, sodium-potassium citrate plus sodium bicarbonate in four and potassium citrate alone in six. Hidrochrolotiazide was associated in nine patients who had hypercalciuria and also nephrocalcinosis. The mean effective dose of sodium bicarbonate was 2.2 ± 1.2 mmol/kg per day. During follow-up, bicarbonate treatment was withdrawn only in the patients with transitory form of distal RTA; all others still required a mean dose of 0.8 ± 0.6 mmol/kg/day at last visit. Among the patients with hypercalciuria, only three remained with urinary calcium excretion at high levels (>4 mg/kg/day) and still continued on hidrochrolothiazide treatment.

b) *Laboratorial findings* - Metabolic acidosis and electrolyte disturbances also improved in all patients (Table 5.2). Serum bicarbonate changed from 14.8 ± 3.9 mmol/L to 24.3 ± 3.4 mmol/L after treatment ($p<0.01$, Figure 5.1) but there was no significant difference between sexes ($p=0.50$). Hypophosphatemia was initially observed in 21.2%, but completely normalized after replacement treatment in all patients. Hypercalciuria completely resolved in 14 among 17 cases (82.4%), clearly improved in one (5.9%) and remained unchanged in two (11.8%) patients. As shown in Figure 5.2, a significant reduction in 24-hour urinary calcium excretion was also observed (4.9 ± 2.9 mg/kg/day at admission vs. 2.7 ± 1.8 mg/kg/day at last visit, $p<0.01$).

TABLE 5.2 - Comparison between laboratorial findings at baseline and at last visit in distal RTA patients.

	At baseline			At last visit			
	Female	*Male*	*Total*	*Female*	*Male*	*Total*	*P value*
Bicarbonate (mmol/L)	12.8 (3.6)	17.0 (2.9)	14.7 (3.9)	23.9 (2.9)	24.7 (3.9)	24.3 (3.4)	*0.0001*
Serum pH	7.29 (0.07)	7.33 (0.04)	7.31 (0.06)	7.35 (0.04)	7.34 (0.05)	7.34 (0.04)	*0.047*
Base excess	-11.7 (4.8)	-6.7 (3.9)	-9.4 (5.1)	-0.9 (2.9)	-0.7 (3.8)	-0.8 (3.3)	*0.0001*
pCO_2	22.0 (11.2)	25.3 (11.2)	23.5 (11.2)	25.7 (24.2)	38.5 (17.2)	31.5 (22.0)	*0.072*
Sodium (mmol/L)	139.5 (4.1)	139.6 (4.3)	139.6 (4.1)	139.1 (2.9)	138.8 (2.7)	139.0 (4.1)	*0.191*
Potassium (mmol/L)	3.6 (1.0)	4.4 (0.5)	4.0 (0.9)	3.7 (0.5)	4.1 (0.5)	4.0 (0.9)	*0.614*

Chloride (mmol/L)	109.7 (7.5)	105.3 (5.3)	107.7 (6.9)	102.7 (4.9)	103.9 (4.6)	103.2 (4.7)	*0.002*
Calcium (mg/dL)	8.2 (3.0)	9.2 (2.6)	8.6 (2.8)	9.1 (2.3)	9.5 (0.5)	9.3 (1.7)	*0.120*
Phosphate (mg/dL)	3.9 (2.1)	4.7 (1.2)	4.2 (1.8)	3.8 (1.6)	4.1 (1.4)	3.9 (1.5)	*0.272*
Magnesium (mg/dL)	0.9 (1.1)	1.6 (0.8)	1.2 (1.0)	1.5 (0.8)	1.7 (0.5)	1.6 (0.7)	*0.053*
Creatinine (mg/dL)	0.5 (0.3)	0.6 (0.4)	0.5 (0.3)	0.9 (1.1)	0.8 (0.5)	0.8 (0.9)	*0.047*
Urea (mg/dL)	23.7 (16.7)	29.6 (16.6)	26.4 (16.8)	25.9 (22.3)	30.5 (23.1)	27.9 (22.4)	*0.610*
Urinary pH	7.3 (1.0)	7.0 (1.0)	7.2 (0.8)	7.3 (0.9)	7.0 (0.8)	7.2 (0.8)	*0.874*
Glomerular Filtration rate * (mL/min)	103.2 (52.7)	101.2 (48.1)	102.3 (49.9)	114.9 (33.6)	117.4 (46.1)	116.0 (39.1)	*0.206*
24-hour urinary calcium (mg/kg/day)	5.6 (3.2)	4.0 (2.1)	4.9 (2.9)	2.5 (1.7)	3.0 (2.0)	2.7 (1.8)	*0.0001*

Data are given as mean, with the standard deviation given in parenthesis. (*) Glomerular filtration rate was estimated by Schwartz formula.

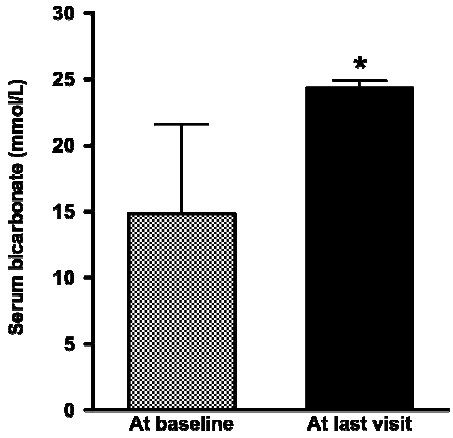

FIGURE 5.1 - *Comparison between bicarbonate levels at baseline and at last visit in distal RTA patients. * p < 0.05 (paired Student T test)*

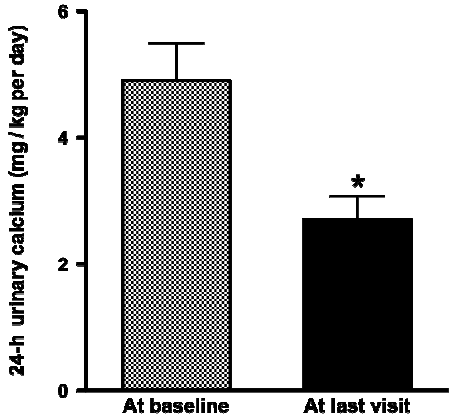

FIGURE 5.2 - *Comparison between 24-hour urinary calcium excretion (mg/Kg/day) at baseline and at last visit in distal RTA patients. * p < 0.05 (paired Student T test)*

5.3.3 Growth parameters analysis

The difference between WAZ and HAZ at last visit and the same parameters at baseline revealed a significant improvement of somatic growth. The mean WAZ was -2.39 (range -5.14 to -0.13, SD 1.34) at baseline and reached -1.08 (range -3.67 to 1.58, SD 1.27) at the end

of follow-up period (p<0.01, Figure 3A). The same occurred for stature whose mean HAZ was -2.47 (range -5.87 to 1.53, SD 1.75) at baseline and achieved -1.57 (range -6.32 to 1.04, SD 1.87) at last visit (p<0.01, Figure 3B). Based on weight/age and stature/age curves, 58.3% of distal RTA patients completely recovered growth and 37.5% clearly improved after treatment but still presented short stature.

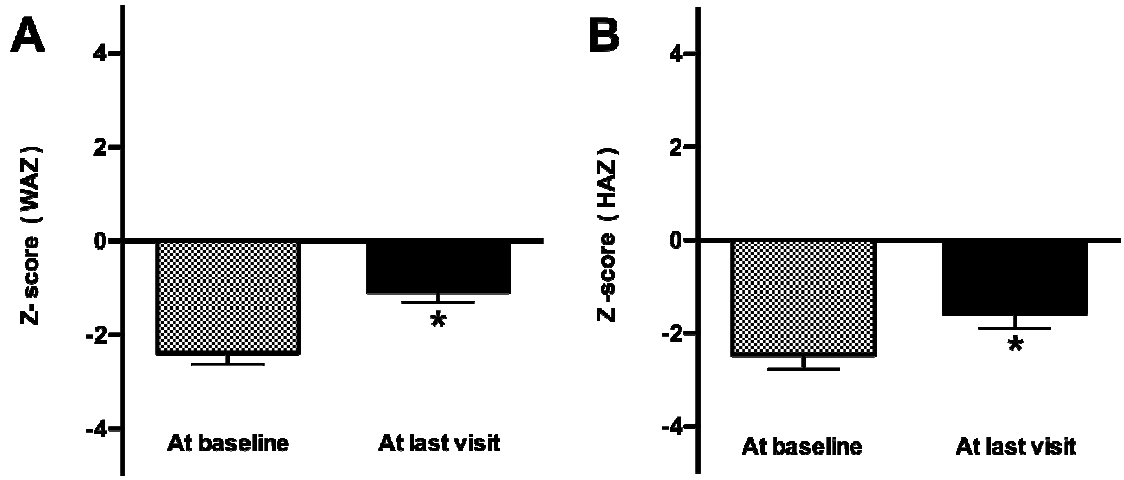

FIGURE 5.3 – *Growth parameters of distal RTA patients at baseline and at last visit. Panel A – Comparison between weight-for-age Z (WAZ) score at last visit and at baseline. Panel B - Comparison between height-for-age Z (HAZ) score at last visit and at baseline. *p<0.05 (paired Student T test)*

In univariate analysis, only bicarbonate levels at admission were significantly associated to HAZ gain. According to our criteria, gender (p=0.059) and nephrocalcinosis (p=0.197) were also included in the regression model. After adjustment, only bicarbonate level at admission was an independent predictor of HAZ improvement (OR=1.3, CI 95% 1.1-1.6, p<0.01). The final model showed that each reduction of 1 mmol/L on bicarbonate levels at admission corresponds to a decrease of approximately 31.2% on the HAZ gain. For WAZ gain, the univariate analysis showed that gender (p=0.001) and bicarbonate levels (p=0.043) were significantly associated to WAZ gain. Among the other variables, etiology (p=0.239) and age 3 (p=0.234) were included in the regression model. After adjustment, only the male gender was negatively associated with WAZ gain in the final model (OR=13.7, CI95% 2.5-74.3, p<0.002).

5.4 Discussion

In this retrospective cohort study we report the clinical outcome of a group of pediatric patients with distal RTA with a prolonged median follow-up of about 10.8 years at a tertiary centre. The main finding regarding our cohort is that the clinical course of distal RTA was relatively benign and the majority of children presented clinical and growth improvement during follow-up.

In our series, primary distal RTA was the commonest form and the mean age at diagnosis was 2.67±3.1 years, which can be considered an early diagnosis as compared with a mean age at diagnosis of 6 years in the series reported by Bajpai et al. [9]. In distal RTA, the clinical features at presentation usually include growth impairment, polyuria, hypercalciuria, nephrocalcinosis, lithiasis and K^+ depletion [1-4, 9, 13]. Accordingly, the most frequent clinical finding in our series was growth impairment in 24 (72%) patients and hypercalciuria in 17 (51%) cases at presentation. Rickets was initially detected in 33.3% of our patients and nephrocalcinosis in 39.4%. In previous series [9, 14], nephrocalcinosis was more frequently observed (60-78.5%) than in our study. This is probably due to the fact that our patients had an earlier diagnosis. In addition, hypercalciuria was well controlled in our patients and none developed chronic renal failure due to the progression of calcium deposition on kidney parenchyma.

At diagnosis, physical examination revealed weight under 3rd percentile in 72.7% patients and 79.2% of them also exhibited height under this score. After treatment, completely growth catch up occurred in 58.3% of distal RTA patients and 37.5% partially recovered weight and height. Bajpai et al. [9] and Nash et al. [12] also found growth impairment at diagnosis and in the former with adequate response to treatment. The evolution of our patients supports the general idea that if distal RTA is detected early in life, adequate correction of acidosis by continuous alkali administration may result in a normal growth, arrest or even prevention of nephrocalcinosis and preservation of renal function [1-4, 19].

More importantly, our study suggests that some aspects should be considered at the first assessment of distal RTA patients. Bicarbonate levels at admission seem to be an independent predictor of height improvement. The regression final model showed that each reduction of 1 mmol/L on bicarbonate levels at admission corresponds to a decrease of approximately 31.2% on height gain. It is known that the alkaline supplementation corrects as much as possible the biochemical abnormalities of RTA and also improves growth in children at all ages.

Furthermore, the normalization of blood pH has a positive effect on bone linear growth by increasing calcium apposition rate and mineralized surface in distal RTA patients [20]. In our point of view, the children early detected with lower levels of bicarbonate at admission should be followed closely and carefully to minimize the effects of acidosis on the final height. In this way, bicarbonate levels at admission might be a marker of a worse prognosis in terms of height.

To our best knowledge, we did not find previous studies which had reported gender differences in clinical and laboratorial data of distal RTA patients. In our series, male sex has a negative effect on final WAZ gain (OR=13.7) despite the fact that, at baseline, females presented a worse WAZ average and, at last visit, no significant correlation of final weight with gender was observed (p=0.46). Indeed, the multivariate model showed that girls with distal RTA have greater chance to gain weight than boys. On the other hand, although HAZ gain was also greater in females, no statistical difference was found in the comparison between genders (p=0.089). These gender differences in growth catch up allow us to suppose that female sex is associated with a better prognosis for distal RTA patients. One could speculate that the variable sex was confounded with other covariates. However, the multivariate model excluded this possibility. In addition, girls exhibited worse metabolic acidosis than boys at admission with lower levels of bicarbonate. A possible explanation for this intriguing observation could be related to the complexity of tubular disorders genetics [21-23].

We are aware of the limitations associated with the retrospective design and the limited number of patients of our study. Nevertheless, some aspects may increase the strength of our findings, such as the long-term follow-up, the utilization of strictly defined inclusion and exclusion criteria and a well-established protocol for the approach of our distal RTA patients.

In conclusion our series clearly showed that primary distal RTA is a chronic disease and therapy should be maintained throughout life. Nevertheless, prognosis is good if diagnosed early in life and appropriate amounts of alkali supplements should be continuously administered. Our series clearly showed adequate treatment restores growth and prevents the progression to nephrocalcinosis at all ages. However, if therapy is delayed to late childhood or adulthood progression to end-stage renal disease may not be avoided. Finally, in our study, we found bicarbonate levels at diagnosis as a good putative height prognosis marker and girls, even with lower bicarbonate levels, will have a better prognosis for distal RTA.

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6 COMENTÁRIOS FINAIS

A grande importância do estudo da ATR distal encontra-se no impacto causado sobre o crescimento pôndero-estatural das crianças acometidas e não tratadas adequadamente [1]. Outro fator relevante é a dificuldade diagnóstica que, ao retardar o inicio do tratamento, pode piorar significativamente o prognóstico. Isso é devido a existência de um extenso número de patologias que podem cursar com sinais e sintomas semelhantes ao da ATR distal [2]. De maneira geral, os pacientes procuram assistência médica com queixas inespecíficas tais como hipodesenvolvimento físico, baixa estatura, poliúria, polidipsia e hidrolabilidade. Quadros de déficit de crescimento são freqüentes, mimetizando uma desnutrição protéico-calórica, muitas vezes associados a raquitismo, considerado carencial [3, 4]. Dessa forma, estas crianças são encaminhadas para serviços de nutrição onde o tratamento instituído raramente surte efeito. São também comuns quadros de desidratação associados à diarréia e vômitos, assim como febre de origem indeterminada, o que proporciona internações freqüentes e conseqüente piora da qualidade de vida dos pacientes [5, 6].

Em nosso estudo, a apresentação clínica mais prevalente foi o déficit de crescimento (72%) seguido por poliúria e polidipsia (24,2%). Esses dados também são encontrados em estudos clínicos semelhantes [3, 4, 6-8]. Hipercalciúria foi detectada em 17 casos ao diagnóstico (51%), nefrocalcinose em 13 (39,4%) e raquitismo em 11 (33,3%). Com relação à etiologia, a ATR distal primária foi a forma mais comum entre nossos pacientes (60,6%), o que também já foi observado em estudos prévios [9, 10].

No entanto, o presente estudo diferencia-se dos anteriores [8-11] por avaliar de forma sistemática os fatores preditores independentes do ganho de peso e de estatura em uma casuística de ATR distal com tempo de seguimento longo. Os resultados deste estudo mostraram que o nível inicial do bicarbonato sanguineo foi um fator preditivo do ganho de estatura, ou seja, a cada redução de 1mmol/L das concentrações de bicarbonato reduz-se em 31,2% a chance de o paciente recuperar um desvio padrão em seu escore Z de altura. Outro achado relevante encontra-se no fato de o sexo masculino exercer efeito negativo sobre o ganho de peso (OR=13,7). É interessante observar que apesar das pacientes do sexo feminino terem apresentado valores médios inferiores para o escore Z peso-idade ao diagnóstico, os meninos tiveram recuperação inferior do peso. Dessa forma, nossos dados sugerem que o sexo feminino está associado a um melhor prognóstico em relação ao ganho de peso. Deve-se

ressaltar ainda que tal achado não foi previamente relatado na literatura e acreditamos que possa estar associado à complexidade genética das tubulopatias.

Após a instituição do tratamento, observamos uma significativa mehora clínica e laboratorial nos nossos pacientes. Destaca-se que 58,3% dos casos de ATR recuperaram completamente o crescimento tanto em peso quanto em estatura e 37,5% obtiveram um ganho signficativo de estatura e peso. Podemos atribuir este sucesso ao diagnóstico precoce, tratamento adequado e acompanhamento regular e rigoroso dessas crianças.

Como limitação, ressalta-se o número reduzido de pacientes. Fato este inerente à raridade da doença estudada. Nosso estudo incluiu 33 crianças e adolescentes. Apesar de numericamente reduzida, pode-se considerar uma amostra representativa se comparada a outras casuísticas [5, 6, 8-11]. Por outro lado, o longo período de seguimento de nossos pacientes (média de 10,8 anos) associado à instituição de um protocolo rigoroso de acompanhamento fortalece nossos achados.

Em resumo, foi mostrado que a detecção precoce, o tratamento adequado e o acompanhamento regular revestem-se de especial importância, já que podem modificar completamente o curso desta doença, não só em relação ao crescimento, mas também provavelmente no que se refere ao dano renal, muitas vezes irreversível [9]. É necessário que novos estudos sejam realizados com o intuito de confirmar ou definir de forma mais apropriada os fatores prognósticos e preditivos da evolução da ATR distal.

Finalmente, o presente estudo abre perspectivas para realização de novas pesquisas tanto em relação aos aspectos clínicos quanto aos moleculares, contribuindo, desse modo, para a melhor compreensão das tubulopatias.

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1972; 80:738-748.

ANEXOS

ANEXO A Parecer do comitê de ética da UFMG (COEP)

Parecer nº 144/02

Interessadas: Profª. Dra. Ana Cristina Simões e Silva e
Critiane Jeannette Crosara Horta
Depto de Pediatria - FM/UFMG

Voto:

O Comitê de Ética em Pesquisa da UFMG – COEP aprovou no dia 04 de setembro de 2002 o projeto de pesquisa intitulado « **Estudo das disfunções tubulares em crianças e adolescentes acompanhados pela unidade de nefrologia pediátrica do HC UFMG** » e o Termo de Consentimento Livre e Esclarecido do referido projeto. O relatório final ou parcial deverá ser encaminhado ao COEP um ano após o início do projeto.

P.P. Marcelo R. Marques (em substituição)
Prof. Dr. Dirceu Bartolomeu Greco
Presidente do COEP/UFMG

ANEXO B Ficha individual para elaboração do banco de dados

NOME:	DN:
Diagnóstico:	GRIM / SAME:

Data										
Idade										
Peso										
Estatura										
PA										
Medicações										
Modificações										
Intercorrências										

EXAMES sg										
Hm										
Hb										
Ht										
Ur										
Cr										
Na										
K										
Cl										
Ca										
P										
Mg										
Ac. Úrico										
FA										
Albumina										
PTH										
pH										
Bic										
BE										
PCO2										
Outros										

URINA rotina										
densidade										
pH										
Elementos Anormais										
URINA 24h										
Volume (mL)										
Proteinuria mg/dl										
g/24h										
Na (mmol/L) / FE%										
K (mmol/L) / FE%										
Cl (mmol/L) / FE%										
Ca (mg/dL) / FE%										
P (mg/dL) / FE%										
Mg (mg/dL) / FE%										
Creatinina(mg/dL)/FE%										
Ca (mg/kg/dia)										
RFG										
Outros										

IMAGENS / OUTROS

Rx					
US					
Outros					

ANEXO C Artigo de Revisão

Current Genomics, 2009, *10*, 000-000

Molecular Pathophysiology of Renal Tubular Acidosis

P.C.B. Pereira, D.M. Miranda, E.A. Oliveira* and A.C. Simões e Silva

Pediatric Nephrology Unit, Department of Pediatrics, School of Medicine – Federal University of Minas Gerais (UFMG), Belo Horizonte, MG, Brazil

Abstract: Renal tubular acidosis (RTA) is characterized by metabolic acidosis due to renal impaired acid excretion. Hyperchloremic acidosis with normal anion gap and normal or minimally affected glomerular filtration rate defines this disorder. RTA can also present with hypokalemia, medullary nephrocalcinosis and nephrolitiasis, as well as growth retardation and rickets in children, or short stature and osteomalacia in adults. In the past decade, remarkable progress has been made in our understanding of the molecular pathogenesis of RTA and the fundamental molecular physiology of renal tubular transport processes. This review summarizes hereditary diseases caused by mutations in genes encoding transporter or channel proteins operating along the renal tubule. Review of the molecular basis of hereditary tubulopathies reveals various loss-of-function or gain-of-function mutations in genes encoding cotransporter, exchanger, or channel proteins, which are located in the luminal, basolateral, or endosomal membranes of the tubular cell or in paracellular tight junctions. These gene mutations result in a variety of functional defects in transporter/channel proteins, including decreased activity, impaired gating, defective trafficking, impaired endocytosis and degradation, or defective assembly of channel subunits. Further molecular studies of inherited tubular transport disorders may shed more light on the molecular pathophysiology of these diseases and may significantly improve our understanding of the mechanisms underlying renal salt homeostasis, urinary mineral excretion, and blood pressure regulation in health and disease. The identification of the molecular defects in inherited tubulopathies may provide a basis for future design of targeted therapeutic interventions and, possibly, strategies for gene therapy of these complex disorders.

Key Words: Renal tubular acidosis, acid-base homeostasis, molecular physiology, tubular transport, gene mutations.

INTRODUCTION

The term Renal Tubular Acidosis (RTA) defines many disorders characterized by metabolic acidosis, secondary to defects in renal tubular reabsorption of bicarbonate (HCO_3^-) and/or in urinary excretion of hydrogen (H^+), while glomerular function is little or not affected [1-6]. All forms of RTA present hyperchloremic metabolic acidosis, with normal anion gap and are chronic diseases with significant impact on the quality of life of affected patients when left untreated, possibly leading to growth failure, osteoporosis, rickets, nephrolithiasis and even renal insufficiency [1-6].

Defects in proximal bicarbonate reclamation or distal acid secretion give rise to the respective clinical syndromes of proximal or distal RTA [1-6]. These disorders can be primary, originating from genetic defects on tubular transport mechanisms [7], or secondary to systemic diseases and to adverse drug reactions [8-12]. The familial conditions exhibit distinct inheritance patterns. Distal RTA can be transmitted as either an autosomal dominant or an autosomal recessive trait, whereas isolated proximal RTA usually occurs as an autosomal recessive disease [6,7,13]. In the past few years, the molecular genetic strategies of positional cloning and candidate gene analysis have been combined to identify the genes responsible for these inherited conditions [6,13]. This review will summarize the mechanisms of acid-base regulation by the kidney and the current understanding of the genetic causes of primary inherited RTA. It will, in addition, evaluate the ability of known functional and biochemical properties of these mutant proteins to explain the pathophysiology of associated renal acidification defects.

BRIEF OVERVIEW OF RENAL ACID-BASE HOMEOSTASIS

The kidney plays two major roles in acid-base homeostasis. First, the filtered bicarbonate load (approximately 4000 mmol/day) must be reabsorbed, mainly in the proximal tubule and beyond in the loop of Henle and distal nephron. This reclamation process in the proximal tubule minimally requires the following: hydrogen (H^+) secretion of an equivalent amount *via* the luminal Na^+/H^+ exchanger (NHE-3) and the vacuolar H^+-ATPase; luminal carbonic anhydrase type IV (CAIV) and cytosolic carbonic anhydrase type II (CAII); and basolateral bicarbonate exit through the electrogenic Na^+-dependent bicarbonate cotransporter (NBC-1) [2,14-17]. Second, the kidney must regenerate new bicarbonate (approximately 50 ± 100 mmol/ day) in the process of acid-secretion, mainly in the collecting ducts, to match the amount of newly produced acid load by systemic metabolism [18,19]. In addition to sufficient buffer in the lumen, this process requires activities of several transport proteins of the acid secreting α-intercalated cells, including the luminal

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vacuolar H^+-ATPase, CA II, and the basolateral chloride-bicarbonate exchanger, AE1 [18,20,21].

Proximal Tubular Bicarbonate Reabsorption

HCO_3^- is freely filtered at the glomerulus and approximately 80 to 90% of this is reabsorbed in the proximal tubule [6]. In the tubular lumen, HCO_3^- combines with H^+ in a reaction catalyzed by CA IV, which is bound to the luminal membrane of proximal tubular cells [2,14,15]. This reaction produces carbonic acid, which is promptly converted to CO_2 and H_2O. The resulting CO_2 rapidly diffuses into the tubular cells and is combined with water to produce intracellular H^+ and HCO_3^-. This intracellular reaction is catalyzed by CA II. HCO_3^- is then cotransported with Na^+ into blood (with a probable stoichiometry of 3 HCO_3^- to 1 Na^+) [6] *via* the NBC-1, located on the basolateral cell membrane. The intracellular H^+ produced by CA II is secreted into the tubular lumen predominantly *via* the NHE-3, situated on the luminal membrane [6,15,22]. This transport process is called facilitated diffusion and depends on the sodium concentration gradient generated by the action of a basolateral membrane Na^+-K^+-ATPase. It should be mentioned that there is minimal net acid excretion in the proximal tubule, since most of the H^+ secretion is coupled with HCO_3^- reabsorption [6,13]. The small amount of remaining H^+ will be buffered by phosphate as titratable acid. HCO_3^- reabsorption is influenced by luminal HCO_3^- concentration and pH, luminal flow rate, peritubular pCO_2, and angiotensin II [2,6,17].

Proximal tubular cells are capable of generating "extra" bicarbonate through the deamination of glutamine to glutamate, then forming α-ketoglutarate and eventually glucose. This metabolic process produces HCO_3^- and NH_4^+: the former reclaimed *via* the basolateral membrane and the latter secreted into the tubular lumen. This pathway can be upregulated in states of chronic acidosis [3,6,15].

The main mechanisms of proximal tubular bicarbonate reabsorption are displayed in Fig. (**1**).

Distal Tubular Hydrogen Secretion

One of the important roles of the collecting duct segment of the nephron is acid secretion, combined with reclamation of the approximately 10% of filtered HCO_3^- that is not reabsorbed by more proximal nephron segments [18]. The average omnivorous human diet in the `Western' world is rich in protein, and generates 1±1.5 mmol hydrogen/kg body weight each day [23]. Urinary acid excretion is therefore essential, and urine pH can drop as low as 4.5. The α-intercalated cell is the main responsible for hydrogen secretion into the urine. In humans at least, hydrogen pumps, called H^+-ATPases, mainly carry out hydrogen secretion [18,19,23]. H^+-ATPases are present at high density on the luminal membrane of α-intercalated cells [18]. Studies in nonhuman mammals show that these H^+-ATPases are also present within specialized intracellular tubulovesicles close to the membrane, allowing additional pumps to be recruited to the membrane quickly in to response to stimuli, such as systemic acidosis, for example [23]. These cells secrete H^+ into the lumen of the distal tubule and collecting duct not only *via* H+-ATPase but possibly also by an exchanger, H^+/K^+-ATPase [7,10]. In addition, the normal function of the luminal H^+-ATPase in α-

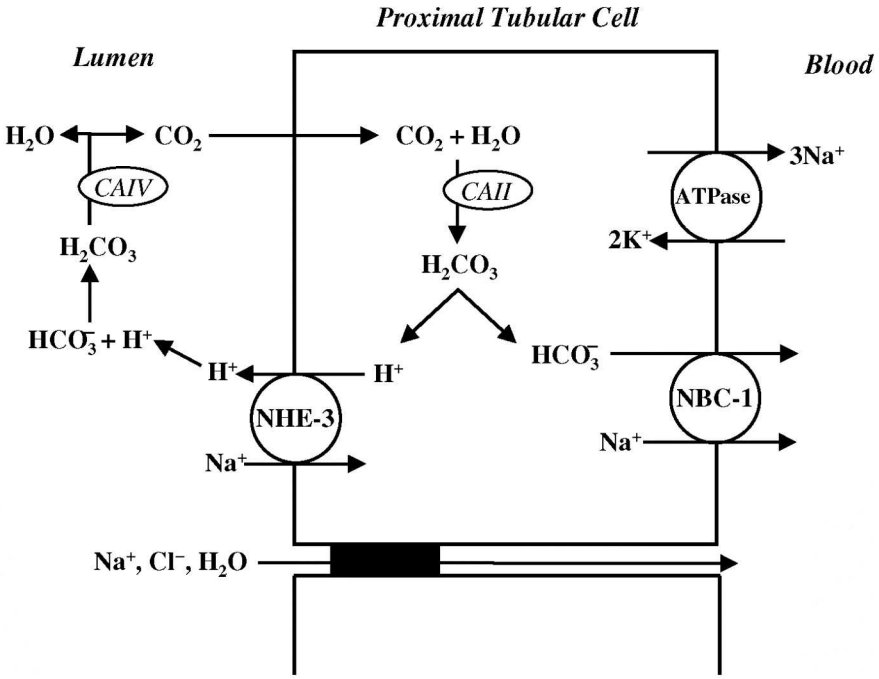

Fig. (1). Schematic model of bicarbonate (HCO_3^-) proximal reabsorption. The intracellular carbonic acid ($H_2CO_3^-$) dissociates into H^+ and HCO_3^- in a reaction catalysed by a cytoplasmic carbonic anhydrase (CAII). At the luminal membrane, H^+ secretion is due to an especific Na^+ – H^+ exchanger (NHE-3), while, at the basolateral membrane, the 1 Na^+ - 3 HCO_3^- cotransporter (NBC-1) is responsible for HCO_3^- transport to the peritubular capilar. The secreted H^+ reacts with filtered HCO_3^- to form luminal H_2CO_3, which is dissociated into H_2O and CO_2 by the action of membrane-bound carbonic anhydrase (CAIV). The generated CO_2 diffuses back into the cell to complete the HCO_3^- reabsorption cycle.

intercalated cells is coupled, in a poorly understood manner, to the electroneutral transport of HCO_3^- back across the basolateral surface into the interstitial fluid, and hence to blood. The transporter responsible for this activity in renal α-intercalated cells is the Cl^-/HCO_3^- exchanger AE1 [7,20,21]. The AE1 exchanger is homologous with the red cell anion exchanger known as 'band 3' (eAE1) [6,24]. After the red cell, the kidney is the next richest source of this protein (kAE1) [24]. Proton secretion varies with systemic pH and it is also aldosterone-dependent and voltage-dependent [24].

Once secreted, net urinary elimination of H^+ depends on its buffering and excretion as titratable acid (mainly phosphate - $HPO_4^{2-} + H^+ \leftrightarrow H_2PO_4^-$), and excretion as NH_4^+ [24]. Notably, the production of NH_4^+ from glutamine by the proximal tubule, and its subsequent excretion in the urine, also generates 'new' bicarbonate, which is added to plasma [24]. Availability of phosphate as a buffer depends on its filtration, whereas NH_4^+ depends on normal function of the proximal tubule, as well as a complex process of secretion, reabsorption, and secretion again along the nephron [24]. The final secretory step for NH_4^+ excretion is 'diffusion trapping' in the collecting duct. Anything that interferes with H^+ secretion in the collecting duct will reduce diffusion trapping and cause a decrease in excretion of both H^+ and NH_4^+ [6,24]. As previously mentioned, chronic metabolic acidosis stimulates renal NH_4^+ synthesis and excretion [3,6,15].

Fig. (**2**) shows renal acidification process in α-intercalated cells of the distal nephron.

CLASSIFICATION AND CLINICAL FEATURES OF RENAL TUBULAR ACIDOSIS

Clinically, RTA is characterized by a normal anion gap, hyperchloremic metabolic acidosis, and associated failure to thrive secondary to growth failure as well as anorexia [13]. Polyuria and constipation can also be seen, although neither may be apparent in the neonatal period [13]. Hyperchloremic metabolic acidosis in pediatric practice is most often associated with diarrheal disease. Both diarrhea and RTA result in hypokalemia. For this reason, in a young infant with diarrhea and underlying RTA, the true diagnosis may be obscured. Thus, inordinately slow resolution of hyperchloremic metabolic acidosis following diarrheal disease should suggest the possibility of an underlying primary RTA [13].

Beyond the difficulties inherent in delineating RTA, RTA can be subcategorized into different disorders with distinctly diverse prognoses [13]. The diagnostic cataloguing of RTA is based on the underlying pathophysiology. The current model of how the nephron reabsorbs HCO_3^- and secretes H^+ has led to a clinical and functional classification of proximal (tubule) versus distal (tubule and collecting duct) forms of RTA [24]. Thus, the main types of RTA are proximal (or type 2) RTA and distal (or type 1) RTA. Type 3 RTA is a mixed type RTA that exhibits both impaired proximal HCO_3^- reabsorption and impaired distal acidification, and more disturbingly osteopetrosis, cerebral calcification and mental retardation [4]. Hyperkalemic (or type 4) RTA is a heterogeneous group of disorders that is characterized by low urine NH_4^+, which is probably caused by the hyperkalemia or by aldosterone deficiency or defective signaling [4].

In distal RTA, distal nephron net acid secretion is impaired. This leads to a high urine pH, even in the presence of systemic acidosis [2,4]. However, there is often no metabolic acidosis and the blood bicarbonate concentration is normal, so-called 'incomplete' distal RTA, and a defect in renal acid excretion must be demonstrated by a failure to lower urine

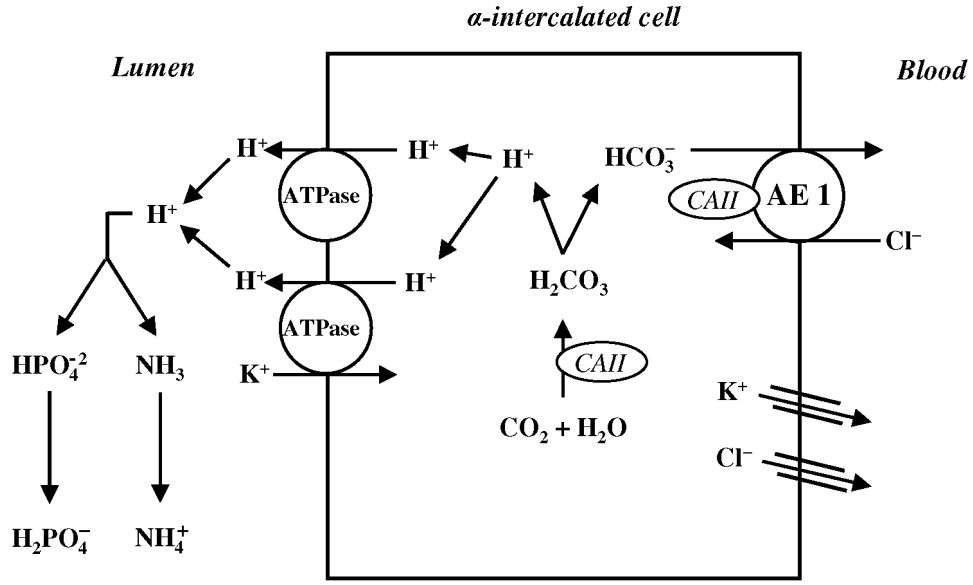

Fig. (2). Schematic model of the α-intercalated cell and the H^+ secretion in cortical collecting tubule. The α-intercalated cell is responsible for H^+ secretion by a vacuolar H^+-ATPase (main pump) and also by a H^+-K^+-ATPase. The luminal ammonia (NH_3) buffers H^+ to form nondiffusible ammonium (NH_4^+) and divalent basic phosphate (HPO_4^-) is converted to the monovalent acid form ($H_2PO_4^-$) in H^+ presence. Intracellularly formed HCO_3^- leaves the cell *via* Cl^- - HCO_3^- exchange, facilitated by an anion exchanger (AE1). Cytoplasmic carbonic anhydrase II (CA II) is necessary to secret H^+.

pH below 5.5 following an NH_4Cl load or a modified furosemide test [2,6,24]. Acquired distal RTA is often secondary to autoimmune diseases, such as Sjogren's syndrome [6,24]. Inherited distal RTA can be essentially of three types: autosomal dominant distal RTA (the commonest form) and autosomal recessive distal RTA with and without sensorineural deafness [24]. In the complete forms of both dominant and recessive distal RTA bone disease is common (rickets or osteomalacia), as well as nephrocalcinosis (often) complicated by renal stone disease. The occurrence of renal stones is attributed to the combination of hypercalciuria, low urinary citrate excretion (due to systemic and intracellular acidosis) and high urine pH, all favouring calcium phosphate stone formation. Hypokalaemia, another characteristic feature, is less troublesome than in the acquired autoimmune form of distal RTA, but it can become symptomatic, especially if a thiazide diuretic is prescribed to reduce hypercalciuria [24]. In recessive distal RTA, some patients suffer from sensorineural deafness, which can be late in onset [24].

Conceptually, the proximal tubule is charged with the task of reclaiming filtered HCO_3^- (~ 85% of the total) [13]. Failure of this process leads to reduction in systemic base, resulting in metabolic acidosis – proximal RTA [13]. Proximal RTA typically manifests as part of a generalized defect of proximal tubule function, namely the renal Fanconi's syndrome (with glycosuria, low molecular weight proteinuria, urinary phosphate wasting, hypophosphataemia and hypouricaemia) [24]. Isolated proximal RTA occurs rarely and usually presents as growth retardation in childhood. Like distal RTA, it can be divided into three types: autosomal recessive proximal RTA with ocular abnormalities, autosomal recessive proximal RTA with osteopetrosis and cerebral calcification, and autosomal dominant proximal RTA [24]. Autosomal recessive proximal RTA with ocular abnormalities is the commonest form of isolated and inherited proximal RTA, but even this is rare. Ocular abnormalities include band keratopathy, glaucoma and cataracts [24]. Short stature is usual; dental enamel defects, mental retardation, hypothyroidism, abnormal pancreatic function and basal ganglia calcification are also features [24,25]. In inherited CA II deficiency, isolated proximal RTA presents with osteopetrosis (due to impaired osteoclast function), cerebral calcification and variable mental retardation [26]. Although this form of inherited RTA is clinically more proximal in type, it can also present with a mixed proximal and distal phenotype, which reflects the presence of CA II in cells all along the renal tubule.

Type 3 RTA can be caused by recessive mutation in the *CA2* gene on chromosome 8q22, which encodes CAII [4] or could involve direct interaction between CA II and the NBC1 [27] or Cl^-/ HCO_3^- exchanger, *SLC26A6* [4,28].

The causes of type 4 RTA include various types of adrenal failure or pseudohypoaldosteronism type 1 (PHA1) due to defects in the mineralocorticoid receptor or the epithelial Na^+ channel, all characterized by salt loss and hypotension [4]. A similar picture may be seen in obstructive uropathy or drug induced interstitial nephritis [4]. Furthermore, a number of drugs may impair signalling in the renin–angiotensin-aldosterone system and cause hyperkalemia and metabolic acidosis (e.g. potassium sparing diuretics, trimethoprim, cyclo-oxygenase inhibitors, angiotensin converting enzyme inhibitors) [4]. Lately, much interest has been given to a group of rare autosomal dominant diseases characterized by hyperkalaemia and acidosis and age-related hypertension [4]. In spite of hypervolaemia, aldosterone is not low and the disorders have been collectively termed pseudohypoaldosteronism type 2 (PHA2) [4].

INHERITED FORMS OF DISTAL RENAL TUBULAR ACIDOSIS

Inherited forms of distal RTA have three variants: autosomal dominant and autosomal recessive with or without deafness. Dominant disease typically presents more mildly in adolescence or adulthood, and recessive variant occurs in infancy/early childhood, where growth retardation is common [6]. In Table **1** we can see the chromosome mapping of distal RTA.

Autosomal Dominant Distal RTA (Distal RTA Type 1a)

Distal RTA occurs with the greatest frequency as an isolated defect, often transmitted as an autosomal dominant trait [13]. In few reported families, the presence of the disorder in several generations suggests an autosomal dominant transmission. Although clinical findings are not different from those observed in autosomal recessive or sporadic cases, in these patients the disease may be diagnosed later (in adolescence or adulthood) [6] or manifest with milder symptomatology.

Autosomal dominant distal RTA has been found to be associated in several kindred with mutations in the *SLC4A1* gene encoding the Cl^-/HCO_3^- exchanger, AE1 [15].

The Electroneutral Anion Exchanger (AE1)

The Cl^-/HCO_3^- anion exchanger, AE1, is a glycoprotein encoded by a gene (*SLC4A1*) present on chromosome 17 q21-22. *SCL4A1* gene is a member of the *SLC4* family com-

Table 1. Chromosome Mapping of the Inherited Distal Renal Tubular Acidosis

Inherited Distal RTA	Gene	Mapping	Protein Encoded
Autosomal dominant	*SLC4A1*	Chromosome 17q21-q22	AE 1 exchanger
Autosomal recessive *(with deafness)*	*ATP6V1B1*	Chromosome 2q13	B1-subunit of H^+-ATPase
Autosomal recessive *(with preserved hearing)*	*ATP6V0A4*	Chromosome 7 q33-q34	a4 isoform subunit of H^+-ATPase

prising 10 genes of which 8 encode bicarbonate ion transporters [6,24,29]. AE1 is an integral membrane glycoprotein containing a long cytoplasmic N-terminus (~ 400 amino acids), 12–13 transmembrane domains (responsible for anion transport and dimerization), and a short cytoplasmic C-terminus (~ 35 amino acids) [30,31]. It is predominantly expressed in the erythrocytes (eAE1) and in the kidney (kAE1).

kAE1 is a truncated isoform of eAE1 with lacking of 65 amino acids at the N-terminus owing to the use of differential transcription and translation start sites [32]. This extra NH2-terminal sequence confers additional roles for eAE1, including facilitation of red cell metabolism and maintenance of erythrocyte structural stability *via* interaction with a glycolytic enzyme complex and cytoskeletal elements, respectively [6]. kAE1 mediates an electroneutral exchange of chloride for bicarbonate at the basolateral membrane of acid secreting α-intercalated cells of the distal nephron and collecting duct [32,33]. This ion exchanger promotes the reabsorption of bicarbonate into the blood. Therefore, eAE1 defect results in morphological changes of red blood cells (RBC) while kAE1 abnormality leads to distal RTA [32].

The physiological role of kAE1 in the regulation of distal nephron acid secretion is well established. In the acidification process of the distal nephron, basolateral kAE1 mediates Na^+-independent, electroneutral Cl^-/HCO_3^- exchange, allowing HCO_3^- to exit the α-intercalated cells in concert with apical H^+ secretion *via* the vacuolar H^+-ATPase.

AE1 Gene (SLC4A1) Mutations

Because of the expression of AE1 in two different cells (RBC and α-intercalated distal tubular cells) with distinct functions, AE1 mutations show pleiotrophic effects resulting in two distinct and seemingly unrelated phenotypes: hereditary spherocytosis (or other forms of erythrocyte abnormalities) and distal RTA [31]. The largest group of mutations in human AE1 is associated with autosomal-dominant red cell dysmorphologies (hereditary spherocytosis – HS; and Southeast Asian ovalocytosis - SAO), where renal acid-base handling is normal [6]. AE1 mutations also result in distal RTA, because the defect in AE1 affects anion Cl^-/HCO_3^- exchanger at the basolateral membrane of the α-intercalated cells in the distal nephron [31].

SAO, a well-known erythrocyte disorder, is caused by a deletion of 27 bp in codons 400-408 in exon 11 (Ex11D27) of AE1 leading to a lack of 9 amino acids in the protein, which is inactive for anion transport.

How can be explained either the absence of red cell abnormalities in patients with distal RTA or the rarity of defects in distal urinary acidification in patients with hematological disorders, when, in both circumstances, mutations in the same *SLC4A1* gene are present? [15]. One exception is the homozygous AE1 mutant V488M (Band 3 Coimbra; GTG → ATG), which presents with severe anemia and renal acidification defect [34,35].

The majority of AE1 mutations apparently cause only erythroid abnormalities without renal phenotype. Most cause autosomal dominant forms of HS and are not encountered in homozygous form, suggesting embryonic lethality [7].

Dominant HS-associated AE1 mutations are generally not associated with distal RTA. Conversely, distal RTA-associated AE1 mutations are also not commonly accompanied with HS. Whereas HS missense mutations are distributed throughout AE1 cytoplasmic and transmembrane domains, distal RTA mutations are restricted to AE1's transmembrane domain. Although, the almost complete segregation between mutations associated with HS and with distal RTA is not fully understood [7].

Autosomal dominant distal RTA was first associated with exon 14 nucleotide substitutions encoding missense mutations in residue 589 (R589), in which the wild-type Arg is converted to His, Ser, or Cys [30,36]. A single base change alters the identical AE1 residue, R589, in eight of the ten reported kindred with dominant distal RTA, supporting the importance of this residue in the normal acidification process. R589 lies at the intracellular border of the sixth transmembrane domain of the protein, adjacent to K590. These basic residues are conserved in all the known vertebrate anion exchanger isoforms and are thought to form part of the site of intracellular anion binding. Arginine at this position is conserved in all vertebrate AE proteins, indicating its functional importance [37].

Three different mutations at this position (R589C, R589H, and R589S) were found in autosomal dominant distal RTA and two *de novo* R589H mutations have also been reported [30,32,36]. A high prevalence of AE1 R589 mutations and the presence of at least two *de novo* mutations at this position suggest that codon 589 (CGC) is a "mutational hotspot" of AE1. The mechanism of recurrent mutations probably involves methylation and deamination altering cytosine (C) to thymine (T) in the CpG dinucleotides [37].

Another missense mutation alters serine to phenylalanine at position 613 [36] within the adjacent transmembrane loop, evidencing the importance of this region of the protein. A further complex mutation results in a C-terminally truncated AE1 protein lacking the last 11 amino acids [29].

AE1 in Autosomal Recessive Distal RTA

Recent gene studies have shown that some of the AE1 mutations are responsible for autosomal recessive distal RTA in several countries in Southeast Asia; these patients may be homozygous for the mutation or be compound heterozygotes of two different AE1 mutations, one of which is usually the SAO mutation [38,39]. The evaluation of the AE1 G701D mutation has provided the first explanation for how any distal RTA-associated AE1 mutation might cause the disease [40].

Recessive distal RTA appears to result from the absence or a very marked deficiency of chloride-bicarbonate exchange activity in the basolateral membrane of the distal α-intercalated cell. In the case of the G701D mutation this occurs because the mutant protein is totally dependent on the presence of glycophorin A (GPA) for its movement to the cell surface. GPA is a glycosylated protein that is associated with band 3 and has a single span across the erythrocyte membrane [38]. Expression in *Xenopus* oocytes demonstrated that GPA completely rescues the cell surface movement of the G701D mutant band 3 to normal levels. This contrasts with normal band 3, which moves to the cell sur-

face even in the absence of GPA, although GPA further enhances this movement. Red blood cells contain GPA but GPA is absent from the kidney, hence individuals homozygous for the G701D mutation have normal levels of band 3 in their red cells. It is proposed that, in homozygotes, the mutant G701D protein does not reach the basolateral membrane of the α-intercalated cell, but is turned over within the cell. In SAO/G701D compound heterozygotes, the SAO protein is presumed to reach the cell surface, but since it is inactive in anion transport, it acts as if it were a band 3 null allele [38].

Autosomal Recessive Distal RTA with Deafness (Distal RTA Type 1b)

Recessive forms of distal RTA are related to mutations in the proton pump in α-intercalated cells. The gene involved (*ATP6V1B1*) is located on chromosome 2q13, and encodes the B1-subunit of H^+-ATPase expressed apically on α-intercalated cells and also in the cochlea and endolymphatic sac [4,23].

In the human cochlea, the H^+-ATPase appears to be required to maintain normal endolymph pH [6] given that the very high potassium concentration (approximately 150 mmol/l) in this closed compartment is not normally accompanied by alkalinity of the endolymph [23]. *ATP6V1B1* expression has also been observed in the male genital tract (with acidification requirement for sperm maturation) [29].

Clinical findings, other than deafness, are identical to those present in patients with sporadic or autosomal recessive distal RTA and normal hearing. There is great variation in the presentation of deafness, from birth to late childhood, it is progressive and does not respond alkali therapy [15]. The defects in B1 cause irreversible hair cell damage in human cochlea because of ambient electrolyte and pH abnormalities [29].

Screening for mutations in this gene revealed fifteen different mutations in kindred. The majority of these mutations are likely to disrupt the structure, or abrogate the production, of the normal B1 subunit protein [29].

The Human Vacuolar H^+-ATPase

The vacuolar-type proton ATPase (V- or H+-ATPase) is a multisubunit pump that is essential for normal acidification of intracellular vesicular structures. In each individual cell, H^+-ATPases may function in a variety of distinct but essential cellular processes. However, the mechanisms by which cells regulate the intracellular trafficking, final destination and activity of these proton pumps are unclear [41].

The H^+-ATPases are composed of two structural domains (membrane-bound V_0 and cytoplasmic or peripheral V_1) each formed of multiple subunits (a–e and A–H, respectively), which are responsible for ATP hydrolysis and proton transport, respectively [6,23]. The mammalian H^+-ATPase is presumed to be similar to that of yeast (in which most of the structural studies have been performed) [23].

Autosomal Recessive Distal RTA with Preserved Hearing (Distal RTA Type 1c)

Individuals without hearing defects carry mutations at chromossome 7 q33-q34. The defective gene is *ATP6V0A4*, which encodes a kidney-specific a4 isoform subunit of H^+-ATPase. The involvement of the a4 subunit in distal RTA shows that it must be essential for proper proton pump function in the kidney [29], but its role is not totally clear.

Site-directed mutagenesis studies of the yeast 'a' subunit ortholog Vph1p (the 'a' subunit in proton pumps localized to the yeast vacuole) have yielded some potential functions [42]. Some mutations showed that this subunit is important for the assembly of the proton pump, whereas other mutations had greater effects on ATPase activity and proton transport. These studies suggest that the 'a' subunit is important for both assembly and function of the pump. [29,42].

INHERITED FORMS OF PROXIMAL RENAL TUBULAR ACIDOSIS

Proximal RTA is caused by a reduction in bicarbonate reabsorption at the proximal tubules, resulting in low renal bicarbonate threshold. The most common proximal RTA in children is secondary to Fanconi Syndrome [2,43]. Rarely, RTA might also be consequence of an inherited or sporadic primary renal disorder.

The acquired proximal RTA follows exposure to drugs or some toxins and the etiopathogenesis is still unknown [2]. Among drugs that cause Fanconi Syndrome are gentamicin, cisplatin, ifosfamide, and sodium valproate [6]. In addition, some hematologic and autoimmune conditions, such as myeloma and Sjogren syndrome respectively, might also course with proximal RTA.

The proximal RTA resulting from Fanconi Syndrome is frequently part of a systemic syndrome. Among systemic disorders that result in RTA, the inheritance pattern is usually autosomal recessive. Some of these disorders are cystinosis, tyrosinaemia, galactosaemia, Fanconi-Bickel syndrome and others (Table **2**) [44]. These syndromes are a heterogeneous group of disorders, which genes are mapped in many chromosome regions.

The RTA non-related to Fanconi Syndrome is a rare disorder and might be sporadic, autosomal dominant or autosomal recessive. The autosomal recessive disorder is associated with ocular abnormalities, frequently coursing with mental retardation. Other clinical features are short stature, dental enamel defects, pancreatitis, and basal ganglia calcification [45]. Loss-of-function mutations in the gene that codifies the NBC-1, the *SLC4A4* gene, were first identified in two Japanese subjects with proximal RTA associated with cataracts, glaucoma and band keratopathy [46]. NBC-1 is formed by 1,035 amino acids; it contains ten transmembrane domains and two cytoplasmic termini, and it is present in kidney, brain, eye, pancreas, heart, prostate, epididymis, stomach, and intestine. In the kidney, NBC-1 is expressed mainly at the basolateral membrane of the proximal tubule. At least two genes encode the NBC proteins. Mutations were identified in the human NBC-1 gene (*SLC4A4*) mapped at chromosome 4p21 [47,48].

Another interesting candidate gene for proximal RTA is the *TASK* gene. TASK2-potassium channel is a member of the tandem-pore domain potassium channel family and is located in pancreas, placenta, lung, small intestine, colon and kidney. TASK2 seems to be important to bicarbonate ab-

Table 2. Chromosome Mapping of the Inherited Fanconi Syndromes

Inherited Fanconi Syndromes	Gene	Mapping
Autosomal recessive	*SLC4A4*	Chromosome 4q21
Dent´s syndrome	*CLCN5*	Chromosome Xp11.22
Cystinosis	*SLC3A1* *SLC7A9*	Chromosome 2p21 Chromosome 19p13.1
Tyrosinaemia type 1	*FAH* gene	Chromosome 15q23-q25
Galactosemia	*GALT* gene	Chromosome 9p13
Wilson´s disease	*ATP7B* gene	Chromosome 13q14.3-q21.1

sorption in renal proximal tubules. Knockout mice for *TASK2* gene course with metabolic acidosis associated with low bicarbonate levels [49]. However, no mutation in these genes was yet identified in individuals with proximal RTA.

Other inherited form of proximal RTA is the one resulting from mutations in the gene *CA2* that encodes CAII. The carbonic anhydrases (CA) are member of a family of zinc metalloenzymes that catalyzes the hydration of CO_2. The human CA2 maps to the chromosome region 8q22. In the kidney, the majority of CA activity is attributable to CA II, which is localized in proximal tubular cells and in α-intercalated cells of the cortical and outer medullary collecting tubules [50]. Due to their localization, this RTA course with some proximal and distal components. In terms of clinical aspects, this RTA present osteopetrosis, cerebral calcification and different levels of mental retardation.

The autosomal dominant proximal RTA was originally described in a large Costa Rican family [51,52], consisting of nine individuals presenting growth retardation and osteomalacia. No gene was found to be associated with this clinical presentation. Recently, another family with isolated proximal RTA inherited as an autosomal dominant disease was described [53]. The father and all four children had RTA with blood bicarbonate levels of 11-14 mEq/L and urine pH of 5.3-5.4 and all presented high bicarbonate fractional excretion. In terms of clinical aspects, they course only with short stature without other organ dysfunction. This family was investigated at the following genes: *CA II, CA IV, CA XIV, NCB1, Na^+/H^+ exchanger (NHE-3), NHE-8*, the regulatory proteins of *NHE3, NHRF1 and NHRF2* and the *Cl-HCO^-_3 exchanger, SLC26A6.* However, no mutation was found in any of the candidate genes studied. The study of these families might clarify other mechanisms involved in renal bicarbonate balance and a genome wide investigation of a pool of these families might result in interesting findings.

INHERITED FORMS OF RENAL TUBULAR ACIDOSIS TYPE 3

Type 3 RTA is a mixed type that exhibits both impaired proximal HCO_3^- reabsorption and distal acidification. The condition is due to an inherited deficiency of CAII caused by a recessive mutation in the *CA2* gene on chromosome 8q22, which encodes this widely expressed enzyme [4,6]. The expression of CAII is affected in bone, kidney (in both proximal and distal nephron segments, explaining the mixed acidosis) and brain.

The mechanisms that underlie the clinical picture in type 3 RTA, apart from much slower conversion of carbonic acid to and from bicarbonate, apparently also involve direct interaction between CA II and the kidney NBC1 [27] or Cl^-/HCO_3^- exchanger, *SLC26A6* (a plasma membrane Cl^-/HCO_3^- exchanger with a suggested role in pancreatic HCO_3^- secretion) [4,28]. Mutation of the identified CAII binding site reduced *SLC26A6* activity, demonstrating the importance of this interaction. [28].

Patients with this deficiency exhibit osteopetrosis and cerebral calcification, as well as a mixed RTA with proximal and distal components [29]. This association of osteopetrosis and RTA is known as Guibaud-Vainsel syndrome or marble brain disease. Osteopetrosis is a condition of increased bone density, but also augmented bone fragility, leading to increased fracture risk, plus intracerebral calcification, intellectual impairment, growth failure, and facial dysmorphism. Excess bone growth leads to conductive deafness and can also cause blindness through compression of the optic nerve [6].

There is a considerable degree of heterogeneity, both in the predominance of proximal or distal acidosis and in the osteopetrotic phenotype [6]. In different kindred, mild or severe mental retardation has also been described.

Different mutations in *CA2* gene have been described; for example, the common 'Arabic' mutation, consisting of loss of the splice donor site at the 5' end of intron 2 [6,29].

INHERITED FORMS OF HYPERKALEMIC RENAL TUBULAR ACIDOSIS

Type 4 RTA is a heterogeneous group of disorders associated with hyperkalemia due to aldosterone deficiency or impairment in aldosterone molecular signaling.

Type 4 RTA might result from a PHA1. Some clinical aspects associated are hyponatremia, hyperkalemia, and elevated plasma aldosterone and plasma renin activity. The inheritance might be autosomal dominant or autosomal recessive [54]. The autosomal dominant is a frequent and mild kidney disorder without any other organ involvement [55].

This disorder seems to be associated to loss-of-function mutations in the mineralocorticoid receptor gene, the *MRL* gene. MRL-knockout mice develop symptoms of pseudohypoaldosteronism. In humans clinical presentation varies from non-symptomatic to important neonatal sodium loss. The recessive inheritance is associated to sodium transport defects in all aldosterone target tissues, not only kidney, but also colon, lungs, salivary and sweat glands. The recessive disorder is more severe and salt wasting is normally more pronounced. However, both types of inheritance might result in the same degree of natriuresis, hyperkalaemia and metabolic acidosis.

Other inherited cause of type 4 RTA includes hyperkalaemia associated with hypertension and low or normal levels of plasma aldosterone [57,58]. This syndrome is called pseudohypoaldosteronism type 2 (PHA2), or Gordon's syndrome, which results in a renal aldosterone resistance inherited as an autosomal dominant pattern [6]. Mutations in the gene of two isoforms of WNK serine-threonine kinases, *WNK4* and *WNK1* genes, were identified in patients with PHA2 [59]. WNKs are serine kinase proteins lacking a lysine residue at the active site, being the WNK type 1 a regulatory protein from *WNK 4*. *WNK4* is found in the distal nephron and controls the sodium and chloride reuptake and inhibits potassium efflux [6].

CONCLUDING REMARKS

Renal tubular acidosis (RTA) is characterized by metabolic acidosis due to renal impaired acid excretion. In this review, we summarized our current understanding of the hereditary diseases caused by mutations in genes encoding transporter or channel proteins operating along the renal tubule. Further molecular studies of inherited tubular transport disorders may shed more light on the molecular pathophysiology of these diseases and may significantly improve our understanding of the mechanisms underlying renal salt homeostasis, urinary mineral excretion, and blood pressure regulation in health and disease. The identification of the molecular defects in inherited tubulopathies may provide a basis for future design of targeted therapeutic interventions and, possibly, strategies for gene therapy of these complex disorders.

ABBREVIATIONS

RTA = Renal Tubulat Acidosis

NHE-3 = Na^+/H^+ exchanger

CAIV = Carbonic anhydrase type IV

CAII = Carbonic anhydrase type II

NBC-1 = Na^+-dependent bicarbonate cotransporter

AE1 = Basolateral chloride-bicarbonate exchanger

eAE1 = Red cell anion exchanger

kAE1 = Kidney anion exchanger

CA2 = Carbonic anhydrase gene

PHA1 = Pseudohypoaldosteronism type 1

PHA2 = Pseudohypoaldosteronism type 2

RBC = Red blood cells

HS = Hereditary spherocytosis

SAO = Southeast Asian ovalocytosis

GPA = Glycophorin A

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ANEXO D Artigo Original

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Clinical course of 33 children with distal renal tubular acidosis

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Original article

Clinical course of 33 children with distal renal tubular acidosis

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Abstract

Distal renal tubular acidosis (RTA) refers to a heterogeneous group of diseases that result from distal tubular dysfunction and can lead to growth retardation, nephrocalcinosis, bone disease and, rarely, chronic kidney disease. This study aimed to describe the clinical course of distal RTA series and to analyze somatic growth by identifying possibly predictive factors of growth improvement. Patients were followed-up from 1984 to 2008 according to our standard protocol. Paired t test was used for comparison between pre and post-treatment results. A logistic regression model was applied to identify variables that were independently associated with the gain of at least one standard deviation (SD) in Z-score for height and weight. A total of 33 distal RTA patients (15 males) were analyzed. Primary disease was the commonest form (60.6%). Based on weight/age and stature/age curves, 58.3% of the patients completely recovered growth after treatment. Bicarbonate levels at admission were an independent predictor of stature gain at last visit and the male sex negatively affected the final weight gain. Metabolic acidosis, electrolyte disturbances, hypercalciuria and nephrocalcinosis also improved during follow-up ($p<0.05$). Our data showed the great impact of treatment on metabolic control and further indicated predictive factors of growth catch-up.

Key words: renal acidification, growth failure, nephrocalcinosis, metabolic acidosis

Introduction

Renal tubular acidosis (RTA), the main cause of tubular dysfunction in pediatric practice, represents a clinical syndrome in which either an inherited (primary) or acquired (secondary) defect in tubular transport mechanisms can lead to failure to maintain the metabolic homeostasis [1-4]. RTA can be basically divided into four categories: distal (type 1), proximal (type 2) with or without Fanconi's syndrome, combined distal and proximal defects (type 3), and hyperkalemic (type 4).

In the past decade, remarkable progress has been made in our understanding of the molecular pathogenesis of hereditary tubulopathies and the physiology of renal tubular transport processes [4-8]. Distal RTA is the commonest type of primary RTA in childhood [1-3], which is characterized by impaired urinary acidification leading to hyperchloremic acidosis with inappropriately alkaline urine [1, 9]. Affected children might course with nephrocalcinosis, nephrolitiasis, failure to thrive, growth retardation, bone disease and, more rarely, chronic renal disease [9, 10]. If detected early in life, therapeutic correction of the acidosis by continuous alkali administration may induce growth catch-up, arrest of nephrocalcinosis and preservation of renal function [2].

There have been a number of studies of distal RTA [1-3, 6, 7, 9-14] but few of them evaluated the outcome of treatment [9-14]. In addition, as expected the majority of the series comprised a few number of pediatric patients [10-12]. Moreover, none of these studies have systematically investigated baseline features associated with growth parameters after treatment. In this regard, the aim of this retrospective cohort study was to describe the clinical course of 33 pediatric patients with distal RTA followed by 1984 to 2008 and to analyze the somatic growth of the patients in order to identify possibly predictive factors of growth improvement.

Patients and Methods

In this retrospective cohort study, data from 33 patients with distal RTA consecutively admitted to our Pediatric Nephrology Unit from 1984 to 2008 were analyzed. Data were compiled from November 1984 to September 2008.

Inclusion criteria

Patients with a confirmed diagnosis of distal RTA based on clinical and laboratorial findings were included in our analysis [1-3, 15-17]. These findings consisted at least of history of polyuria or polydipsia and/or failure to thrive accompanied by normal anion gap, hyperchloremic metabolic acidosis with abnormally high urine pH (>5.5 in the presence of blood pH<7.30) and normal or near normal glomerular filtration rate [1-3].

Exclusion criteria

Patients whose follow up were not enough to confirm the diagnosis of distal RTA and those whose medical records had insufficient data for analysis were automatically excluded from the study.

Study protocol

Medical records of 33 patients diagnosed with distal RTA were reviewed from a database of all patients with RTA admitted to our Unit during study period. All distal RTA patients followed a systematic protocol, including: blood gas analysis and concomitant spot urine pH to confirm the acidification defect, complete metabolic evaluation (serum and 24-hour urine electrolytes and nitrogen waste levels measurements), images (renal ultrasonography, X-rays) and specific exams to define secondary cases of distal RTA (screening inborn metabolic diseases, autoimmune, hematological and endocrine disorders). The visits were scheduled periodically at intervals of about two to six months, depending on the clinical and metabolic condition

of each patient. A complete examination was performed on each occasion including clinical and laboratory evaluation. The data analyzed were obtained at diagnosis and at the time of the last visit.

The following variables were studied: gender, age at diagnosis, clinical presentation (polyuria, polydipsia, failure to thrive, metabolic acidosis, nephrocalcinosis and signs rickets were evaluated as present or absent), etiology of distal RTA, follow-up duration, height, weight, laboratorial data (blood gas analysis and serum levels of creatinine, urea, uric acid and electrolytes, urinary pH and 24-hour urine samples for electrolytes and creatinine measurements). Anthropometric data were collected in all visits. Weight was measured to the nearest 100 g using a baby scale for infants and a beam scale for older children. Length was checked using a measuring board of standard design for children younger than two years and height was measured using a wall-mounted scale for those aged two years or older. Glomerular filtration rate was estimated by the Schwartz formula [16]. Bone X-ray and ultrasound scan evaluated the presence of rickets and nephrocalcinosis, respectively. Rickets was defined radiologically as widened and irregular epiphyseal-metaphyseal junctions or evidence of bone softening in the long bones and nephrocalcinosis as diffuse calcification of the renal pyramids [18]. Image studies were periodically repeated to pursue the evolution of these complications in all positive cases.

Treatment was based on standard guidelines, which generally included alkali replacement therapy, electrolyte supplementation and control of hypercalciuria. Patients were treated with sodium-potassium citrate or bicarbonate. Basically, the amount of base given daily per kg of body weight to young infants was 5 to 8 mmol/kg per day (bicarbonate or citrate), 3 to 4 mmol/kg per day and 1 to 2 mmol/kg per day, to children and adolescents, respectively. Potassium citrate alone could also be used in a mean

amount of 4 mmol/kg per day [2, 3, 14]. Supplements of potassium were given according to blood biochemistry findings. Patients with persistent hypercalciuria (>4 mg/kg/day) despite correction of acidosis were started on hydrochlorthiazide (1 to 2 mg/kg/day). Medication was chosen and doses was adjusted individually to achieve plasma bicarbonate in the range of 20-24 mEq/L and to successfully revert most of the urinary abnormalities, including hypercalciuria.

Statistical analysis

Data were analyzed with SPSS (release 15, SPSS Inc., Chicago, Illinois) and were presented as mean and standard deviation (SD) for continuous variables and as proportions for categorical variables. Growth parameters were expressed as Z-score. Weight-for-age (WAZ) and height-for-age (HAZ) Z-scores were used to assess weight and stature, respectively. These parameters were calculated with the public domain software EPI-INFO (version 6.0). The normality of the distribution was evaluated by the Kolmogornov–Smirnov test for each parameter. The delta HAZ or delta WAZ was calculated by the following equation: HAZ or WAZ at last visit – HAZ or WAZ at baseline. The Mann-Whitney or Kruskal-Wallis nonparametric test was used to compare medians of delta HAZ or delta WAZ. Paired t-test was used to analyze possible changes in normally distributed continuous variables from entry to follow-up.

To further analyze the association between baseline factors and growth improvement, the response variable was set as an increase of at least one SD in WAZ or HAZ at last visit. The following variables were assessed as independent predictors: gender; etiology (primary/secondary and transitory disease); nephrocalcinosis (present/absent); and bicarbonate levels (continuous variable). Age at diagnosis was assesses as a continuous variable and also as a categorical variable using two cut-off points (≤ 15 months and > 15 months, based on the median value; ≤ 42 months and > 42

months, based on the third quartile value). The analysis was conducted in two steps. In the first step, univariate analysis was performed by the chi-square test with Yates correction for comparison of proportions between growth parameters and clinical features and Mann Whitney U-test to compare growth parameters with continuous variables at admission. Then, a logistic regression model was applied to identify variables that were independently associated with the gain of at least one standard deviation (SD) in HAZ and WAZ. Only those variables that were found to present different proportions in univariate analysis ($p<0.25$) were included in the regression model. Next, using a backward elimination strategy, those variables that retained a significant independent association ($p<0.05$) were included in the final models.

Ethical aspects

The Ethics Committee of the Federal University of Minas Gerais approved the study. Informed consent was obtained from all included subjects and their parents.

Results

Clinical and laboratorial characteristics at baseline

Clinical characteristics - We analyzed data from 33 patients classified as distal RTA among 48 patients with RTA (68.8%). The clinical characteristics at baseline were displayed in Table 1. These 33 patients comprised 15 males and 18 females with mean age at diagnosis of 2.67±3.1 years (one month to 10 years). Primary disease was the commonest form of distal RTA (n=20, 60.6%) and in three patients it was associated to deaf. The transitory form of distal RTA was detected in six patients (18.2%) and distal RTA due to an underlying disease corresponded to seven patients (21.2%). The etiologies of secondary distal RTA were uropathies (n=3, 9.1%), primary hyperoxaluria type 2 (n=2, 6%) and autoimmune diseases (n=2, 6%). The main related symptoms

were growth retardation (63.4%), polyuria (24.2%) and polydipsia (24.2%). The physical examination revealed growth impairment in 24 patients (72.7%), since all of them presented weight under 3rd percentile and 79.2% also exhibited height under this score. Females presented lower baseline values of WAZ (p=0.025) and HAZ (p=0.016) than males. The image evaluation showed nephrocalcinosis in 13 patients (39.4%) and rickets in 11 (33.3%).

Table 1

Laboratorial characteristics - Main laboratorial findings at diagnosis included metabolic acidosis with low bicarbonate levels (14.8 ± 3.9 mmol/L) and high urinary pH (7.2 ± 0.83) in all patients, hypopotassemia (< 3.5 mmol/L) in eight (24.2%), hypophosphatemia (< 3 mg/dL) in seven (21.2%) and hypercalciuria (>4 mg/kg/day) in 17 (51.5%). Estimated glomerular filtration rate (102.3 ± 49.9) were within the normal limits in all patients. Females presented lower initial bicarbonate (p=0.001), base excess (p=0.005), serum calcium (p=0.016) and potassium levels (p=0.009) than males. No more differences were detected in the comparison between genders.

Clinical and laboratorial characteristics at last visit

Clinical findings - The mean duration of follow-up was 10.8 years (1.4 to 26 years). Twenty-eight patients (84.8%) were followed for more than five years. Only one patient abandoned the treatment after 14 years of follow-up. Some symptoms at presentation such as dehydration, persistent fever and recurrent infections were not reported at last visit. Polyuria and polydipsia improved in six patients but remained unchanged in two. Among 13 patients with nephrocalcinosis at baseline, eight of them clearly improved, in one the alteration was no more detected and it remained stable in four. None of them worsened the nephrocalcinosis. Among 11 patients with rickets at diagnosis, six had their X-rays completely normalized and, in five, the improvement

was evident. Only two patients developed chronic kidney disease due to the progression of their underlying disease (complex uropathies in both cases). These two cases did not exhibit hypercalciuria or nephrocalcinosis.

The treatment consisted of sodium bicarbonate alone in 18 children, potassium citrate and sodium bicarbonate in five patients, sodium-potassium citrate plus sodium bicarbonate in four and potassium citrate alone in six. Hidrochrolotiazide was associated in nine patients who had hypercalciuria and also nephrocalcinosis. The mean effective dose of sodium bicarbonate was 2.2 ± 1.2 mmol/kg per day. During follow-up, bicarbonate treatment was withdrawn only in the patients with transitory form of distal RTA; all others still required a mean dose of 0.8 ± 0.6 mmol/kg/day at last visit. Among the patients with hypercalciuria, only three remained with urinary calcium excretion at high levels (>4 mg/kg/day) and still continued on hidrochrolothiazide treatment.

Laboratorial findings - Metabolic acidosis and electrolyte disturbances also improved in all patients (Table 2). Serum bicarbonate changed from 14.8 ± 3.9 mmol/L to 24.3 ± 3.4 mmol/L after treatment ($p<0.01$, Figure 1) but there was no significant difference between sexes ($p=0.50$). Hypophosphatemia was initially observed in 21.2%, but completely normalized after replacement treatment in all patients. Hypercalciuria completely resolved in 14 among 17 cases (82.4%), clearly improved in one (5.9%) and remained unchanged in two (11.8%) patients. As shown in Figure 2, a significant reduction in 24-hour urinary calcium excretion was also observed (4.9 ± 2.9 mg/kg/day at admission vs. 2.7 ± 1.8 mg/kg/day at last visit, $p<0.01$).

Table 2

Figure 1

Figure 2

Growth parameters analysis

The difference between WAZ and HAZ at last visit and the same parameters at baseline revealed a significant improvement of somatic growth. The mean WAZ was -2.39 (range -5.14 to -0.13, SD 1.34) at baseline and reached -1.08 (range -3.67 to 1.58, SD 1.27) at the end of follow-up period ($p<0.01$, Figure 3A). The same occurred for stature whose mean HAZ was -2.47 (range -5.87 to 1.53, SD 1.75) at baseline and achieved -1.57 (range -6.32 to 1.04, SD 1.87) at last visit ($p<0.01$, Figure 3B). Based on weight/age and stature/age curves, 58.3% of distal RTA patients completely recovered growth and 37.5% clearly improved after treatment but still presented short stature.

Figure 3

In univariate analysis, only bicarbonate levels at admission were significantly associated to HAZ gain. According to our criteria, gender ($p=0.059$) and nephrocalcinosis ($p=0.197$) were also included in the regression model. After adjustment, only bicarbonate level at admission were an independent predictor of HAZ improvement (OR=1.3, CI 95% 1.1-1.6, $p<0.01$). The final model showed that each reduction of 1 mmol/L on bicarbonate levels at admission corresponds to a decrease of approximately 31.2% on the HAZ gain. For WAZ gain, the univariate analysis showed that gender ($p=0.001$) and bicarbonate levels ($p=0.043$) were significantly associated to WAZ gain. Among the other variables, etiology ($p=0.239$) and age 3 ($p=0.234$) were included in the regression model. After adjustment, only the male gender was negatively associated with WAZ gain in the final model (OR=13.7, CI95% 2.5-74.3, $p<0.002$).

Discussion

In this retrospective cohort study we report the clinical outcome of a group of pediatric patients with distal RTA with a prolonged median follow-up of about 10.8 years at a tertiary centre. The main finding regarding our cohort is that the clinical course of distal RTA was relatively benign and the majority of children presented clinical and growth improvement during follow-up.

In our series, primary distal RTA was the commonest form and the mean age at diagnosis was 2.67±3.1 years, which can be considered an early diagnosis as compared with a mean age at diagnosis of 6 years in the series reported by Bajpai et al. [9]. In distal RTA, the clinical features at presentation usually include growth impairment, polyuria, hypercalciuria, nephrocalcinosis, lithiasis and K^+ depletion [1-4, 9, 13]. Accordingly, the most frequent clinical finding in our series was growth impairment in 24 (72%) patients and hypercalciuria in 17 (51%) cases at presentation. Rickets was initially detected in 33.3% of our patients and nephrocalcinosis in 39.4%. In previous series [9, 14], nephrocalcinosis was more frequently observed (60-78.5%) than in our study. This is probably due to the fact that our patients had an earlier diagnosis. In addition, hypercalciuria was well controlled in our patients and none developed chronic renal failure due to the progression of calcium deposition on kidney parenchyma.

At diagnosis, physical examination revealed weight under 3rd percentile in 72.7% patients and 79.2% of them also exhibited height under this score. After treatment, completely growth catch up occurred in 58.3% of distal RTA patients and 37.5% partially recovered weight and height. Bajpai et al. [9] and Nash et al. [12] also found growth impairment at diagnosis and in the former with adequate response to treatment. The evolution of our patients supports the general idea that if distal RTA is detected early in life, adequate correction of acidosis by continuous alkali administration may

result in a normal growth, arrest or even prevention of nephrocalcinosis and preservation of renal function [1-4, 19].

More importantly, our study suggests that some aspects should be considered at the first assessment of distal RTA patients. Bicarbonate levels at admission seem to be an independent predictor of height improvement. The regression final model showed that each reduction of 1 mmol/L on bicarbonate levels at admission corresponds to a decrease of approximately 31.2% on height gain. It is known that the alkaline supplementation corrects as much as possible the biochemical abnormalities of RTA and also improves growth in children at all ages. Furthermore, the normalization of blood pH has a positive effect on bone linear growth by increasing calcium apposition rate and mineralized surface in distal RTA patients [20]. In our point of view, the children early detected with lower levels of bicarbonate at admission should be followed closely and carefully to minimize the effects of acidosis on the final height. In this way, bicarbonate levels at admission might be a marker of a worse prognosis in terms of height.

To our best knowledge, we did not find previous studies which had reported gender differences in clinical and laboratorial data of distal RTA patients. In our series, male sex has a negative effect on final WAZ gain (OR=13.7) despite the fact that, at baseline, females presented a worse WAZ average and, at last visit, no significant correlation of final weight with gender was observed (p=0.46). Indeed, the multivariate model showed that girls with distal RTA have greater chance to gain weight than boys. On the other hand, although HAZ gain was also greater in females, no statistical difference was found in the comparison between genders (p=0.089). These gender differences in growth catch up allow us to suppose that female sex is associated with a better prognosis for distal RTA patients. One could speculate that the variable sex was

confounded with other covariates. However, the multivariate model excluded this possibility. In addition, girls exhibited worse metabolic acidosis than boys at admission with lower levels of bicarbonate. A possible explanation for this intriguing observation could be related to the complexity of tubular disorders genetics [21-23].

We are aware of the limitations associated with the retrospective design and the limited number of patients of our study. Nevertheless, some aspects may increase the strength of our findings, such as the long-term follow-up, the utilization of strictly defined inclusion and exclusion criteria and a well-established protocol for the approach of our distal RTA patients.

In conclusion our series clearly showed that primary distal RTA is a chronic disease and therapy should be maintained throughout life. Nevertheless, prognosis is good if diagnosed early in life and appropriate amounts of alkali supplements should be continuously administered. Our series clearly showed adequate treatment restores growth and prevents the progression to nephrocalcinosis at all ages. However, if therapy is delayed to late childhood or adulthood progression to end-stage renal disease may not be avoided. Finally, in our study, we found bicarbonate levels at diagnosis as a good putative height prognosis marker and girls, even with lower bicarbonate levels, will have a better prognosis for distal RTA.

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Figure legends

Figure 1 - Comparison between bicarbonate levels at baseline and at last visit in distal RTA patients. * $p < 0.05$ (paired Student T test)

Figure 2 - Comparison between 24-hour urinary calcium excretion (mg/Kg/day) at baseline and at last visit in distal RTA patients. * $p < 0.05$ (paired Student T test)

Figure 3 – Growth parameters of distal RTA patients at baseline and at last visit. Panel A – Comparison between weight-for-age Z (WAZ) score at last visit and at baseline. Panel B - Comparison between height-for-age Z (HAZ) score at last visit and at baseline. *$p<0.05$ (paired Student T test)

Figures

Figure1

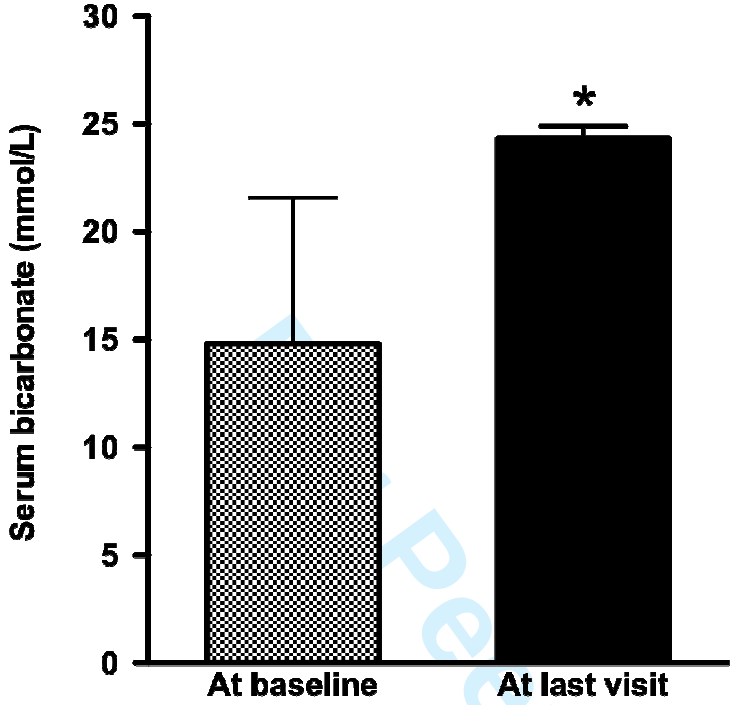


Figure 2

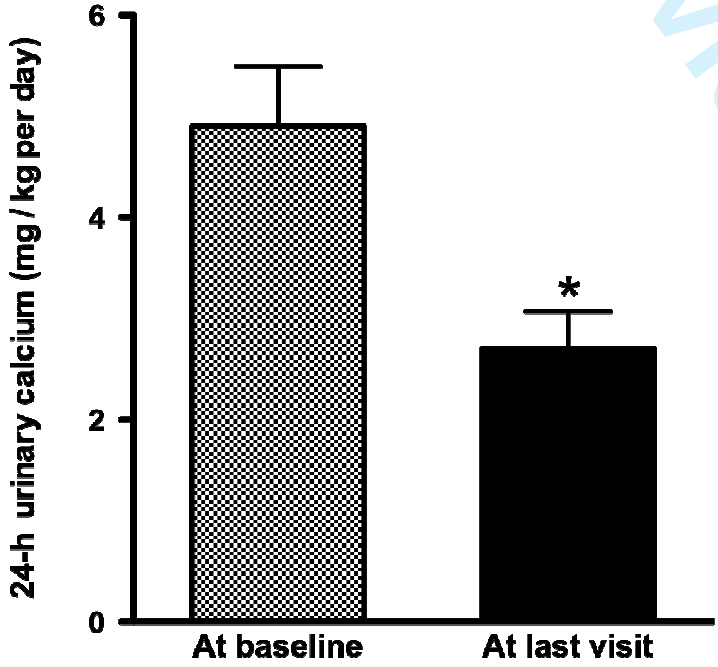
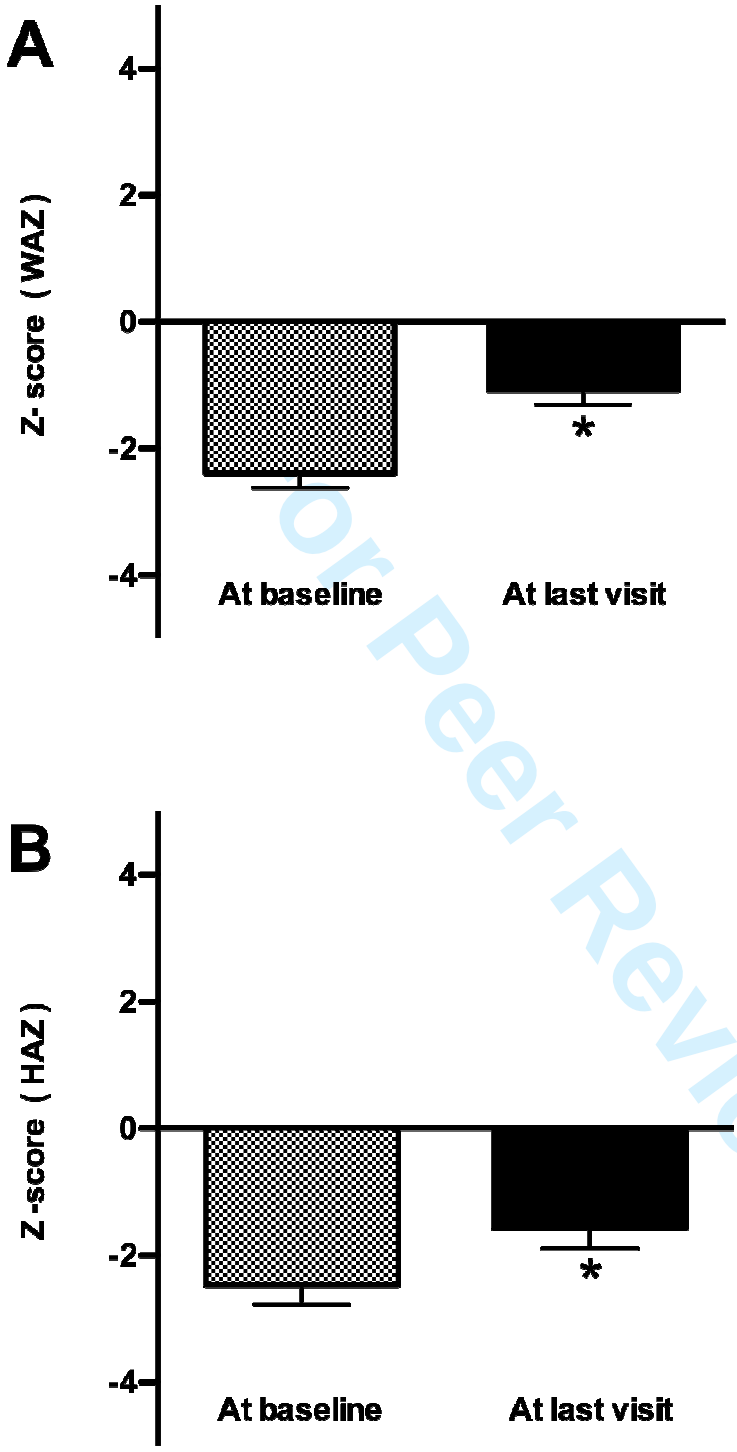


Figure 3



Tables

Table1. Clinical characteristics of distal RTA patients at baseline.

Features	Female	Male	Total
	18	15	33
Age (*years*)*	2.95 (3.3)	2.33 (2.8)	2.67 (3.1)
Etiology (*n*)			
Primary	13	7	20
Secondary	2	5	7
Transitory	3	3	6
Family history (*n*)			
Present	9	3	12
Absent	9	12	21
Presentation (*n*)			
Growth retardation	12	8	21
Polyuria / Polydipsy	5	3	8
Acidosis	3	2	5
Nephrolitiasis	2	0	2
Persisting fever	2	0	2
Hematuria	2	0	2
Recurrent infections	0	1	1
Dehydration	0	1	1
Image evaluation findings (*n*)			
Nephrocalcinosis	7	6	13
Rickets	9	2	11
Follow-up duration (*years*)*	12.4 (6.7)	8.9 (4.8)	10.8 (6.1)

(*) Data are given as mean, with the standard deviation given in parenthesis

Table 2 - Comparison between laboratorial findings at baseline and at last visit in distal RTA patients.

	At baseline			At last visit			
	Female	*Male*	*Total*	*Female*	*Male*	*Total*	*P value*
Bicarbonate (mmol/L)	12.8 (3.6)	17.0 (2.9)	14.7 (3.9)	23.9 (2.9)	24.7 (3.9)	24.3 (3.4)	*0.0001*
Serum pH	7.29 (0.07)	7.33 (0.04)	7.31 (0.06)	7.35 (0.04)	7.34 (0.05)	7.34 (0.04)	*0.047*
Base excess	-11.7 (4.8)	-6.7 (3.9)	-9.4 (5.1)	-0.9 (2.9)	-0.7 (3.8)	-0.8 (3.3)	*0.0001*
pCO_2	22.0 (11.2)	25.3 (11.2)	23.5 (11.2)	25.7 (24.2)	38.5 (17.2)	31.5 (22.0)	*0.072*
Sodium (mmol/L)	139.5 (4.1)	139.6 (4.3)	139.6 (4.1)	139.1 (2.9)	138.8 (2.7)	139.0 (4.1)	*0.191*
Potassium (mmol/L)	3.6 (1.0)	4.4 (0.5)	4.0 (0.9)	3.7 (0.5)	4.1 (0.5)	4.0 (0.9)	*0.614*
Chloride (mmol/L)	109.7 (7.5)	105.3 (5.3)	107.7 (6.9)	102.7 (4.9)	103.9 (4.6)	103.2 (4.7)	*0.002*
Calcium (mg/dL)	8.2 (3.0)	9.2 (2.6)	8.6 (2.8)	9.1 (2.3)	9.5 (0.5)	9.3 (1.7)	*0.120*
Phosphate (mg/dL)	3.9 (2.1)	4.7 (1.2)	4.2 (1.8)	3.8 (1.6)	4.1 (1.4)	3.9 (1.5)	*0.272*
Magnesium (mg/dL)	0.9 (1.1)	1.6 (0.8)	1.2 (1.0)	1.5 (0.8)	1.7 (0.5)	1.6 (0.7)	*0.053*
Creatinine (mg/dL)	0.5 (0.3)	0.6 (0.4)	0.5 (0.3)	0.9 (1.1)	0.8 (0.5)	0.8 (0.9)	*0.047*
Urea (mg/dL)	23.7 (16.7)	29.6 (16.6)	26.4 (16.8)	25.9 (22.3)	30.5 (23.1)	27.9 (22.4)	*0.610*
Urinary pH	7.3 (1.0)	7.0 (1.0)	7.2 (0.8)	7.3 (0.9)	7.0 (0.8)	7.2 (0.8)	*0.874*
Glomerular Filtration rate * (mL/min)	103.2 (52.7)	101.2 (48.1)	102.3 (49.9)	114.9 (33.6)	117.4 (46.1)	116.0 (39.1)	*0.206*
24-hour urinary calcium (mg/kg/day)	5.6 (3.2)	4.0 (2.1)	4.9 (2.9)	2.5 (1.7)	3.0 (2.0)	2.7 (1.8)	*0.0001*

Data are given as mean, with the standard deviation given in parenthesis. (*) Glomerular filtration rate was estimated by Schwartz formula.