Universidade Federal de Minas Gerais
Programa de Pós-Graduação em Ciências da Saúde

Avaliação dos níveis urinários de citocinas pró-inflamatórias e do fator de crescimento e transformação do tipo beta em pacientes com hidronefrose diagnosticada intraútero

MARIANA AFFONSO VASCONCELOS

BELO HORIZONTE
2010

MARIANA AFFONSO VASCONCELOS

Avaliação dos níveis urinários de citocinas pró-inflamatórias e do fator de crescimento e transformação do tipo beta em pacientes com hidronefrose diagnosticada intraútero

Dissertação apresentada ao Programa de Pós-Graduação da Faculdade de Medicina da Universidade Federal de Minas Gerais, área de concentração em saúde da Criança e do Adolescente

Orientador: Eduardo Araújo de Oliveira

Co-Orientadora: Ana Cristina Simões e Silva

Faculdade de Medicina
2010

UNIVERSIDADE FEDERAL DE MINAS GERAIS
FACULDADE DE MEDICINA
PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE
ÁREA DE CONCENTRAÇÃO: SAÚDE DA CRIANÇA E DO ADOLESCENTE

Dedico esta dissertação aos meus exemplos de vida, Dra. Maria Aparecida Affonso Vasconcelos e Antonio Eustaquio Vasconcelos que sempre me estimularam a dar este grande passo, por renunciarem de sonhos para investirem na minha formação e por estarem sempre ao meu lado. Muito obrigada!

AGRADECIMENTOS

Ao professor Eduardo Araújo Oliveira, pelos incentivos, desde a graduação, a seguir a carreira acadêmica, pelos ensinamentos, paciência e confiança.

À professora Ana Cristina Simões e Silva por estar sempre presente, pelo apoio, compreensão e pelo detalhismo na revisão da dissertação.

Ao professor José Maria Penido Silva, meu mestre, meu muito obrigada por acreditar no meu potencial e por ter me apoiado em momentos difíceis da carreira.

À professora Maria Candida Ferrarez Bouzada Viana, pela dedicado cuidado clínico dos pacientes e pela coordenação do projeto junto às fontes financiadoras.

À equipe de nefrologia pediátrica, em especial, à professora Eleonora Moreira Lima pelos ensinamentos.

À doutora Roberta Maia de Castro Romanelli pela amizade e por sempre me estimular a seguir meus ideais.

Às acadêmicas Flávia, Juliana e Letícia, pelo bom desempenho nas tarefas atribuídas.

À pesquisadora Kátia Daniela Silveira, sempre disposta, pela a ajuda incondicional nas dosagens.

Aos funcionários do laboratório central que cederam espaço e auxiliaram nas coletas.

À minha irmã Marisa por me despertar o interesse pela pesquisa.

Ao meu irmão Daniel pelo carinho.

À minha avó Hercília, uma fortaleza, exemplo de pessoa.

Aos meus amigos, seria injusto citar apenas alguns nomes, por estarem sempre por perto me incentivando.

Aos pacientes que participaram do estudo, sem eles essa dissertação não seria possível.

A todas as pessoas que, direta ou indiretamente, contribuíram para a execução dessa dissertação de mestrado.

APOIO:

Este projeto foi aprovado pelo edital Universal 2007 da FAPEMIG, sob a coordenação da Professora Maria Candida Ferrarez Bouzada Viana

Este projeto contou com o apoio do CNPq, nas modalidades de bolsa de iniciação científica e bolsas de produtividade em pesquisa.

LISTA DE ABREVIATURAS E SIGLAS

- **AUC:** area under the curve.
- **CCL2/MCP-1:** Monocyte chemotactic protein-1
- **CCL3/MIP-1α:** Macrophage inflammatory protein 1 alfa
- **CCL5/RANTES**: regulated on activation, normal T Expressed and Secreted.
- **CEMEFE:** Centro de Medicina Fetal.
- **COEP:** Comitê de Ética em Pesquisa.
- **DAP:** diâmetro anteroposterior.
- **DMSA:** dimercaptosuccicinic acid/ cintilografia estática.
- **DTPA:** dietilenoaminopentacetic acid/ cintilografia dinâmica.
- **ELISA:** enzyme-linked immunoassay/ ensaio imunoenzimático.
- **IGF-1:** insulin-growth factor-1
- **IL-1:** interleucina-1/ interleukin-1.
- **IL-6:** interleucina-6/ interleukin-6.
- **IL-8/CXCL-8:** interleucina-8/ interleukin-8.
- **IP-10:** ɣ-interferon-inducible protein-10.
- **JUP:** junção ureteropélvica.
- **MIP-2:** macrophage inflammatory protein-2.
- **mRNA:** RNA mensageiro
- **NGF:** nerve growth factor
- **PNH:** prenatal hydronephrosis.
- **RAS:** sistema renina-angiotensina/ renin-angiotensin system.

- **ROC:** Receiver-operating curve.
- **RPD:** renal pelvic dilatation.
- **RVU:** refluxo vesicoureteral.
- **SFU:** Society of Fetal Urology.
- **TGF-β1:** fator de crescimento e transformação-β1/ transforming and growth factor-β1
- **UFMG:** Universidade Federal de Minas Gerais
- **UCM:** uretrocistografia miccional.
- **UE:** urografia excretora
- **UPJO:** ureteropelvic junction obstruction.
- **US:** ultra-sonografia/ ultrasound
- **UTI:** urinary tract infection.
- **VCUG:** voiding cystourethrogram.
- **VEGF:** vascular endothelial growth factor.
- **VUP:** válvula de uretra posterior.
- **VUR:** vesicoureteral reflux.

SUMÁRIO

1. INTRODUÇÃO

O diagnóstico intrauterino é definido, segundo a OMS, como "todas aquelas ações no pré-natal que tenham como objetivo o diagnóstico de um defeito congênito, entendido como toda anomalia do desenvolvimento morfológico, estrutural, funcional ou molecular presentes ao nascimento (ainda que possa manifestar tardiamente), externa ou interna, familiar ou esporádica, hereditária ou não, única ou múltipla" [1]. As anormalidades envolvendo o trato geniturinário podem ser suspeitadas em 1 a cada 100 gestações, dependendo do critério adotado [2-3, 5]. A dilatação da pelve renal é a uropatia mais comum detectada intraútero [4].

As uropatias são uma das causas mais frequentes de doença renal crônica na infância e adolescência, junto com as glomerulonefrites crônicas, em todos os continentes [9]. São responsáveis por 20 a 35% das causas que levam à doença renal crônica em crianças, como pode ser observado na série de casos ilustrada na figura 1. No Brasil, dois estudos mostraram que as uropatias são responsáveis por um percentual aproximado de 30% de doença renal crônica na infância e adolescência [10, 11].

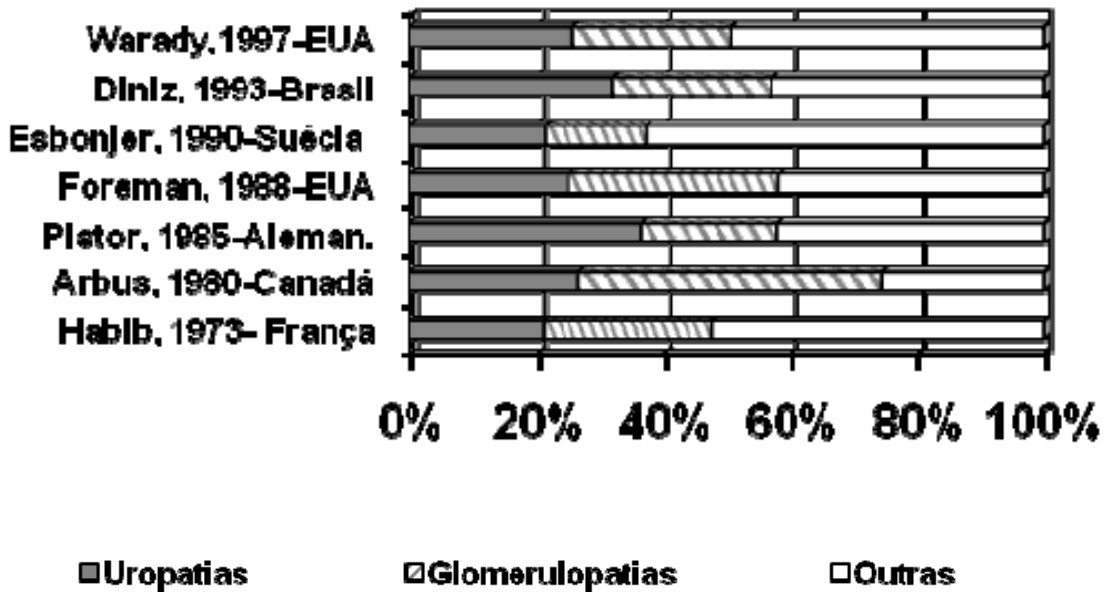


Figura 1- Causas de doença renal crônica nas crianças e adolescentes em vários países.

Ressalte-se que dentre as etiologias da insuficiência renal crônica, são as uropatias, possivelmente, as únicas em que o tratamento precoce, evitando uma sobrecarga de pressão e episódios infecciosos sobre o parênquima renal, pode prevenir ou adiar uma perda da função renal [7]. Modelos experimentais foram utilizados para demonstrar essa possível prevenção do dano renal com o tratamento precoce das uropatias obstrutivas. Em uma série de estudos, em fetos de carneiro, foi demonstrada que a obstrução no primeiro trimestre de gestação resulta em marcante fibrose intersticial e uma desorganização do parênquima características dos quadros de displasia. A recuperação da função renal foi diretamente proporcional ao tempo de alívio da obstrução e inversamente proporcional ao tempo que o rim permaneceu obstruído [14-17].

Poucos estudos abordam os fatores associados à evolução da função renal que, em última análise, serão os determinantes de uma boa qualidade de vida para essas crianças. Neste estudo, vários fatores relacionados ao ultrassom pré-natal, às condições maternas e do recém-nascido foram sistematicamente obtidos durante o seguimento desses pacientes. Esses fatores também têm sido relacionados a eventos adversos, tais como insuficiência renal e óbito [12, 13].

Nos últimos anos, temos estudado o curso clínico, a história natural e os fatores preditivos de mau prognóstico em relação à função renal. Temos contribuído na avaliação prospectiva, em longo prazo, das mais freqüentes uropatias diagnosticadas intraútero: obstrução de junção pieloureteral [38, 39], refluxo vesicoureteral [26, 33], rim multicístico [30, 32, 35, 36, 37], válvula de uretra posterior [28, 31] e megaureter primário [24, 25]. Em uma série de estudos, demonstramos o impacto do diagnóstico fetal das anomalias do trato urinário na abordagem, tratamento e prognóstico dessas crianças [6, 7, 18-40]. Assim, uma série de fatores clínicos, laboratoriais, e ultrassonográficos têm sido estabelecidas como parâmetros de grande utilidade na abordagem desses lactentes. No entanto, deve ser reconhecido que faltam marcadores de maior acurácia, menos invasivos e de melhor custo/benefício na abordagem desses pacientes. Recentemente, Galanakis et al [42] demonstraram que os níveis urinários de Interleucina 8 (IL-8/CXCL-8), produzida pelas células epiteliais do trato urinário em resposta a um estímulo inflamatório, permaneciam elevados em lactentes com refluxo vesicoureteral mesmo na ausência de infecção do trato urinário. Esses autores mostraram que o ponto de corte de 5 pg de IL-8(CXCL-8)/mmol de creatinina na urina foi de alta sensibilidade e adequada especificidade no diagnóstico do refluxo vesicoureteral. A elevação dos níveis urinários das citocinas, incluindo interleucina-1 (IL-1),

interleucina-6 (IL-6) e IL-8/CXCL-8 tem sido observada em pacientes com infecção urinaria [41-44]. Haraoka et al [45] demonstraram que os níveis urinários de IL-8/CXCL8 eram maiores em crianças com refluxo vesicoureteral e dano renal. Outros marcadores também têm sido utilizados nas anomalias do trato urinário. O fator de transformação e crescimento do tipo beta (TGF-β1) 1 é um mediador de fibrose renal em uropatia obstrutiva. O TGF-β1 é uma citocina que estimula a síntese de matriz extracelular e inibe sua degradação [48]. A fibrose intersticial desenvolve como resultado de uma ausência de balanço entre a síntese de matriz extracelular, seu depósito e degradação [48]. O sistema renina-angiotensina (RAS) encontra-se ativado após o início de obstrução ureteral, e a angiotensina II pode contribuir para a perda precoce do rim obstruído [47]. Angiotensina II induz diretamente e indiretamente a produção de TGF-β1 [47]. A expressão do RNA mensageiro (mRNA) para TGF-beta1 aumenta em rins obstruídos [48]. Alguns autores têm demonstrado que há um consistente aumento de TGF-β1 em crianças com uropatia obstrutiva [49-51]. No entanto, não há estudos de lactentes portadores de uropatia identificada por meio da detecção de hidronefrose fetal.

A proposta deste estudo transversal, desenvolvido pela Unidade de Nefrologia Pediátrica do Departamento de Pediatria da UFMG foi de avaliar marcadores capazes de auxiliar no diagnóstico e na definição do prognóstico de pacientes com hidronefrose diagnosticada intraútero e assim facilitar o seu manejo clínico e cirúrgico.

Finalmente, é importante explicar que essa dissertação de Mestrado foi elaborada conforme o modelo aprovado pelo Programa de Pós-Graduação em Ciências da Saúde (Faculdade de Medicina, UFMG), que permite sua confecção em formato de artigos

científicos a serem submetidos a revistas médicas. Sendo assim, a apresentação do trabalho seguiu a seguinte estrutura:

1. Seção de Introdução

2. Seção de Revisão da Literatura, apresentada sob a forma do artigo de revisão: *Cytokines in congenital anomalies of kidney and urinary tract.*

3. Seção de Objetivos

4. Seção de Metodologia

5. Seção de Resultados e Discussão, apresentada sob a forma do artigo original: *Urinary levels of transforming growth factor beta-1 and interleukins in patients with prenatally detected uropathies.*

Observações:

As tabelas e figuras estão dispostas no final de cada artigo.

As referências bibliográficas estão dispostas ao final de cada artigo ou seção de acordo com as normas do periódico para o qual o artigo será submetido. As referências listadas no final de cada seção estão dispostas em ordem de citação e seguem as normas de Vancouver.

REFERÊNCIAS BIBLIOGRÁFICAS

1. Carrera JM, Alegre M, Torrents M. Anomalías nefrourológicas. In: Carrera JM, ed. Diagnóstico prenatal. Barcelona: Salvat Editores, 1987:465-483.
2. Carr MC. Prenatal management of urogenital disorders. Urol Clin North Am. 2004 Aug;31:389-97, vii.
3. Morin L, Cendron M, Crombleholme TM, Garmel SH, Klauber GT, D'Alton ME. Minimal hydronephrosis in the fetus: clinical significance and implications for management. J Urol. 1996 Jun;155:2047-9.
4. Chudleigh T. Mild pyelectasis. Prenat Diagn. 2001 Nov;21:936-41.5.
5. Goncalves LF, Jeanty P, Piper JM. The accuracy of prenatal ultrasonography in detecting congenital anomalies. Am J Obstet Gynecol 1994; 171:1606-12.
6. Colodny AH. Antenatal diagnosis and management of urinary abnormalities. Pediatr Clin North Am 1987; 34:1365-81.
7. Oliveira EA. Estudo prospectivo das anomalias do trato urinário diagnosticadas no feto pelo ultra-som. Depto. de Pediatria. Belo Horizonte: UFMG, 1992:260.
8. Oliveira EA. Fatores prognósticos do óbito, da insuficiência renal e da presença de obstrução uretral: uma análise multivariada. Depto. de Pediatria. Belo Horizonte: UFMG, 1998:263.
9. Gusmano R, Perfumo F. Worldwide demographic aspects of chronic renal failure in children. Kidney Int Suppl 1993; 41:S31-5.
10. Diniz JSS. Insuficiência renal crônica na criança: Relato de uma experiência no HC/UFMG. Rev Med Minas Gerais 1993; 3:127-133.

11. Garcia C, Goldani J, Garcia V. Paediatric dialysis and renal transplantation in the state of Rio Grande do Sul, Brazil. Pediatr Nephrol 1992; 6:74-7.

12. Simoes e Silva AC, Silva JM, Diniz JS, Pinheiro SV, Lima EM, Vasconcelos MA, Pimenta MR, Oliveira EA. Risk of hypertension in primary vesicoureteral reflux. Pediatr Nephrol. 2007 Mar; 22:459-62.

13. Penido Silva JM, Oliveira EA, Diniz JS, Bouzada MC, Vergara RM, Souza BC. Clinical course of prenatally detected primary vesicoureteral reflux.Pediatr Nephrol. 2006 Jan;21:86-91

14. Harrison MR, Golbus MS, Filly RA, et al. Fetal surgery for congenital hydronephrosis. N Engl J Med 1982; 306:591-3.

15. Harrison MR, Golbus MS, Filly RA, et al. Fetal hydronephrosis: selection and surgical repair. J Pediatr Surg 1987; 22:556-8.

16. Harrison MR, Nakayama DK, Noall R, de Lorimier AA. Correction of congenital hydronephrosis in utero II. Decompression reverses the effects of obstruction on the fetal lung and urinary tract. J Pediatr Surg 1982; 17:965-74.

17. Harrison MR, Ross N, Noall R, de Lorimier AA. Correction of congenital hydronephrosis in utero. I. The model: fetal urethral obstruction produces hydronephrosis and pulmonary hypoplasia in fetal lambs. J Pediatr Surg 1983; 18:247-56.

18. Oliveira EA, Cabral AC, Pereira AK, et al. Outcome of fetal urinary tract anomalies associated with multiple malformations and chromosomal abnormalities. Prenat Diagn 2001; 21:129-34.

19. Oliveira EA, Cabral ACV, Leite HV, et al. Estudo prospectivo da hidronefrose fetal diagnosticada por ultra-som: uma contribuição na prevenção ao dano renal na infância. Radiol Bras 1998; 31:75-81.

20. Oliveira EA, Cabral ACV, Leite HV, et al. Hidronefrose fetal: abordagem pós-natal e seguimento. Jornal de Pediatria 1997; 73:252-8.

21. Oliveira EA, Diniz JS, Cabral AC, et al. Prognostic factors in fetal hydronephrosis: a multivariate analysis. Pediatr Nephrol 1999; 13:859-64.

22. Oliveira EA, Diniz JS, Cabral AC, et al. Prognostic factors in fetal hydronephrosis: a multivariate analysi. Pediatr Nephrol 1999; 13:859-64.

23. Oliveira EA, Diniz JS, Cabral AC, et al. Predictive factors of fetal urethral obstruction: a multivariate analysis. Fetal Diagn Ther 2000; 15:180-6.

24. Oliveira EA, Diniz JS, Rabelo EA, et al. Primary megaureter detected by prenatal ultrasonography: conservative management and prolonged follow-up. Int Urol Nephrol 2000; 32:13-8.

25. Oliveira EA, Diniz JS, Rabelo EA, et al. Primary megaureter detected by prenatal ultrasonography: conservative management and prolonged follow-up [In Process Citation]. Int Urol Nephrol 2000; 32:13-8.

26. Oliveira EA, Diniz JS, Silva JM, Rabelo EA, Pontes AK, Souza MF. Features of primary vesicoureteric reflux detected by investigation of fetal hydronephrosis. Int Urol Nephrol 1998; 30:535-41.

27. Oliveira EA, Diniz JS, Vilasboas AS, Rabelo EA, Silva JM, Filgueiras MT. Multicystic dysplastic kidney detected by fetal sonography: conservative management and follow-up. Pediatr Surg Int 2001; 17:54-7.

28. Oliveira EA, Diniz JSS, Rabelo EAS, et al. Seguimento a longo prazo da válvula de uretra posterior detectada intra-útero. J Bras Nefrol 2000.

29. Oliveira EA, Diniz JSS, Rabelo EAS, et al. Primary megaureter detected by prenatal ultrasonography: conservative management and prolonged follow-up. Int Urol Nephrol 2000.

30. Oliveira EA, Diniz JSS, Vilasboas AS, Rabelo EAS, Silva JMP, Filgueiras MTF. Multicystic dysplastic kidney detected by fetal sonography: conservative management and follow-up. Pediatr Surg Int 2001; 17:54-7.

31. Oliveira EA, Rabelo EA, Pereira AK, et al. Prognostic factors in prenatally-detected posterior urethral valves: a multivariate analysis. Pediatr Surg Int 2002; 18:662-7.

32. Oliveira EA, Silva AS, Rabelo EA, Filgueiras MT, Pereira AK, Mesquita FM. Spontaneous improvement of hypertension in multicystic dysplastic kidney: a case report. Pediatr Nephrol 2002; 17:954-958.

33. Penido Silva JM, Oliveira EA, Diniz JS, Bouzada MC, Vergara RM, Souza BC. Clinical course of prenatally detected primary vesicoureteral reflux. Pediatr Nephrol 2006; 21:86-91.

34. Pereira AK. Uropatias fetais: avaliação dos critérios de diagnóstico morfológico e funcional. Tese de Doutorado. Depto. de Ginecologia e Obstetrícia. Belo Horizonte: UFMG, 1999:160.

35. Rabelo EA, Oliveira EA, Diniz JS, et al. Natural history of multicystic kidney conservatively managed: a prospective study. Pediatr Nephrol 2004; 19:1102-7.

36. Rabelo EA, Oliveira EA, Silva GS, Pezzuti IL, Tatsuo ES. Predictive factors of ultrasonographic involution of prenatally detected multicystic dysplastic kidney. BJU Int 2005; 95:868-71.

37. Rabelo EA, Oliveira EA, Silva JM, et al. [Conservative management of multicystic dysplastic kidney: clinical course and ultrasound outcome]. J Pediatr (Rio J) 2005; 81:400-4.

38. Apocalypse GT, Oliveira EA, Rabelo EA, et al. Outcome of apparent ureteropelvic junction obstruction identified by investigation of fetal hydronephrosis. Int Urol Nephrol 2003; 35:441-8.

39. Apocalypse GT. Obstrução de junção ureteropélvica identificada na investigação de hidronefrose fetal: um estudo retrospectivo. Faculdade de Medicina. Belo Horizonte: UFMG, 2002:122.

40. Bouzada MC, Oliveira EA, Pereira AK, et al. Diagnostic accuracy of postnatal renal pelvic diameter as a predictor of uropathy: a prospective study. Pediatr Radiol 2004; 34:798-804.

41. Bouzada MC, Oliveira EA, Pereira AK, et al. Diagnostic accuracy of fetal renal pelvis anteroposterior diameter as a predictor of uropathy: a prospective study. Ultrasound Obstet Gynecol 2004; 24:745-9.

42. Galanakis E, Bitsori M, Dimitriou H, Giannakopoulou C, Karkavitsas NS, Kalmanti M. Urine Interleukin-8 as a Marker of Vesicoureteral Reflux in Infants. Pediatrics 2006.

43. Sedor JR, Nakazato Y, Konieczkowski M. Interleukin-1 and the mesangial cell. Kidney Int 1992; 41:595-9.

44. Hedges S, Stenqvist K, Lidin-Janson G, Martinell J, Sandberg T, Svanborg C. Comparison of urine and serum concentrations of interleukin-6 in women with acute pyelonephritis or asymptomatic bacteriuria. J Infect Dis 1992; 166:653-6.

45. Martins SM, Darlin DJ, Lad PM, Zimmern PE. Interleukin-1B: a clinically relevant urinary marker. J Urol 1994; 151:1198-201.

46. Haraoka M, Senoh K, Ogata N, Furukawa M, Matsumoto T, Kumazawa J. Elevated interleukin-8 levels in the urine of children with renal scarring and/or vesicoureteral reflux. J Urol 1996; 155:678-80.

47. Klahr S. Urinary tract obstruction. Semin Nephrol 2001; 21:133-45.

48. Vuruskan H, Caliskan Z, Kordan Y, Ozakin C, Yavascaoglu I, Oktay B. Elevated plasma concentrations of transforming growth factor-beta 1 in patients with unilateral ureteral obstruction. Urol Res 2005; 33:465-9.

49. Roberts AB, McCune BK, Sporn MB. TGF-beta: regulation of extracellular matrix. Kidney Int 1992; 41:557-9.

50. El-Sherbiny MT, Mousa OM, Shokeir AA, Ghoneim MA. Role of urinary transforming growth factor-beta1 concentration in the diagnosis of upper urinary tract obstruction in children. J Urol 2002; 168:1798-800.

51. Monga M, Gabal-Shehab LL, Stein P. Urinary transforming growth factor-beta1 levels correlate with bladder outlet obstruction. Int J Urol 2001; 8:487-9.

52. Furness PD, 3rd, Maizels M, Han SW, Cohn RA, Cheng EY. Elevated bladder urine concentration of transforming growth factor-beta1 correlates with upper urinary tract obstruction in children. J Urol 1999; 162:1033-6.

2. ARTIGO DE REVISÃO

Review article:

Cytokines in congenital anomalies of kidney and urinary tract

Mariana A. Vasconcelos, MD, Ana C. Simões e Silva, MD, PhD, Eduardo A. Oliveira, MD, PhD

Pediatric Nephrourology Unit, Department of Pediatrics, Federal University of Minas Gerais (UFMG), Belo Horizonte, MG, Brazil

Corresponding author:

Eduardo A. Oliveira.

Rua Engenheiro Amaro Lanari 389 / 501

Belo Horizonte - Minas Gerais

Postal Code: 30.310.580

E-mail: eduolive@medicina.ufmg.br

Abstract

Fetal hydronephrosis is the most common anomaly detected on antenatal ultrasound, affecting 1–5% of pregnancies. Postnatal investigation has the major aim in detecting infants with severe urinary tract obstruction and clinically significant urinary tract anomalies among the heterogeneous universe of patients. Imaging techniques clearly contributes to this purpose. However, sometimes, these exams are invasive, very expensive and not sufficient to precisely define the best approach as well as the prognosis. Recently, biomarkers have become a focus of clinical research as potentially useful diagnostic tools in pediatric urological diseases. In this regard, recent studies suggest a role for cytokines and chemokines in the pathophysiology of fetal hydronephrosis. Some authors proposed that the evaluation of these inflammatory mediators might help the management of postnatal uropathies. Therefore, the aim of this article is to revise general aspects of cytokines and their relationship with fetal hydronephrosis by including experimental and clinical evidence.

Introduction

Fetal hydronephrosis is the most common anomaly detected on antenatal ultrasound, affecting 1–5% of pregnancies[1-2]. Despite their high frequency of occurrence, there is little consensus on the management of infants with prenatal hydronephrosis (PNH). In particular, the management of those with isolated antenatal hydronephrosis is still a source of controversy[3].

There has been a number of studies discussing the significance of fetal renal pelvic dilatation (RPD) as an indicator of urinary tract anomalies[4-7]. The degree of PNH varies from mild to severe, and possibly, the degree of PNH should correlate with the severity of the underlying etiology[1-2]. Recently, Lee et al.[2] demonstrated in a meta-analysis that the risk of any postnatal pathology per degree of PNH was 11.9% for mild, 45.1% for moderate, and 88.3% for severe. More specifically, the risk of ureteropelvic junction obstruction (UPJO) increased significantly with greater degrees of PNH, but the risk of vesicoureteral reflux (VUR) was not significantly different among all severity groups. Most studies also have shown that a single postnatal US is unable to predict the presence or severity of VUR [6, 8-10]. Consequently, postnatal management is heterogeneous, with some centers advocating detailed investigations including voiding cystourethrography (VCUG) in all cases and others indicating a less intensive approach[11-16]. Therefore, in spite of advances, the issue of postnatal diagnostic management of antenatal hydronephrosis remains a challenging problem[17-18].

Renal pelvic dilatation can be an early sonographic sign of urinary tract obstruction or a marker of other abnormalities such as renal duplication or VUR which, are not be easily detected by US during pregnancy. Therefore, the pregnant is now presented to the

urologist or pediatric nephrologist before the baby is even born, with a presumptive diagnosis rather than a symptom[19]. It is the reason why infants diagnosed with congenital hydronephrosis must routinely undergo postnatal imaging evaluation. Classically, the prenatal diagnosis of hydronephrosis leads to postnatal investigations, including sonography, VCUG and isotopic renography[17, 20]. Ultrasonography has typically assumed a more important role in the surveillance of children with hydronephrosis but this exam is not used alone to establish the presence of obstruction. Presently diuretic renography is the most widely used investigation to evaluate renal function and urine flow. However, the test is invasive and the use of ionizing radiation remains a concern, especially in infants and young children. Moreover, repeat tests are often required, especially when the excretion curve is equivocal [21]. Postnatal investigation has the major aim in detecting infants with severe urinary tract obstruction and clinically significant urinary tract anomalies among the heterogeneous universe of patients. Imaging techniques clearly contributes to this purpose. However, some of these exams are invasive and very expensive. Furthermore, sometimes imaging techniques are not sufficient to precisely define the indication of surgical approach as well as to determine the prognosis [21].

Recently, biomarkers have recently become a focus of clinical research as potentially useful diagnostic tools in pediatric urological diseases [22]. Biomarkers are any tests that help to distinguish between two or more biological states and guide further clinical decision making [23]. For instance, preliminary investigations looking at urinary concentrations of transform growth factor-beta 1 (TGF-β1) have suggested that this biomarker might be useful in detecting urinary tract obstruction and clinically relevant urinary tract anomalies among the heterogeneous universe of patients [24].

The obstructive nephropathy is not a simple result of mechanical impairment to urine flow but a complex syndrome that results in alterations of both glomerular hemodynamics and tubular function usually caused by the interaction of a variety of vasoactive factors and cytokines that are activated in response to obstruction. The cytokines play a role in the development and progression of fibrotic and sclerotic changes in the obstructed kidney [25]. A large numbers of events can initiate apoptosis, several of which may be related to obstructive nephropathy, such as hypoxia, ischemia, cytokines, growth factors, angiotensin II and mechanical stretch [26]. However, it should be pointed out that the biochemical, cellular and molecular mechanisms of the obstructive uropathies are still largely unknown [26, 27]. The comprehension of this process will certainly help in the management of fetal hydronephrosis. In this regard, recent studies suggest a role for cytokines and chemokines in the pathophysiology of fetal hydronephrosis [26, 27, 28]. Indeed, the evaluation of these inflammatory mediators might help the management of postnatal uropathies. The aim of this article is to revise general aspects of cytokines and their relationship with fetal hydronephrosis by including experimental and clinical studies.

Cytokines: general concepts and characteristics

Cytokines are redundant secreted proteins with growth, differentiation, and activation functions that regulate and determine the nature of immune responses including the control the immune cell trafficking and the cellular arrangement of immune organs. These mediators are involved in virtually every facet of immunity and inflammation, including innate immunity, antigen presentation, bone marrow differentiation, cellular recruitment and activation, and adhesion molecule expression. A cascade of responses is triggered in response to cytokines, and several cytokines acting together are required to

express their optimal function. Numerous cytokines have both inflammatory and anti-inflammatory properties [29].

Chemokines constitute a large family of low molecular-weight cytokines whose main action is the recruitment and activation of leukocyte subsets in various models of inflammation—the word "chemokine" is a contraction of the terms "chemoattractant" and "cytokine" [30].

Cytokines in renal diseases

A number of studies have shown the relation between renal diseases and cytokines production [26, 30, 33, 34, 35, 36]. Indeed, the measurement of urinary, plasma and renal tissue levels of cytokines has been used to monitor and diagnosis various urological and nephrological diseases [30, 36, 37].

Tubular epithelial cells can be a rich source of inflammatory chemokines including CCL5/RANTES (Regulated on activation, normal T Expressed and Secreted), CCL2/MCP-1 (Monocyte chemotactic protein-1), CCL3/MIP-1α (Macrophage inflammatory protein 1 alfa), CX3CL1/fractalkine and CKCL8/IL8 (Interleukin 8) [31]. Tubular epithelial cells are also targets for chemokines, since these cells respond to CCL2/MCP1 stimulation by releasing interleukin-6 (IL-6) and intracellular adhesion molecule-1 [32]. Messenger RNA of chemokines receptors can also be detected in other regions like podocytes and glomeruli [30]

Interleukin-1 β (IL-1β) is primarily synthesized by cells of the mononuclear phagocyte lineage, but this cytokine is also produced by endothelial cells and neutrophils. The most important biological activity is its ability to active T lymphocytes and to augment B-cell proliferation thus increasing immunoglobulin synthesis [29]. Sheu et al [34] suggested that IL-1β could be used for early detection of acute pyelonephritis in febrile children and as an indicator of risk for subsequent development of renal scarring in these patients. The

levels of IL1-β were significantly reduced in children with renal scarring, probably indicating a protective function [34].

Interleukin-8 (IL-8) is a chemokine responsible for neutrophil infiltration into the urinary tract with an important role in acute inflammation [35]. Gene polymorphisms of IL-8 seem to increase the susceptibility for acute pyelonephritis. For instance, the presence of the IL-8-251A allele in the genotype of children with urinary tract infection without vesicoureteral reflux has increased the risk of pyelonephritis [38].

Interleukin-6 (IL-6) mediates T-cell activation, growth and differentiation. This pro-inflammatory cytokine is responsible for the induction of pyrexia and production of acute phase proteins [29]. Sheu et al. [35] found that there is a significant elevation of serum and urinary levels of IL-6 and IL-8 in children with acute pyelonephritis when compared to children with lower urinary tract infection. This finding is consistent with the hypothesis that the release of IL-6 from the urinary tract leads to systemic host responses [35].

Transforming growth factor-β (TGF-β) is a fibrogenic cytokine that stimulates extracellular matrix proteins deposition and renal scarring formation. On the other hand, concerning immune system regulation, TGF-β exerts anti-inflammatory effects by inhibiting the proliferation of many different cell types [29]. Monga et al. [39] have studied 17 men with bladder outlet obstruction and 6 non-obstructed subjects and showed that, in the obstructed ones, the urinary levels of TGF- β were significantly higher than in non-obstructed.

Cytokines in hydronephrosis – Experimental studies

Animal models have been frequently used to understand histopathological changes, mechanisms and therapeutic approaches of obstructive nephropathies [37, 40, 41, 42]. The

majority of the reported animal models utilized rats and mice, but rabbits, pigs and sheep were also used [27].

Models of experimental postnatal unilateral ureteral obstruction have been developed in newborn rat pups that continue to exhibit active nephrogenesis in the postnatal period [27]. A partial unilateral ureteral obstruction was surgically created by entrapping the ureter in the animal psoas muscle, whereas the complete obstruction was produced by surgically campling and occluding the ureter [27]. In rats, the major part of nephrogenesis occurs within 7 to 10 days after birth [43, 44]. Some models have used animals with congenital uropathies, while others have evaluated animals submitted to surgery after birth [43].

The induction of ureteral obstruction in newborn rats clearly interferes with ongoing nephrogenesis and this procedure usually leads to substantial renal damage [43]. This kind of experimental model mimics human ureteral obstruction at the second and third trimester of pregnancy; however, significant renal damage is less common in infants [45].

The main features found in obstructive models are tubular cell apoptosis, mesenchymal myocyte transformation and decreased glomerular endowment and glomerular injury [26, 44, 46]. The understanding of the pathophysiolocal mechanisms and the molecular events is important to define the moment of intervention. [27]. Figure 1 shows the main mechanisms involved in obstructive models.

INSERT FIGURE 1

Obstructed kidneys exhibited an elevation in Angiotensin II activity, which, in turn, decreases renal blood, causes ischemia and kidney growth arrest. Although, renal blood flow usually normalizes 6 weeks after the relief of temporary obstruction, renal growth remains altered, suggesting that other factors are responsible for growth impairment [43] such

as the reduction in cell proliferation, the increase in cell apoptosis and the progression of interstitial fibrosis [44].

Chevalier et al [44] have studied neonatal rats submitted to unilateral ureteral obstruction or sham operation at one day of age, with relief five days later. In additional groups of neonatal rats, the operation was at 14 days, with relief at 19 days [44]. Three months following relief of unilateral ureteral obstruction during days 14 to 19, renal growth was decreased by 50%, compared to a 30% reduction following relief of unilateral ureteral obstruction during days 1 to 5. The number of glomeruli was reduced by approximately 50% regardless of the timing of obstruction, but glomerular size was reduced only in rats with unilateral ureteral obstruction from days 14 to 19 [44]. This study shows that, in the period immediately following nephrogenesis, the kidney is particularly susceptible to long-term injury from temporary unilateral obstruction. This suggests that a delay in relief of significant ureteral obstruction should be avoided if diagnosed in the perinatal or neonatal period [44]. The same group has also evaluated neonatal rats that underwent unilateral ureteral obstruction at one day of age whose obstruction was released at days 1, 2, 3 or 5 following the operation [47]. The growth of the obstructed kidney decreased linearly according to the duration of ureteral obstruction, while the contralateral kidney developed compensatory hypertrophy [47]. Indeed, contralateral renal hypertrophy should be considered as an important sign of advanced obstructive uropathy [48]. In summary, these animal models reveal that renal growth and function are impaired in proportion to the severity and duration of obstruction.

The microscopic alterations of obstructed kidneys are, initially, increased of tubular diameter secondary to tubular dilation and cell proliferation. Next begins the apoptosis of tubular cell followed by the apoptosis of interstitial compartment [49]. There is a gradual, but

continuous, apoptosis and proliferation of fibroblasts and inflammatory cells [49, 50]. Tubular cell apoptosis contributes to renal growth impairment [49], whereas proliferation of interstitial fibroblasts with myofibroblast transformation leads to excess deposition of the extracellular matrix and renal fibrosis [46]. Phenotypic transition of resident renal tubular cells, endothelial cells, and pericytes has also been implicated in this process.

A variety of intrarenal factors lead to progressive interstitial fibrosis, including growth factors and cytokines, such as Angiotensin II, MCP-1, TGF- β and adhesion molecules, which are produced by the hydronephrotic kidney [26]. Altered renal expression of growth factors and cytokines modulate cell death by apoptosis or phenotypic transition of glomerular, tubular, and vascular cells. Mediators of cellular injury include hypoxia, ischemia, and reactive oxygen species, while fibroblasts undergo myofibroblast transformation with increased deposition of extracellular matrix. On the other hand, a number of endogenous antifibrotic counter-regulatory molecules have been identified, opening the possibility of enhancing the kidney's own defenses against progressive fibrosis [26, 51].

Cytokines as TGF-β and TNF-α and chemokines like MCP-1, RANTES, macrophage inflammatory protein-2 (MIP-2) and ɣ-interferon-inducible protein (IP-10) have been evaluated in experimental hydronephrosis [25, 26, 27, 28].

TGF-β is highly involved in tubulointerstitial fibrosis. This cytokine increases matrix synthesis, collagen deposition and tubular aptotosis, upregulates the integrin-matrix adhesion, and inhibits matrix degradation [28, 41, 52]. Resident renal tubular cells and interstitial cells may be responsible for TGF-β production; however, interstitial fibroblast cells seem to be the major source of TGF-β during the process of interstitial fibrosis [54]. In this regard, Mizuno et al [53] found that the increased expression of TGF-β was correlated to

fibrotic changes of interstitial regions in kidneys of mice subjected to unilateral ureteral obstruction. Accordingly, Seseke et al. [46] also detected the association between interstitial fibrosis and increased renal expression of TGF-β mRNA in an inbred strain of rats with congenital hydronefrosis. In addition, Zhou et al. [48] reported a marked elevation of renal TGF-β level in parallel to fibrotic changes of congenital and surgical ureteral obstruction in rats. Indeed, TGF-β expression increased significantly after completing nephrogenesis [43].

The role of TGF-β in obstructive nephropathies was also evidenced in other animal species. Seremites and Maizels [52] have studied rabbit pups submitted to left partial ureteral constriction and human specimens of renal pelvis and ureter derived from cases of isolated renal obstruction managed by pyeloplasty and nepherectomy or of isolated vesicoureteral reflux managed by ureteral reimplantation. These authors have detected significantly higher expression of TGF-β mRNA in obstructed pelvis than in non-obstructed ones. This elevation in TGF-β mRNA expression was correlated to muscle hypertrophy and increased collagen deposition, both representing the process of renal pelvis remodeling in response to obstruction. The lower level of TGF-β mRNA expression may be a sign of less remodeling due to a steady state of obstruction. The expression of TGF-β mRNA emerges as a good predictor of early obstruction [52].

The molecular pathways for TGF-β receptor-mediated effects were also evaluated in experimental hydronephrosis [27]. In this context, Smad 3 is a protein responsible for signaling downstream of the TGF-β receptors [56.] Sato et al. [55] have studied mice with genetic deletion of Smad3 and the wild type controls. The right proximal ureter was exposed and double ligated at 6-8 weeks of age. In the absence of Smad3, the formation of fibroblasts was blocked, clearly indicating a connection between fibrosis and TGF-β in obstructive uropathies [55].

TNF-α may play a role in initiating tubulointerstitial injury in obstructed kidney [26.] TNF-α stimulates the production of chemotactic factors by resident cells and upregulates MCP-1 in human mesangial cells [26]. The increase of TNF-α at early stages of obstruction stimulates the production of chemoattractants for monocytes, which in turn contributes to leukocyte infiltration in obstructed kidneys [26]. Misseri et al. [57] have studied the expression of TNF-α mRNA in rats submitted to progressive degrees of left ureteral obstruction. Renal cortical TNF-α mRNA expression and protein production reached a peak at 3 days of ureteral obstruction. The TNF-α production, localized primarily to renal cortical cells, was not associated with significant inflammatory cell infiltrate [57]. Indeed, TNF-α might participate in initiating tubulointerstitial injury in the obstructed kidney by upregulating chemoattractants for monocytes and by producing leukocytes infiltration [28].

MCP-1 is an inflammatory chemokine that attracts and activates monocytes, T-cells and natural killer cells [29, 30]. Stephan et al [45] produced partial or complete ureteral obstruction in 28-day-old Wistar rats. These authors found that MCP-1 mRNA expression was moderately increased in partial ureteral obstruction, whereas kidneys without significant damage did not show any up-regulation. The study qualifies MCP-1 mRNA expression as a prognostic marker of partial ureteral obstruction [45].

In relation to chemokines, Vielhauer et al [58] found an increased expression of the CC chemokines, MCP-1/CCL2 and RANTES/CCL5, at sites of progressive tubulointerstitial damage in murine obstructive nephropathy model. It was also observed an interstitial infiltration of macrophages and T lymphocytes, which differentially expressed the CCR2 receptors. These data suggest that CCR2- and CCR5-positive monocytes and CCR5-positive lymphocytes are attracted by locally released MCP-1 and RANTES, resulting in chronic interstitial inflammation [58]. Crisman et al [59] detected the

expression of MCP-1, RANTES and IP-10 at 1 day of unilateral ureteral obstruction in mice. At 7 days, RANTES became the most abundant chemokine in the obstructed kidney and the cortical tubular cells significantly contributed to this elevation [59].

In an experimental model of congenital hydronephrosis, the alteration was observed in 75% of transgenic animals with overexpression of IL-9 and was dependent on the presence of IL-4 and IL-13 [60].

The study of cytokines in hydronephrosis might provide new insights for the treatment or novel ways to blunt renal damage in obstructive nephropathy. For instance, animals with right ureter obstruction treated with spironolactona exhibited less fibrosis than control group [42]. Since Angiotensin II contributes at least in part to the increased expression of TNF-α mRNA in obstructed kidney [26], the use of Angiotensin converting enzyme inhibitors emerges as an effective way in preventing renal fibrosis [40]. Another rational approach to blunt renal fibrosis is to block growth factors effects. In this regard, Isaka et al [54] showed that interstitial fibrosis could be blocked by TGF-β1 antisense oligodexoxynuceloutides. Additionally, the modulation of nitric oxide, epidermal growth factor (EGF) and hepatocyte growth factor seems to be a good strategy to treat obstructive nephropathy in the future [51, 53, 61].

Cytokines in hydronephrosis - Clinical studies

It should be pointed out that few data about the role of cytokines in hydronephrosis were provided by clinical studies and the majority of them evaluated ureteropelvic junction obstruction (UPJO) and vesicoureteral reflux (VUR).

Ureteropelvic junction obstruction

UPJO is the most common cause of severe hydronephrosis in children [62]. UPJO is unilateral in 90% of cases and may result from intrinsic narrowing at the junction between

ureter and renal pelvis or extrinsic compression by an accessory lower pole artery of the kidney [20]. The degrees of hydronephrosis vary among patients with UPJO. The histological changes may vary from the absence of abnormalities to renal dysplasia with glomerulosclerosis and extensive interstitial fibrosis and tubular atrophy [63]. The UPJO area is consistently inflamed and has varying degrees of fibrosis and muscular hypertrophy [63].

Postnatal differentiation between obstructive and non-obstructutive hydronephrosis is quite difficult. Several studies have been made in patients with UPJO in order to find out noninvasive biomarkers to allow the diagnosis and treatment of these patients. In this regard, cytokines and growth factors have been studied in UPJO [37]. The most relevant results were obtained with MCP-1, EGF and TGF-β.

Healthy children, who underwent nepherectomy because of tumor or trauma, presented high expression of EGF mRNA in renal tissue, whereas MCP-1 mRNA was normally undetectable. On the other hand, in UPJO patients, MCP-1 gene expression was strikingly increased at the tubulointerstitial level, while the EGF gene expression was markedly reduced. The interstitial mononuclear cell infiltrate in UPJO patients was strictly correlated with the degree of tubulointerstitial damage [64, 65]. Accordingly, the urinary concentrations of EGF were reduced in UPJO patients, whereas the MCP-1 levels were increased [64]. After surgical correction, there was a significant reduction in urinary levels of MCP-1 accompanied by a marked increase in EGF concentration. These two cytokines could be useful for the follow-up of obstructed patients [64].

Palmer et al. [66] have studied patients who undergoing pyeloplasty (UPJO patients), ureteral reimplantation (VUR patients) or circumcision/orchiopexy and measured urinary levels of TGF-β1 collected in bladder and pelvis. TGF-β1 concentrations were detected in all groups without significant differences in bladder samples. In contrast, the level of this

cytokine was significantly elevated in the renal pelvis of children with UPJO when compared to the level obtained in the bladder of control group, of VUR group and of UPJO patients [66]. More recently, Furness et al. [67] have measured urinary levels of TGF-β1 collected in the bladder and renal pelvis of patients with UPJO. Urinary levels of TGF-β1 in children with UPJO were 4-fold higher than in healthy controls and samples obtained in renal pelvis had a 2-fold increase in cytokine concentrations when compared to bladder samples. In addition, if a cutoff point of 61pg/ mg .creatinine was considered, a 92% of sensitivity was obtained for the urinary measurement of TGF-β1 in bladder [67]. The main concern of this study was the lack of correlation to patients with dilated non-obstructed uropathy conservatively managed.

El-Sherbiny et al. [68] have compared urinary TGF-β1 levels between obstructed and non-obstructed patients with grade 3 hydronephrosis. In obstructed patients, urinary concentrations of TGF-β1 measured in renal pelvis were 4-fold higher than the measurements in the bladder, which were, in turn, 3-fold higher than in healthy controls samples. There was also a trend in decreasing bladder TGF-β1 levels 3 months after surgical correction of obstruction. Furthermore, the measurement of urinary levels of TGF-β1 had 80% of sensibility and 82% of specificity for the recognition of obstruction [68]. At the same hospital in Egypt, Taha et al [69] have evaluated 35 children with UPJO submitted to pyeloplasty who had grade 3 or higher hydronephosis. These authors have found significantly elevated levels of TGF-β1 in UPJO group compared to healthy controls. The presence of high baseline urinary levels of TGF-β1 in younger children significantly increased the diagnostic accurancy of this measurement. In addition, there was a decrease of TGF-β-1 concentration 1 month after of pyeloplasty that reached statistical significance

1 year after surgery [69]. The difference in the results obtained in both Egyptian studies might be due to time-point of the measurements: 3 versus 12 months after pyeloplasty.

Older children normally have lower urinary levels of TGF-β1 in the bladder probably due to the reduction or the steady-state production of this cytokine in long-term obstruction [67, 68, 69]. In Canada, Almodhen et al [24] have evaluated the role of TGF-β in the diagnosis and longitudinal follow-up of a homogeneous group of newborns with prenatal unilateral hydronephrosis. These authors showed that in the conservatively-managed group the decrease in hydronephrosis grade through time was associated with a similar decrease in urinary concentrations of TGF-β1 [24]. This result indicates the utility of urinary measurement of TGF-β1 for monitoring patients with congenital hydronephrosis. In the surgical-treated group, urinary concentrations of TGF-β1 significantly decreased after pyeloplasty during a mean follow-up of 7 months. At a cutoff point of 17 pg/ mmol of creatinine, the measurement of urinary TGF-β1 in the first 3 months of life had 82% of sensibility and 86% of specificity in predicting surgery [24].

Table 1 resumes the principal studies about UPJO and cytokines.

Table 1

Vesicoureteral reflux

VUR is a congenital anomaly that increases the risk of repeated pyelonephritis and, consequently, can result in renal scarring, renin-mediated hypertension, and, in some cases, renal insufficiency [70, 71]. VUR is a heterogeneous condition that can be primary or associated with multicystic kidney, hypodysplasic kidneys, renal agenesia and renal or ureteral ectopia.

Kidneys with reflux nephropathy have disjointed glomeruli from proximal tubules, interstitial infiltration with chronic inflammatory cells and periglomerular fibrosis. Dysplasic feature is one of the characteristics of congenital reflux nephropathy. The main findings are areas of mesenchymal tissue containing primitive tubules [73].

There were found associations between gene polymorphisms of TNF-α and of TGF-β and VUR [74, 75, 76, 77]. Some of these polymorphisms were also associated to reflux nephropathy and progressive renal damage [76, 77]. These associations could help in understanding the mechanisms of reflux nephropathy and could allow the detection of patients at risk of severe renal damage.

TNF-α and TGF-β are abundant in the smooth muscle cell of the ureter of VUR patients [78]. On the other hand, patients without VUR have higher expression of growth promoting factors like insulin-growth factor-1 (IGF-1), nerve growth factor (NGF) and vascular endothelial growth factor (VEGF) than those with VUR [78]. In this regard, Chertin et al [73] have showed that the reduced production of EGF associated with high expression of MCP-1 might cause an over production of proinflammatory and profibrotic cytokines that trigger apoptosis, ultimately leading to tubular atrophy and renal dysfunction in reflux nephropathy [73].

The inflammatory process in VUR is ongoing despite the occurrence or not of urinary tract infection (UTI). The elevated urinary level of interleukin-8 (IL-8) in children with reflux and without UTI might contribute to reflux nephropathy [79, 80]. Haraoka et al [80] have found a significant difference between urinary levels of IL-8 in children with and without renal scarring and in patients with and without VUR. This finding suggests that urinary IL-8 measuments could be useful to detect VUR patients with more pronounced renal damage and who need strict follow-up [80]. Galanakis et al [79] proposed the use of IL-8 as a biomarker

for the diagnostic of VUR. A cutt-off concentration of 5 pg of IL-8/μmol of creatinine has a sensitivity of 88% and a specificity of 69% [79].

The IL-6 may also be involved in the pathogenesis of reflux nephropathy. IL-6 induces B and T cells activation and differentiation during inflammation [29]. Ninan et al [81] have detected a significant elevation of urinary IL-6 levels in patients with VUR. In addition, Wang et al [82] have found that urinary IL-6 was significantly higher in children with severe bilateral renal scarring than in those with mild scarring and normal controls. Gokce et al [83] have related high urinary levels of IL-6 with the presence of VUR and increased IL-8 concentrations with renal scarring. Concerning serum measurements of cytokines, Jutley et al [84] have detected significant elevation of IL-6 and TNF-α in patients with reflux nephropathy when compared to those without reflux nephropathy or to healthy controls.

Since the main histological alteration in reflux nephropathy is renal fibrosis, Sabasiñska et al [85] have measured urinary levels of TGF-β1 in patients with VUR. These authors have found that urinary concentrations of TGF-β1 were increased in high-grade reflux and in bilateral cases [85].

Table 2 shows studies about VUR and cytokines.

Table 2

Concluding Remarks

Congenital obstructive nephropathy accounts for a great fraction of chronic kidney disease in children. Genetic and nongenetic factors responsible for the lesions are largely unidentified, and attention has been focused on minimizing obstructive renal injury and optimizing long-term outcomes. The renal response to urinary tract obstruction is complex and involves a wide array of interacting molecules. Elucidation of these interactions will

lead to the identification of biomarkers that will allow a more precise prediction to the response to surgical intervention and, hopefully, to novel therapies to prevent renal deterioration.

Acknowledgements. This study was partially supported by CNPq (Brazilian National Research Council) and FAPEMIG.

Conflicts of interest: none

REFERENCES

1. Estrada CR, Jr. Prenatal hydronephrosis: early evaluation. Curr Opin Urol 2008;18:401-3.

2. Lee RS, Cendron M, Kinnamon DD, Nguyen HT. Antenatal hydronephrosis as a predictor of postnatal outcome: a meta-analysis. Pediatrics 2006;118:586-93.

3. Mallik M, Watson AR. Antenatally detected urinary tract abnormalities: more detection but less action. Pediatr Nephrol 2008;23:897-904.

4. Cohen-Overbeek TE, Wijngaard-Boom P, Ursem NT, Hop WC, Wladimiroff JW, Wolffenbuttel KP. Mild renal pyelectasis in the second trimester: determination of cut-off levels for postnatal referral. Ultrasound Obstet Gynecol 2005;25:378-83.

5. Bouzada MC, Oliveira EA, Pereira AK, et al. Diagnostic accuracy of fetal renal pelvis anteroposterior diameter as a predictor of uropathy: a prospective study. Ultrasound Obstet Gynecol 2004;24:745-9.

6. Coplen DE, Austin PF, Yan Y, Blanco VM, Dicke JM. The Magnitude of Fetal Renal Pelvic Dilatation can Identify Obstructive Postnatal Hydronephrosis, and Direct Postnatal Evaluation and Management. J Urol 2006;176:724-7.

7. Gramellini D, Fieni S, Caforio E, et al. Diagnostic accuracy of fetal renal pelvis anteroposterior diameter as a predictor of significant postnatal nephrouropathy: second versus third trimester of pregnancy. Am J Obstet Gynecol 2006;194:167-73.

8. Anderson NG, Abbott GD, Mogridge N, Allan RB, Maling TM, Wells JE. Vesicoureteric reflux in the newborn: relationship to fetal renal pelvic diameter. Pediatr Nephrol 1997;11:610-6.

9. Dias CS, Bouzada MC, Pereira AK, et al. Predictive factors for vesicoureteral reflux and prenatally diagnosed renal pelvic dilatation. J Urol 2009;182:2440-5.

10. Phan V, Traubici J, Hershenfield B, Stephens D, Rosenblum ND, Geary DF. Vesicoureteral reflux in infants with isolated antenatal hydronephrosis. Pediatr Nephrol 2003;18:1224-8.

11. Coelho GM, Bouzada MC, Pereira AK, et al. Outcome of isolated antenatal hydronephrosis: a prospective cohort study. Pediatr Nephrol 2007;22:1727-34.

12. Coelho GM, Bouzada MC, Lemos GS, Pereira AK, Lima BP, Oliveira EA. Risk factors for urinary tract infection in children with prenatal renal pelvic dilatation. J Urol 2008;179:284-9.

13. Farhat W, McLorie G, Geary D, et al. The natural history of neonatal vesicoureteral reflux associated with antenatal hydronephrosis. J Urol 2000;164:1057-60.

14. Ismaili K, Hall M, Donner C, Thomas D, Vermeylen D, Avni FE. Results of systematic screening for minor degrees of fetal renal pelvis dilatation in an unselected population. Am J Obstet Gynecol 2003;188:242-6.

15. Moorthy I, Joshi N, Cook JV, Warren M. Antenatal hydronephrosis: negative predictive value of normal postnatal ultrasound--a 5-year study. Clin Radiol 2003;58:964-70.

16. Tibballs JM, De Bruyn R. Primary vesicoureteric reflux--how useful is postnatal ultrasound? Arch Dis Child 1996;75:444-7.

17. Toiviainen-Salo S, Garel L, Grignon A, et al. Fetal hydronephrosis: is there hope for consensus? Pediatr Radiol 2004;34:519-29.

18. Leung VY, Chu WC, Metreweli C. Hydronephrosis index: a better physiological reference in antenatal ultrasound for assessment of fetal hydronephrosis. J Pediatr 2009;154:116-20.

19. Ismaili K, Hall M, Piepsz A, Alexander M, Schulman C, Avni FE. Insights into the pathogenesis and natural history of fetuses with renal pelvis dilatation. Eur Urol 2005;48:207-14.

20. Hubert KC, Palmer JS. Current diagnosis and management of fetal genitourinary abnormalities. Urol Clin North Am 2007;34:89-101.

21. Piepsz A. Antenatally detected hydronephrosis. Semin Nucl Med 2007;37:249-60.

22. Lee RS. Biomarkers for pediatric urological disease. Curr Opin Urol 2009;19:397-401.

23. LaBaer J. So, you want to look for biomarkers (introduction to the special biomarkers issue). J Proteome Res 2005;4:1053-9.

24. Almodhen F, Loutochin O, Capolicchio JP, Jednak R, El-Sherbiny M. The role of bladder urine transforming growth factor-beta1 concentrations in diagnosis and management of unilateral prenatal hydronephrosis. J Urol 2009;182:292-8; discussion 8.

25. Wen JG, Frøkiær J, Jørgensen TM, Djurhuus JC. Obstructive nephropathy: an update of the experimental research. Urol Res. 1999; 27:29-39.

26. Klahr S, Morrisey. Obstructive nephropathy and renal fibrosis. Am J Renal Physiol. 2002; 283: F861-F875.

27. Matsell DG, Tarantal AF. Experimental models of fetal obstructive nephropathy. Pediatric Nephrology. 2002; 17: 470-476.

28. Klahr S. Obstructive nephropathy. Kidney international. 1998; 54: 286-300.

29. Borish LC, Steinke JW. Cytokines and chemokines. J Allergy Clin Immunol. 2003; 111(2): S460-S465.

30. Sereger S, Alpers CE. Chemokines and chemokine receptors in renal pathology. Current Opinion in Nephrology and Hypertension. 2003; 12:243-249.

31. Sereger S, Nelson PJ, Schlondorff D. Chemokines, chemokine receptors, and renal disease: from basic science to pathophysiologic and therapeutic studies. J Am Soc Nephrol 2000; 11:152-176.

32. Viedt C, Dechend R, Fei J, et al. MCP-1 induces inflammatory activation of human tubular epithelial cells: involvement on transcription factors, nuclear factor-kappaB and activating protein-1. J Am Soc Nephrol 2002; 13:1534-1547.

33. Gröne, HJ, Cohen CD, Gröne E, Schimidt C, Kretzler M, Schlöndorff D, Nelson PJ. Spatial and temporally restricted expression of chemokines and chemokine receptors in the developing human kidney. J Am Nephrol. 2002; 13: 957-967.

34. Sheu JN, Chen MC, Cheng SL, Lee IC, Chen SM, Tsay GJ. Urine interleukin-1β in children with acute pyelonephritis and renal scarring. Nephrology. 2007; 12: 487-493.

35. Sheu JN, Chen MC, Lue KH, Cheng SL, Lee IC, Chen SM, Tsay GJ. Serum and urine of interleukin-6 and interleukin-8 in children with acute pyelonephritis. Cytokine. 2006; 36:276-282.

36. Souto MFO, Teixeira AL, Russo RC, Penido MGMG, Silveira KD, Teixeira MM, Simões e Silva AC. Immune mediators in idiopathic nephrotic syndrome: Evidence for a relationship between interleukin 8 and proteinuria. Pediatr Res. 2008;64(6):637-42.

37. Decramer S, Bascands Jl, Schanstra JP. Non-invasive markers of ureteropelvic junction obstruction. World Journal of Urology. 2007; 25:457-465.

38. Artifoni L, Negrisolo S, Montini G, Zuchetta P, Molinari PP, Cassar W, Destro R, Anglani F, Rigamonti W, Zacchello G, Murer L. Interleukin-8 and CXCR1 receptor functional polymorphisms and susceptibility to acute pyelonephritis. The Journal of Urology.2007; 177: 1102-1106.

39. Monga M, Gabal-Shehab LL, Stein P. Urinary transforming growth factor-β1 levels correlate with bladder outlet obstruction. International Journal of Urology. 2001; 8:487-489.

40. Klahr S, Morrisey JJ. Comparative study of ACE inhibitors and angiotensin II receptor antagonists in international scarring. Kidney International. 1997; 52(63): S111-S114.

41. Miayjima A, Chen J, Lawrence C, Ledbetter S, Soslow RA, Stern J, Jha S, Pigato J, Lemer ML, Poppas DP, Vaughan Jr ED, Felsen D. Antibody to transforming growth factor-β ameliorates tubular apoptosis in unilateral obstruction. Kidney International. 2000; 58:2301-2313.

42. Trachtman H, Weiser AC, Valderrama E, Morgado M, Palmer LS. Prevention of renal fibrosis by spironolactona in mice with complete unilateral ureteral obstruction. The Journal of Urology. 2004; 172:1590-1594.

43. Seseke F, Thelen P, Heuser M, Zöller G, Ringert RH. Impaired nephrogenesis in rats with congenital obstrutive uropathy. The Journal of Urology. 2001; 165:2289-2292.

44. Chevalier RL, Thornhill BA, Chang AY, Cachat F, Lackey A.Recovery from release of ureteral obstruction in the rat: Relationship to nephrogenesis. Kidney International. 2002; 61: 2033-2043.

45. Stephan M, Conrad S, Eggert T, Heuer R, Fernandez S, Huland H. Urinary concentration and tissue messenger RNA expression of monocyte chemoattractant protein

as an indicator of the degree of hydronephrotic atrophy in partial ureteral obstruction. The Journal of Urology. 2002; 167:1497-1502.

46. Seseke F, Thelen P, Hemmerlein B, Kliese D, Zöller G, Ringert RH. Histologic and molecular evidence of obstructive uropathy in rats with hereditary congenital hydronephrosis. Urol Res. 2000; 28: 104-109.

47. Chevalier RL, Thornhill BA, Wolstenholme JT, Kim A. Unilateral ureteral obstruction in early development alters renal growth: dependence on the duration of obstruction. The Journal of Urology. 1999; 161: 309-313.

48. Zhou, Y, Takahashi G, Shinagawa T, Okuhara T, Yonamine K, Aida Y, Tadokoro M. Increased transforming growth factor-β1 and tubulointersticial fibrosis in rats with congenital hydronephrosis. International Journal of Urology. 2002; 9: 491-500.

49. Truong LD, Petrusevska G, Yang G, Gurpinar T, Shappell S, Lechago J, Rouse D, Suki WN. Cell apoptosis and proliferation in experimental chronic obstructive uropathy. Kidney International. 1996; 50: 200-207.

50. Truong LD, Choi YJ, Tsao CC, Ayala G, Sheikh-Hamad D, Nassar G Suki WN. Renal cell apoptosis in chronic obstructive uropathy: the role of caspases. Kidney International. 2001; 60:924-934.

51. Chevalier RL, Goyal S, Wolstenholme JT, Thornhill BA. Obstructive nephropathy in the neonatal rat is attenuated by epidermal growth factor. Kidney International. 1998; 54: 38-47.

52. Seremetis GM, Maizels M. TGF-β1 mRNA expression in the renal pelvis after experimental and clinical ureteropelvic junction obstruction. The Journal of Urology. 1996; 156: 261-266.

53. Mizuno S, Matsumoto K, Nakamura T. Hepatocyte growth factor suppresses interstitial fibrosis in a mouse model of obstructive nephropathy. Kidney International. 2001; 59: 1304-1314.

54. Isaka Y, Tsujie M, Ando T, Nakamura H, Kaneda Y, Imai E, Hori M. Transforming growth factor-β1 antisense oligodeoxynucleotides block interstitial fibrosis in unilateral ureteral obstruction. Kidney International. 2000; 1885-1892.

55. Sato M, Muragaki Y, Saika S, Roberts AB, Ooshima A. Targeted disruption of TGF-β1/ Smad3 signaling protects against renal tubulointerstitial fibrosis induced by unilateral ureteral obstruction. Journal of Clinical Investigation, 2003; 112(10): 1486-1494.

56. Derynck R, Zhang Y, Feng XH. Smads: transcriptional activators of TGF-β responses. Cell. 1998; 95: 737-740.

57. Misseri R, Meldrum DR, Dagher P, Hile K, Rink RC, Meldrum KK. Unilateral ureteral obstruction induces renal tubular cell production of tumor necrosis factor-α independent of inflamatory cell infiltration. The Journal of Urology. 2004; 172; 1595:1599.

58. Vielhauer V, Anders HJ, Mack M, Cihak J, Strutz F, Sangasswinger M, Luckow B, Gröne HJ, Schlöndorff. Obstructive nephropathy in the mouse: progressive fibrosis correlates with tubulointerstitial chemokine expression and accumulation of CC chemokine receptor 2- and 5-positive leukocytes. J Am Soc Nephrol. 2001; 12:1173-1187.

59. Crisman JM, Richards LL Valach DP, Valach DF, Franzoni DF, Diamond JR. Chemokine expression in the obstructed kidney. Exp Nephrol. 2001; 9: 241-248.

60. Lauder AJ, Jolin HE, Smith P, Berg JG, Jones A, Wisden W, Smith KGC, Dasvarma A, Fallon PG, Mckenzie ANJ. Lymphomagenesis, hydronephrosis, and autoantibodies result from dysregulation of IL-9 and are differentially dependent on Th2 cytokines. The Journal of Immunology. 2004; 113-122.

61. Hochberg D, Johnson CW, Chen J, Cohen D, Stern, Vaughan Jr ED, Poppas D, Felsen D. Intertitial fibrosis of unilateral ureteral obstruction is exacerbated in kidneys of mice lacking the gene for inducible nitric oxide synthase. Laboratory Investigation. 2000; 11: 1721-1728.

62. Bulas DI, Fonda JS. Prenatal evaluation of fetal anomalies. Pediatric Radiology. 1997; 44:537-553.

63. Zhang PL, Peters CA, Rosen S. Ureteropelvic junction obstruction: morphological and clinical studies. 2000; 14:820-826.

64. Grandaliano G, Gesualdo L, Bartoli F, Ranieri E, Monno Rafaela, Leggio A, Paradies G, Caldarulo E, Infante B, Schena FP. MCP-1 and EGF renal expression and urine excretion in human congenital obstructive nephropathy. Kidney International. 2000; 58:182-192

65. Bartoli F, Gesualdo L, Paradies G, Caldarulo E, Infante B, Grandaliano G, Monno R, Leggio S, Salzillo F, Schena FP, Leggio A. Renal expression of monocyte chemotactic protein-1 and epidermal growth factor in children with obstructive hydronephrosis. Journal of Pediatric Surgery. 2000; 35:569-572.

66. Palmer LS, Maizels M, Kaplan WE, Firlit CF, Cheng EY. Urine levels of transform growth factor-beta 1 in children with ureteropelvic junction obstruction. Pediatric Urology. 1997; 50(5): 769-773.

67. Furness PD, 3rd, Maizels M, Han SW, Cohn RA, Cheng EY. Elevated bladder urine concentration of transforming growth factor-beta1 correlates with upper urinary tract obstruction in children. J Urol. 1999 Sep;162(3 Pt 2):1033-6.

68. El-Sherbiny MT, Mousa OM, Shokier AA, Ghoneim MA. Role of urinary transforming growth factor-β1 concentration in the diagnosis of upper urinary tract obstruction in children. The Journal of Urology. 2002; 168: 1798-1800.

69. Taha MA, Shokeir AA, Osman HG, El-Aziz AEAFA, Farahat SE. Pelvi-ureteric obstruction in children: the role of urinary transform growth factor-β1 and epidermal growth factor. Pediatric Urology. 2007; 99:899-903.

70. Elder, J. S., C. A. Peters, et al. Pediatric vesicoureteral reflux Guidelines Panel summary report on the management of primary vesicoureteral reflux in children. J Urol. 1997; 157: 1846-1851.

71. Silva, J. M., J. S. Santos Diniz, et al. Clinical course of 735 children and adolescents with primary vesicoureteral reflux. Pediatr Nephrol. 2006;21: 981-988.

73. Chertin B, Farkas A, Puri P. Epidermal growth factor and monocyte chemotactic peptide-1 expression in reflux nephropathy. European Urology. 2003; 44:144-149.

74. Yim HE, Bae IS, Yoo KH, Hong YS, Lee JW. Genetic control of VEGF and TGF-β1 gene polymorphisms in childhood urinary tract infection and vesicoureteral reflux. Pediatric Research. 2007; 62 (2):183-187.

75. Kuronda S, Solari V, Puri P. Association of Transform growth factor-β1 gene polymorphism with familial vesicoureteral reflux. The Journal of Urology. 2007; 178:1650-1653.

76. Lee-Chen GJ, Liu PK, Lai YC, Juang HS, Huang SY, Lin CY. Significance of the tissue kallikrein promoter and transforming growth factor-β1 polymorphisms with renal progression in children with vesicoureteral reflux. Kidney International. 2004; 65:1467-1472.

77. Solari V, Ennis S, Cascio S, Puri P. Tumor necrosis factor-α gene polymorphism in reflux nephropathy. The Journal of Urology. 2004; 172:1604-1606.

78. Schwentner C, Oswald J, Lunacek A, Pelzer AE, Fritsch H, Schlenck B, Karatzas A, Bartsch G, Radmayr C. Extracellular Microenvironment and cytokine profile of the ureterovesical junction in children with vesicoureteral reflux. 2008; 180: 674-700.

79. Galanakis E, Bitsori M, Dimitriou H, Giannakopoulou C, Karkavitsas NS, Kalmanti M. Urine Interleukin-8 as a Marker of Vesicoureteral Reflux in Infants. Pediatrics. 2006 Apr 3.

80. Haraoka M, Senoh K, Ogata N, Furukawa M, Matsumoto T, Kumazawa J. Elevated interleukin-8 levels in the urine of children with renal scarring and/or vesicoureteral reflux. The Journal of Urology. 1996; 155:678-680.

81. Ninan GK, Juntley RS, Eremin O. Urinary Cytokines as markers of reflux nephropathy. The Journal of Urology. 1999 November; 162:1739-1742.

82. Wang J, Konda R, Sato H, Sakai K, Ito S, Orikasa S. Clinical significance of urinary interleukin-6 in children with reflux nephropathy. The Journal of Urology. 2001 January; 165:210-214.

83. Gokce I, Alpay H, Biyikli, Unluguzel G, Dede F, Topuzoglu A. Urinary levels of interleukin-6 and interleukin-8 in patients with vesicoureteral reflux and renal parenchymal scar. Pediatric Nephrology.2010.

84. Jutley RS, Youngson GG, Eremin O, Ninan GK. Serum Profile in reflux nephropathy. Pediatr Surg Int. 2000; 16:64-68.

85. Sabasiñska A, Zoch-Zwierz W, Wasilewska A, Porowski T. Laminin and Transform growth factor beta-1 in children with vesicoureteral reflux. Pediatric Nephrology. 2008; 23:769-774.

FIGURE 1

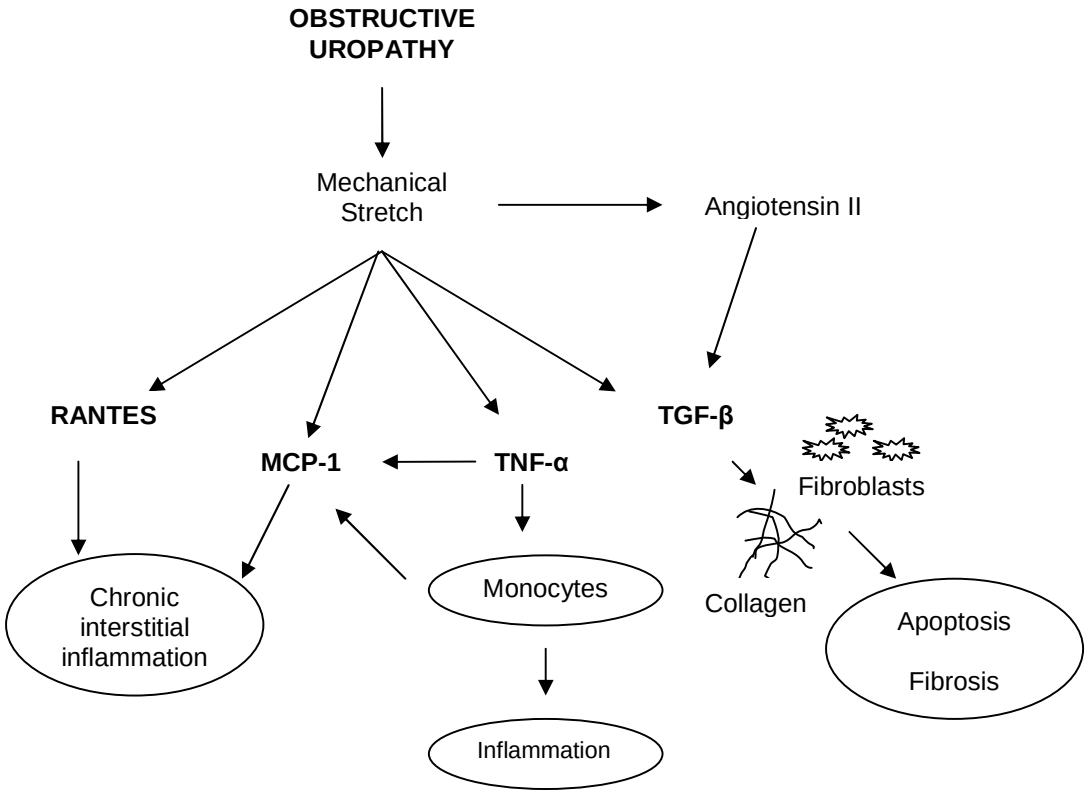


Figure 1: Main mechanisms involved in obstructive models

Table 1: Recent studies on urinary cytokines in patients with ureteropelvic junction obstruction

Author	Year	Ref	Age of patients	Cytokine	Study / control group (N)	Sensitivity	Specificity	Conclusions
Palmer	1997	66	4.6 years (1 month to 11 years)	TGF-β1	13 / VUR (11) and healthy children (19)	-	-	Pelvic urinary TGF-β_1 levels are elevated compared to the control group
Furness	1999	67	Median: 2.1 years	TGF-β1	30 / Healthy children (19)	92%	-	Bladder urinary TGF-β_1 levels are significantly elevated
Grandaliano	2000	64	1 months to 13 years	MCP-1; EGF	24 / Healthy children (15)	-	-	Bladder urinary EGF levels are reduced in UPJO, while MCP-1 levels are elevated
El-Sherbiny	2002	68	5.2 ± 4.7 years	TGF-β1	15 / Dilated non obstructed kidneys (11)	80%	82%	Elevated bladder urinary TGF-β_1 levels in obstructed kidneys decreased after surgery.
Taha	2007	69	Median: 5.9 years	TGF-β1; EGF	35 / Healthy children (30)	100% (TGF-β1)	80% (TGF-β1)	Bladder urinary TGF-β_1 levels are significantly elevated, while no significant differences are detected in EGF levels.
Almodhen	2009	24	14 ± 6 months	TGF-β1	42 / -	82%	86%	Bladder urinary TGF-β1 levels can predict the need for surgery

Ref: reference number

Table 2: Recent studies on urinary cytokines in patients with vesicoureteral reflux (VUR)

Author	Year	Ref	Age of patients	Cytokine	Study/ control group (N)	Sensitivity	Specificity	Conclusions
Haraoka	1996	80	Mean age 6.7 years	IL-8	32 / -	-	-	Levels of IL-8 are elevated in patients with VUR or renal scarring
Ninan	1999	81	5 months to 13.33 years	IL-6; TNF-α	17 / Healthy children (15)	-	-	Levels of IL-6 and TNF-α receptor-1 are elevated in reflux associated with renal damage
Wang	2001	82	Mean age 14.6 years	IL-6	66 / Healthy children (28)			Levels of IL-6 are elevated in severe bilateral renal scarring
Galanakis	2007	79	1 month to 2 years	IL-8	24 / History ITU but no VUR (14); No ITU, no VUR (21)	88%	69%	Levels of IL-8 are elevated in VUR patients
Sabasiñska	2008	85	6.23±4.15 years	TGF-β1	54 / Healthy children (27)	-	-	Highest urinay concentrations of TGF-β1 are detected in grade IV and V reflux
Gokce	2010	83	1 month 16 years	IL-6; IL-8	87 / Healthy children (27)	-	-	IL-6 levels are elevated in VUR and IL-8 levels in renal scarring

Ref: reference number

3. OBJETIVOS

Objetivo Geral:

Avaliação dos níveis urinários de citocinas pró-inflamatórias e do fator de crescimento e transformação do tipo beta em crianças e adolescentes com hidronefrose diagnosticada intraútero.

Objetivos específicos:

Os objetivos específicos desse projeto foram:

1. Determinar as concentrações urinárias de IL-6, TNF-α (fator de necrose tumaoral alfa, tumor necrosis factor-α) e TGF-β1, expressas em valores absolutos e relativos à creatinina urinária, em crianças e adolescentes portadores de anomalias do trato urinário, subdivididos em portadores de hidronefrose idiopática, de uropatia e de displasia/ hipoplasia renal;
2. Comparar esses parâmetros obtidos nos pacientes portadores de hidronefrose idiopática e portadores de uropatias clinicamente significativas;
3. Comparar esses marcadores nos pacientes com captação normal versus captação reduzida à cintilografia renal estática (DMSA);
4. Verificar se existe correlação das determinações urinárias de IL-6, TNF-α e TGF-β1 com a ocorrência de infecção urinária.

4. PACIENTES E MÉTODOS

4.1 DELINEAMENTO, POPULAÇAO E LOCAL DE ESTUDO

Trata-se de um estudo transversal de uma coorte de crianças e adolescentes que tiveram o diagnóstico de dilatação da pelve renal intraútero e que foram acompanhados desde o nascimento no Ambulatório Bias Fortes de atendimento terciário da Unidade de Nefrologia Pediátrica do Departamento de Pediatria da Faculdade de Medicina da Universidade Federal de Minas Gerais.

4.1.1 PERÍODO DE ESTUDO

Foram coletados exames do período de janeiro de 2008 a janeiro de 2009.

4.1.2 CRITÉRIOS DE INCLUSÃO

Foram incluídos pacientes que apresentavam o diâmetro ântero-posterior da pelve renal medido na seção transversa do hilo renal fetal ≥ 5mm no terceiro trimestre da gestação [2-4].

4.1.3 CRITÉRIOS DE EXCLUSÃO

- Foram excluídos pacientes com aneuploidia ou malformações múltiplas.
- Foram excluídos os pacientes que se apresentarem agudamente doentes no momento da coleta, tais como, aqueles com infecção não controlada, insuficiência renal crônica (IRC) estágios maior que 3, desequilíbrio hemodinâmico e /ou metabólico agudo.

4.1.4 ASPECTOS ÉTICOS

Os pacientes e os responsáveis por eles foram devidamente esclarecidos sobre a natureza do estudo e o que seria feito, tendo resguardado seu direito, em caso de recusa, de receberem a avaliação e o tratamento indicados (para maiores detalhes, ver termo de consentimento em anexo). Além disso, as amostras de urina dos pacientes, que aceitaram participar da pesquisa, foram colhidas simultaneamente a outros exames de sangue e urina que fazem parte da avaliação clínica rotineira dessas crianças e adolescentes portadores de hidronefrose fetal.

Este projeto de pesquisa foi aprovado pela Câmara Departamental do Departamento de Pediatria da Faculdade de Medicina da UFMG (vide parecer em anexo) e pelo cômite de ética do Hospital das Clínicas e da UFMG (COEP) – Protocolo número ETIC 0487/06 e DEP 143/06, respectivamente (vide parecer em anexo).

4.2 DEFINIÇÕES E CLASSIFICAÇÃO DA DILATAÇÃO DA PELVE RENAL

As seguintes definições foram adotadas no presente estudo:

Uropatia significativa – diagnóstico de uma entidade nosológica bem definida através da combinação da análise dos exames de imagem: uretrocistografia miccional, urografia excretora, cintilografia renal estática e dinâmica, exames ecográficos seriados. Foram considerados como uropatia significativa: obstrução da junção ureteropélvica, refluxo vesicoureteral, megaureter primário, ureterocele, duplicação pieloureteral. Outras malformações foram também consideradas, como: rim em ferradura, displasia multicística e rim hipoplásico. Na análise estatística, os pacientes foram divididos em três grupos: hidronefrose idiopática, uropatia e displasia renal. A análise foi realizada também

dividindo-se os pacientes em dois grupos: uropatas (com uropatia significativa e rins displásicos) e não-uropatas (com hidronefrose idiopática).

Obstrução de junção ureteropélvica - foi considerada quando houve dilatação da pelve renal associada a padrão de excreção intermediário ou obstrutivo, independentemente da captação renal relativa pelo $^{99tm-}$DMSA.

Obstrução de junção ureteropélvica cirúrgica - quando houver dilatação da pelve renal associada a padrão de excreção intermediário ou obstrutivo, com captação renal relativa pelo $^{99tm-}$DMSA < 40%.

Lesão renal – presença de qualquer alteração da morfologia e/ou crescimento dos rins e também presença de captação renal relativa pelo $^{99tm-}$DMSA < 45%. Para avaliação do crescimento renal foram utilizados os gráficos de Han & Bancock [5].

Hidronefrose – é o termo que descreve a dilatação do sistema coletor renal, não implicando na presença de obstrução [6]. Hidronefrose idiopática – dilatação da pelve renal sem causa definida.

Classificações da hidronefrose - para avaliação da dilatação renal serão vários pontos de corte do diâmetro ântero-posterior (DAP): 1) dilatação leve - ≥ 5 mm e < 10 mm 2) dilatação moderada - ≥ 10 mm e < 15mm 3) dilatação acentuada - ≥ 15 mm para construir intervalos para análises. As unidades renais também foram classificadas de acordo com a classificação da SFU (Society of Fetal Urology) [7]. Quando bilateral, foi considerada a unidade renal com dilatação maior e/ou com classificação em grau maior pela SFU.

Infecção do trato urinário – sinais e sintomas como febre, disúria e hematúria associados à urocultura positiva (>100.000 col/ml), com o crescimento de uma única

bactéria gram-negativo. A urina foi colhida em saco coletor em caso de crianças sem controle da micção e jato médio naquelas que já apresentam controle da micção. Todas as coletas foram realizadas no laboratório após anti-sepsia adequada.

4.3 INVESTIGAÇÃO CLÍNICA E POR IMAGENS DOS FETOS E RECÉM-NASCIDOS PORTADORES DE DILATAÇÃO DA PELVE RENAL NA ULTRA-SONOGRAFIA FETAL

As gestantes nas quais se evidenciou alteração no trato urinário fetal foram encaminhadas para avaliação no Centro de Medicina Fetal (CEMEFE) do Hospital das Clínicas da UFMG e submetidas a estudos ecográficos seriados, em intervalos variáveis de acordo com a indicação clínica. Para a análise foi considerado o último exame realizado no terceiro trimestre de gestação, quando uma ou duas unidades renais fetais apresentaram DAP ≥ 5mm. Como referência foi usada o maior valor para a inclusão do recém-nascido neste estudo [2-4].

Dinâmica do exame ultra-sonográfico fetal:

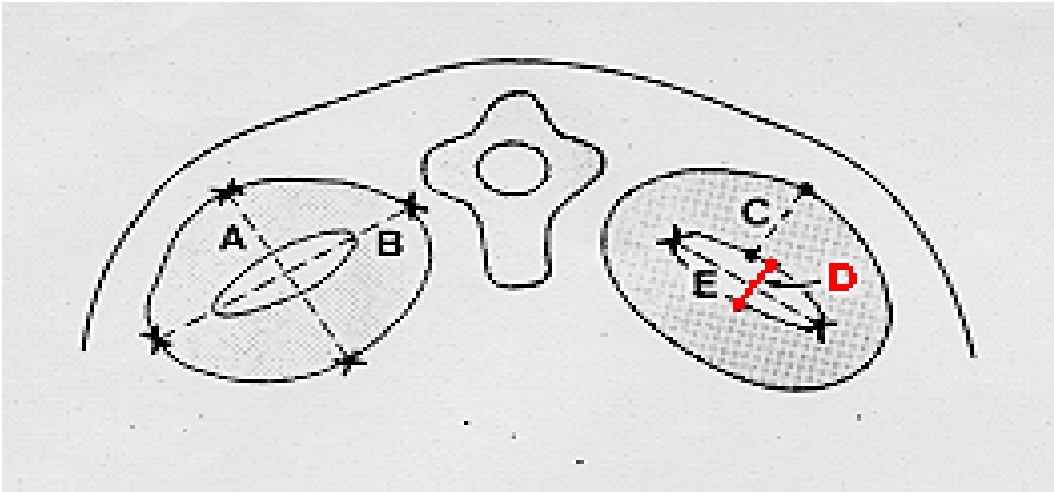
A) Morfologia fetal geral:

O estudo segmentar do feto foi realizado considerando-se:

- Visibilização do crânio, face, membros superiores e inferiores, órgãos internos do tórax, abdome, buscando malformações associadas;
- Biometria fetal – para identificar a idade gestacional e o padrão de crescimento fetal;
- Avaliação da placenta e do cordão umbilical;
- Avaliação do volume do líquido amniótico através da medida do ILA, considerando-se o valor normal entre oito e 18 [8,9].

B) **Morfologia do trato urinário:**

- Estudo das unidades renais – localização anatômica, relações anatômicas, contorno, ecogenicidade, volume e estruturas anatômicas. Presença de cistos, seu tamanho e distribuição foram também descritos. As seguintes mensurações foram obtidas: comprimento renal (diâmetro longitudinal, no plano longitudinal) e o ântero-posterior no corte transversal;
- O diagnóstico ultrassonográfico de dilatação da pelve foi realizado através da DAP da pelve renal no corte transversal. A Figura 3 mostra esquematicamente as principais dimensões renais mensuradas através da ultra-sonografia fetal;
- Estudos das vias urinárias (ureter e uretra) – foram avaliadas a presença de dilatações tubulares irregulares e anecóicas em suas respectivas topografias, caracterizando-se a presença de megaureter e megauretra. Foi definida como megabexiga quando a bexiga fetal, estrutura arredondada e centralizada no abdome fetal, manteve-se persistentemente com diâmetro longitudinal > 5mm; também foi avaliada a espessura da parede vesical, considerando-se anormal valor > 2mm.



Adaptado de CORTEVILLE *et al.*,1991[23]

Figura 3. Corte transversal esquemático mostrando ambas as unidades renais do feto. Linha A: Diâmentro antero-posterior renal; Linha B: diametro transversal do rim; Linha D: diâmetro ântero-posterior da pelve renal; Linha E: espessura do parenquima renal.

PERÍODO PÓS-NATAL

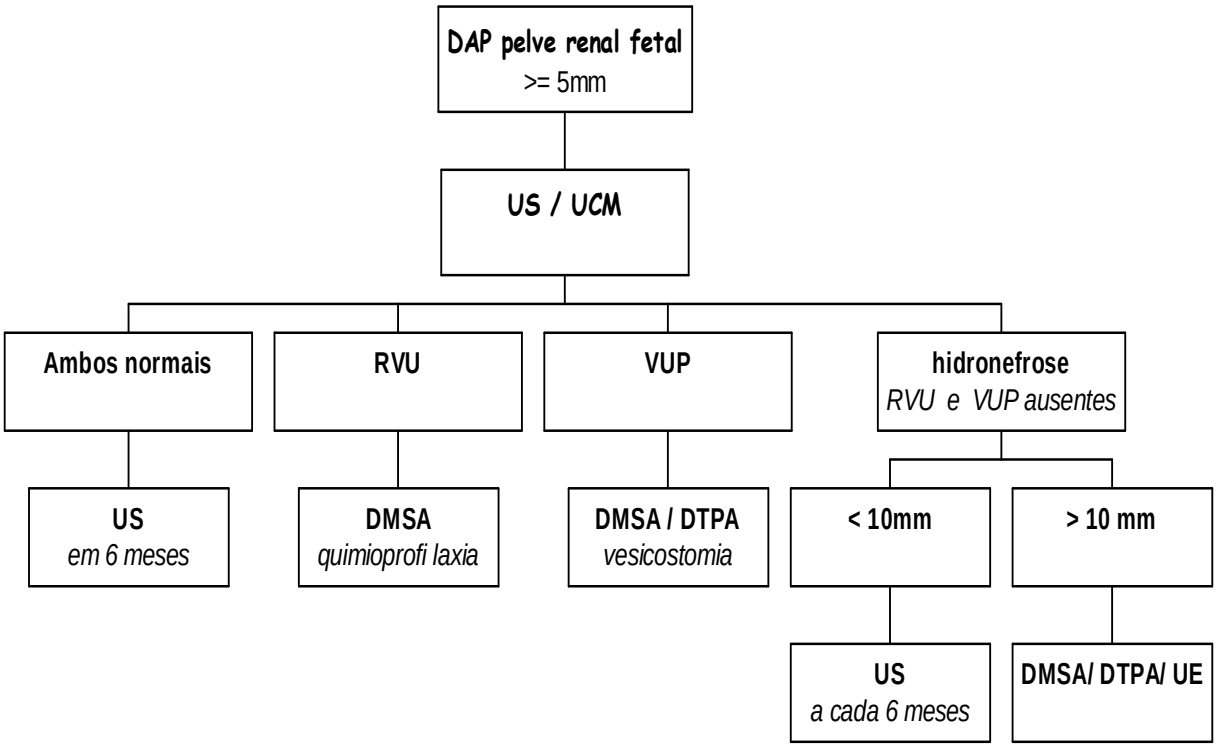
Exame físico

Logo após o nascimento, por volta de três horas de vida, os recém-nascidos foram submetidos a exame físico pelos pediatras assistentes da Maternidade Otto Cirne do Hospital das Clínicas da UFMG. Dados perinatais como peso e estatura ao nascimento e os escores de Apgar foram obtidos.

Todos os pacientes foram acompanhados no Ambulatório Bias Fortes, anexo do Hospital das Clínicas (UFMG), sendo a periodicidade das consultas de seis em seis meses nos dois primeiros anos de vida e anual a partir desse período. Esse seguimento foi flexível o suficiente para se adaptar às condições clínicas dos pacientes. A mensuração da pressão arterial foi realizada em todas as visitas.

Investigação por imagens pós-natais

A avaliação por imagens do trato urinário será obtida em todos os neonatos portadores de dilatação da pelve renal, de acordo com o algoritmo da Figura 4.



US: ultrassonografia; UCM: uretrocistografia miccional; RVU: refluxo vesicoureteral; VUP: válvula de uretra posterior; DMSA: cintilografia estática; DTPA: cintilografia dinâmica; UE: urografia excretora

Figura 4. Algoritmo para avaliação do trato urinário do neonato portador de hidronefrose fetal.

A) Ultrassonografia pós-natal:

O primeiro ultrassom pós-natal foi realizado após a primeira semana de vida, exceto para os casos suspeitos de grave hidronefrose bilateral, quando foi realizado logo após o nascimento. Foram realizados exames ecográficos a cada seis meses para os casos leves a moderados de hidronefrose até o desaparecimento da doença. Os casos mais graves tiveram vigilância mais frequente. Se submetidos à intervenção cirúrgica, a avaliação foi realizada quatro meses após o procedimento.

Os exames foram realizados em equipamento Siemens (Sonoline Prima SLC) utilizando-se transdutor de 5 MHz, na posição supina. A mensuração foi obtida nas seções longitudinais e transversais de ambas as unidades renais. As seguintes mensurações renais foram registradas: comprimento no corte longitudinal, diâmetro transversal e ântero-posterior dos rins no corte transversal. O volume renal foi calculado de acordo com a fórmula proposta por Han & Babcock[5].

A pelve renal foi mensurada pelo DAP no corte transversal. A razão entre o DAP da pelve renal e diâmetro antero-posterior do rim (DAP/DR) foi calculada para todas as unidades renais [10, 11]. A gravidade da hidronefrose foi graduada de acordo com a escala padronizada pela SFU [7]. Todas as medidas da pelve renal foram realizadas quando a criança estava com a bexiga vazia [12-15]. Na Figura 5 podem ser observadas, de forma esquemática, as mensurações das unidades renais e das pelves renais.

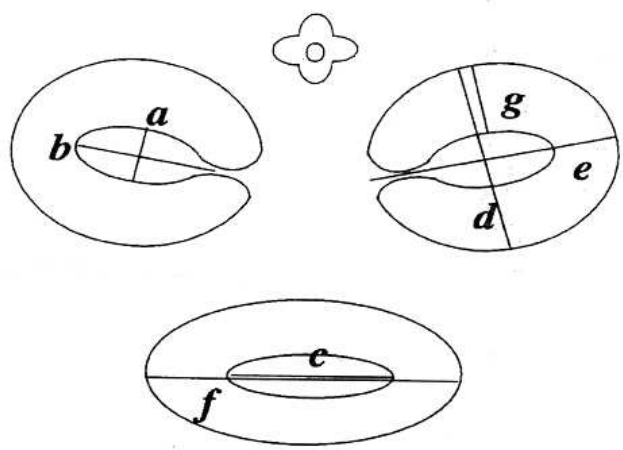


Figura 5. Dimensões da pelve e das unidades renais mensuradas pela ultra-sonografia: (a) diâmetro ântero-posterior da pelve renal; (b) diâmetro

transverso da pelve renal; (c) diâmetro longitudinal da pelve renal; (d) diâmetro ântero-posterior do rim; (e) diâmetro transversal do rim; (f) diâmetro longitudinal do rim; (g) espessura do parênquima renal.

B) Uretrocistografia miccional:

A avaliação contrastada do trato urinário baixo foi obtida no primeiro mês de vida, sempre precedida de cobertura antibiótica profilática com ¼ da dose terapêutica de cefalosporina de primeira geração (cefalexina ou cefadroxila) em dose única [16]. Os exames foram realizados no Serviço de Radiologia do Hospital das Clínicas da UFMG segundo técnicas e normas padronizadas por radiologistas que desconheciam, as dimensões da pelve renal à ultra-sonografia.

C) Cintilografia renal:

A morfologia do parênquima renal foi estudada utilizando-se radioisótopos: ^{99}Tc-ácido dimercaptosuccínico (DMSA) para quantificar a captação do parênquima renal e ^{99}Tc-ácido dietilenotriaminopentacético (DTPA) para a avaliação do fluxo e excreção renal. Se a dilatação de pelve renal foi ≥ 10mm, foram realizadas cintilografia estática e cintilografia dinâmica após o primeiro mês de vida. Nos casos de RVU, apenas a cintilografia estática foi realizada.

Os estudos foram feitos após o primeiro mês de vida, ou antes, se a condição clínica do paciente assim o exigisse [17]. Os estudos cintilográficos foram realizados nos setores de Medicina Nuclear do Hospital Felício Rocho e da Santa Casa de Misericórdia de Belo Horizonte, conforme técnicas e normas padronizadas. Os resultados dos estudos foram avaliados por examinadores que desconheciam a evolução clínica dos pacientes em questão. A captação relativa, comparativa entre as duas unidades renais, foi calculada de

acordo com a equação utilizada por Konda *et al.* [18]. As unidades renais dilatadas foram classificadas em um dos três grupos funcionais, dependendo da captação relativa ao DMSA. A classificação de acordo com a captação do DMSA foi: 1) função gravemente acometida - menos de 20% de captação relativa do radioisótopo; 2) moderadamente acometida – captação relativa entre 20 e 39%; 3) função renal preservada - captação relativa acima de 40% [18,19]. As unidades renais foram também classificadas dentro de um dos três grupos, dependendo de interpretação subjetiva das curvas DTPA [20,21]. O grupo I foi definido como uma unidade não obstruída, com uma curva descendente; o grupo II foi classificado como um padrão intermediário, com uma curva plana; e o grupo III foi classificado como uma curva ascendente no renograma (unidade obstruída). Na Figura 6 estão representados os três grupos de acordo com a curva de excreção do DTPA.

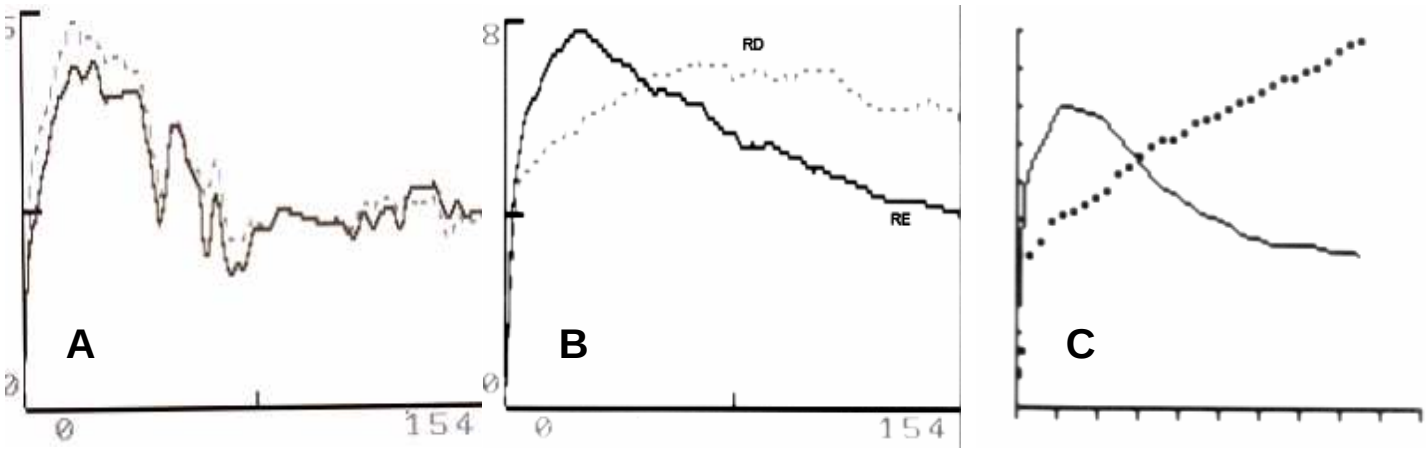


Figura 6. Curvas representativas dos três grupos de acordo com a excreção do DTPA: (A) ambas as unidades com excreção normal – grupo I; (B) uma unidade renal com padrão intermediário (linha pontilhada) – grupo II; (C) uma unidade renal com padrão obstrutivo (linha pontilhada) – grupo III.

D) Urografia excretora:

Esse exame foi empregado apenas para os casos suspeitos de megaureter primário e ureterocele ectópica para os quais não foi possível o diagnóstico através da ultra-sonografia, UCM e cintilografia renal. Foi realizado após o segundo mês de vida no Serviço de Radiologia do Hospital das Clínicas da UFMG, também segundo técnicas e normas padronizadas.

Exames laboratoriais

A) Urina rotina e urocultura:

Amostras de urina de todos os recém-nascidos foram obtidas para urinálise e cultura após o primeiro dia de vida e, subsequentemente, em cada visita clínica ou quando houve suspeita clínica de infecção urinária. Os exames foram coletados no Laboratório Central do Hospital das Clínicas da UFMG, conforme técnicas padronizadas.

B) Dosagem sérica de uréia e creatinina:

As concentrações séricas das escórias nitrogenadas foram determinadas inicialmente após 72 horas de vida e o ritmo de filtração glomerular foi estimado, de acordo como a fórmula proposta por Schwartz et al. [22]. Novas determinações dos níveis séricos de uréia e creatinina foram, sequencialmente, obtidas durante o seguimento. Quando a suspeita diagnóstica foi válvula de uretra posterior ou outra condição na qual o estado geral estivesse comprometido, esses exames foram feitos logo após o nascimento e repetidos em 72 horas.

4.4. Determinação das concentrações urinárias de citocinas e creatinina

Coleta das amostras urina

Amostras de urina (volume mínimo 10 ml) foram obtidas a partir de coleta por amostra única de 7 às 9 am e, seguir, centrifugadas a 2100 r.p.m. por 10 minutos. O sobrenadante foi armazenado a -80ºC.

Ensaio imunoenzimático (ELISA) para TGF-β1, IL-6 e TNF-α humano na urina (R&D Systems):

Os ensaios imunoenzimáticos foram realizados por meio de kits ultrassensíveis da R&D Systems (Minneapolis, EUA) para medida de IL-6 e TNF-α. Para medida do TGF-β1 foi utilizado kit do tipo Quantikine (R&D Systems, Minneapolis, EUA), a as amostras foram ativadas antes do ensaio. Resumidamente, os anticorpos de captura específicos (fornecido no kit) foram diluídos em tampão fosfato (PBS) e adicionados a cada poço de placas de poliestireno (96 poços). As placas foram, então, incubadas a 4ºC por 12 horas. Em seguida, foram submetidas a quatro ciclos de lavagem com PBS e Tween 20 a 0,05% (Sigma). As placas foram então bloqueadas com albumina sérica bovina (BSA) 1% e PBS, e incubadas por uma hora em temperatura ambiente. Um novo procedimento de lavagem foi feito, como descrito acima. Em seguida, as amostras foram adicionadas às placas e incubadas por 12 horas a 4ºC, sendo depois submetidas a novos ciclos de lavagem. Os anticorpos de detecção, diluídos em PBS, foram adicionados, e foi feita incubação por duas horas em temperatura ambiente, com novo procedimento de lavagem em seguida. O reagente de cor (fenilenediamina) foi adicionado a cada poço e as placas deixadas no escuro por 15 minutos. A reação foi parada com a adição de 1M H_2SO_4 aos poços. A absorbância

foi lida em um leitor de placas (Emax, Molecular Devices, MN, EUA), ajustado no comprimento de onda de 492nm. Todas as amostras foram analisadas em um único ensaio para evitar a variabilidade interensaio. Nossa variabilidade intra-ensaio foi inferior a 3% e os limites de detecção foram 6 pg/ ml para o TGF-β1, 0.039 pg/ ml para IL-6 e 0.106 pg/ ml para TNF- α. Os valores obtidos para cada citocina medida foram expressos em termos absolutos (pg/ml) e em termos relativos (pg/mg de creatinina), considerando-se as concentrações de creatinina obtidas nas mesmas amostras de urina.

Determinação de creatinina na urina.

As dosagens de creatinina urinária foram realizadas nas mesmas amostras utilizadas para determinação das citocinas urinárias. Foi utilizado o pelo método fotocolorimétrico, baseado na reação da creatinina com a solução de picrato em meio alcalino. Para a realização dessas dosagens será utilizado o Kit 35 E do LABTEST Sistemas para Diagnóstico, segundo o protocolo abaixo.

(a) Reagentes

Ácido pícrico - 44.4 mmol/ l.

Tampão contendo 0.25 mmol/ l de hidróxido de sódio, 25 mmol/ l de fosfato de sódio, 24 mmol/ l de tetraborato de sódio e surfactantes não reativos.

Padrão - Creatinina 4 mg/ dl.

Acidificante - Ácido acético 11.2 mmol/ l.

(b) Amostra

A urina coletada foi diluída de 1:5. Foram feitas duplicatas de todas as amostras de urina, para verificar a reprodutibilidade das leituras obtidas com esse ensaio.

(c) Curva padrão

Foi verificada a linearidade do ensaio, através da construção de curvas padrão, utilizando, inicialmente, cinco pontos experimentais que correspondem às concentrações de creatinina de 0.5, 1.0, 2.0, 4.0 e 8.0 mg/ dl. Depois de confirmada a linearidade do ensaio, a curva padrão foi obtida a partir da leitura de três diferentes concentrações de creatinina (1.0, 2.0 e 4.0 mg/dl), sendo as duas primeiras obtidas pela diluição do padrão de creatinina contendo 4.0 mg/ dl. A curva padrão foi realizada em todos os ensaios, sendo determinado um fator de calibração (inclinação da reta obtida a partir dos três pontos experimentais), que permitirá a transformação das leituras das absorbâncias para concentrações em mg/ dl para cada ensaio.

(d) Ensaio

1- Preparados em tubos de ensaio conforme descrito abaixo:

	Branco	Padrão 1.0	Padrão 2.0	Padrão 4.0	Amostras
Tampão	2.0 ml	2.187 ml	2.125 ml	2.0 ml	2.0 ml
Amostras	-	-	-	-	0.25 ml
Água	0.25 ml	-	-	-	-
Padrão 4.0	-	63 µl	0.125 ml	0.25 ml	-
Ác. Pícrico	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml

2- Misturados e colocados em banho-maria a 37°C por 10 minutos.

3- Determinados as absorbâncias em 510 nm ou filtro verde (500 a 540), acertando o zero do espectofotômetro com o branco do ensaio. Essa primeira leitura de absorbância correspondeu à absorbância total e foi denominada A1.

4- Após a leitura do branco, padrões e amostras, adicionados no branco e nas amostras 100 µl do acidificante, a fim de detectar a presença de cromógenos que podem também reagir com o ácido pícrico.

5- Misturados e deixados em temperatura ambiente por 5 minutos.

6- Feita nova leitura das absorbâncias em 510 nm, acertando o zero do espectofotômetro com o branco do ensaio. Essa segunda leitura corresponde à reação do ácido pícrico com os cromógenos em pH = 5, após adição do acidificante e foi denominada A2.

(e) Cálculos

Determinação da creatinina urinária:

U = (A1- A2) x F x D onde U é o valor da creatinina urinária em mg/dL e D corresponde ao fator de diluição das amostras de urina.

4.5 CONDUTA CLÍNICA

Os dados clínicos referentes à gestante, ao parto e ao recém-nascido foram registrados em prontuário próprio. O tratamento realizado foi indicado pela uropatia específica em cada caso, não sendo definido *a priori*, já que este não foi o delineamento da pesquisa. Basicamente, dois tipos de intervenção foram realizados: profilaxia de infecção urinária e abordagem cirúrgica para os casos de obstrução significativa das vias urinárias. Foi instituída antibioticoterapia profilática, no intuito de prevenir a infecção do trato urinário, com cefalosporina de primeira geração (50 mg/dia) nos dois primeiros meses de vida e sulfametoxazol+trimetoprim (2mg/kg/dia de timetoprim) a partir do terceiro mês de vida. A profilaxia foi mantida de acordo com os seguintes parâmetros:

1) presença de obstrução significativa de vias urinárias até a correção cirúrgica;

2) presença de RVU até a resolução do mesmo;

3) presença de dilatação leve da pelve renal leve (DAP < 10 mm) até a realização da UCM.

Para os casos de uropatias obstrutivas, uma abordagem não cirúrgica foi tentada em pacientes com dilatação pélvica leve/moderada, unidades renais com função preservada (45%) e um padrão não obstrutivo ao DTPA. Pacientes com dilatação grau IV (SFU), função moderada ou gravemente acometida e curva de padrão obstrutivo ao DTPA foram tratados cirurgicamente. Ambos os grupos foram acompanhados prospectivamente. As ultra-sonografias do trato urinário e os renogramas foram realizados aproximadamente quatro a seis meses após a cirurgia ou após a avaliação inicial.

A cada retorno foram reavaliados: crescimento, evolução clínica, pressão arterial, adesão à profilaxia e presença ou não de infecção do trato urinário (urina rotina e urocultura). As medidas de uréia e creatinina séricas foram obtidas de seis em seis meses no primeiro ano, e, a seguir anualmente ou mais freqüentemente conforme a exigência clínica.

As crianças recebem alta do acompanhamento ambulatorial quando os parâmetros clínicos e laboratoriais mantiverem-se satisfatórios e a dilatação da pelve renal desapareceu. É deixada em aberto a possibilidade de retorno se qualquer problema relacionado ao trato urinário aparecesse.

4.6. ANÁLISE ESTATÍSTICA

Foi feita a análise estatística baseada na apresentação descritiva dos dados, utilizando-se medidas-síntese como a média e desvio-padrão, mediana e intervalo interquartílico, além da distribuição percentual das variáveis categóricas. As variáveis foram avaliadas quanto à distribuição paramétrica ou não paramétrica pelo teste de Kolmogornov-Smirnov Para avaliar a associação entre as medidas de interesse e as respostas em estudo, foi utilizado o teste do Qui-Quadrado para comparação de proporções.

Para as comparações de medianas de dois grupos foi utilizado o teste não-paramétrico de Mann-Whitney e, para comparação médias de vários grupos, foi utilizado o teste não-paramétrico de Kruskal Wallis. O teste de Spearman foi usado para testar correlações. A receiver-operating curve (ROC) foi utilizada para avaliar a acurácia diagnóstica das medidas dos níveis urinários de TGF-β, IL-6 e TNF-α em discriminar crianças com ou sem uropatia. Similarmente, suas acurácias diagnósticas foram usadas para discriminar crianças com e sem lesão renal. A área sob a curva foi interpretada como a probabilidade de um paciente selecionado randomicamente com uropatia apresentar níveis elevados de TGF-β1, IL-6 e TNF-α em comparação a um paciente randomicamente selecionado sem uropatia. Foram consideradas diferenças estatisticamente significativas aquelas cujo valor *p* for inferior a 0,05.

REFERÊNCIAS BIBLIOGRÁFICAS

1. Pereira AK. Uropatias fetais: avaliação dos critérios de diagnóstico morfológico e funcional. Tese de Doutorado. Depto. de Ginecologia e Obstetrícia. Belo Horizonte: UFMG, 1999:160.
2. Dudley JA, Haworth JM, McGraw ME, Frank JD, Tizard EJ. Clinical relevance and implications of antenatal hydronephrosis. Arch Dis Child Fetal Neonatal Ed 1997; 76:F31-4.
3. Jaswon MS, Dibble L, Puri S, et al. Prospective study of outcome in antenatally diagnosed renal pelvis dilatation. Arch Dis Child Fetal Neonatal Ed 1999; 80:F135-8.
4. Brogan PA, Chiyende J. Antenatally diagnosed renal pelvis dilatation. Arch Dis Child Fetal Neonatal Ed 2000; 82:F171.
5. Han BK, Babcock DS. Sonographic measurements and appearance of normal kidneys in children. AJR Am J Roentgenol 1985; 145:611-6.
6. Tripp BM, Homsy YL. Neonatal hydronephrosis--the controversy and the management. Pediatr Nephrol 1995; 9:503-9.
7. Fernbach SK, Maizels M, Conway JJ. Ultrasound grading of hydronephrosis: introduction to the system used by the Society for Fetal Urology. Pediatr Radiol 1993; 23:478-80.
8. Phelan JP, Ahn MO, Smith CV, Rutherford SE, Anderson E. Amniotic fluid index measurements during pregnancy. J Reprod Med 1987; 32:601-4.

9. Phelan JP, Smith CV, Broussard P, Small M. Amniotic fluid volume assessment with the four-quadrant technique at 36- 42 weeks' gestation. J Reprod Med 1987; 32:540-2.

10. Kleiner B, Callen PW, Filly RA. Sonographic analysis of the fetus with ureteropelvic junction obstruction. AJR Am J Roentgenol 1987; 148:359-63.

11. Gotoh H, Masuzaki H, Fukuda H, Yoshimura S, Ishimaru T. Detection and assessment of pyelectasis in the fetus: relationship to postnatal renal function. Obstet Gynecol 1998; 92:226-31.

12. Arger PH, Coleman BG, Mintz MC, et al. Routine fetal genitourinary tract screening. Radiology 1985; 156:485-9.

13. Grignon A, Filion R, Filiatrault D, et al. Urinary tract dilatation in utero: classification and clinical applications. Radiology 1986; 160:645-7.

14. Grignon A, Filiatrault D, Homsy Y, et al. Ureteropelvic junction stenosis: antenatal ultrasonographic diagnosis, postnatal investigation, and follow-up. Radiology 1986; 160:649-51.

15. Jones DA, Lupton EW, George NJ. Effect of bladder filling on upper tract urodynamics in man. Br J Urol 1990; 65:492-6.

16. Elder JS. Antenatal hydronephrosis. Fetal and neonatal management. Pediatr Clin North Am 1997; 44:1299-321.

17. de Bruyn R, Gordon I. Postnatal investigation of fetal renal disease. Prenat Diagn 2001; 21:984-91.

18. Konda R, Sakai K, Ota S, Abe Y, Hatakeyama T, Orikasa S. Ultrasound grade of hydronephrosis and severity of renal cortical damage on 99m technetium

dimercaptosuccinic acid renal scan in infants with unilateral hydronephrosis during followup and after pyeloplasty. J Urol 2002; 167:2159-63.

19. Ransley PG, Dhillon HK, Gordon I, Duffy PG, Dillon MJ, Barratt TM. The postnatal management of hydronephrosis diagnosed by prenatal ultrasound. J Urol 1990; 144:584-7; discussion 593-4.

20. Gonzalez R, Schimke CM. Ureteropelvic junction obstruction in infants and children. Pediatr Clin North Am 2001; 48:1505-18.

21. Gonzalez R, Chiou R. The diagnosis of upper urinary tract obstruction in children: comparison of diuresis renography and pressure flow studies. J Urol 1985; 133:646-9.

22. Schwartz GJ, Brion LP, Spitzer A. The use of plasma creatinine concentration for estimating glomerular filtration rate in infants, children, and adolescents. Pediatr Clin North Am 1987; 34:571-90.

23. Corteville JE, Gray DL, Crane JP. Congenital hydronephrosis: correlation of fetal ultrasonographic findings with infant outcomeAm J Obstet Gynecol. 1991 Aug;165(2):384-8.

5. RESULTADOS E DISCUSSÃO

Original Article

Urinary levels of transforming growth factor beta-1 and of inflammatory cytokines in patients with fetal hydronephrosis

Mariana A. Vasconcelos[1], Maria Candida F. Bouzada[1], Katia D. Silveira[2], Letícia R. Moura[1], Fabiana F. Santos[1], Juliana M. Oliveira[1], Flávia F. Carvalho[1], Mauro M. Teixeira[2], Ana Cristina Simões e Silva[1], Eduardo A. Oliveira[1]

[1]Pediatric Nephrourology Unit, [2]Immunopharmacology Laboratory

Department of Pediatrics, Hospital das Clínicas, Federal University of Minas Gerais

Belo Horizonte, MG, Brazil

Key words: fetal hydronephrosis - vesicoureteral reflux - urinary tract infection -

Acknowledgements. This study was partially supported by CNPq (Brazilian National Research Council) and FAPEMIG.

Conflicts of interest: none

Correspondence:

Eduardo A. Oliveira
Rua Engenheiro Amaro Lanari 389 / 501
Belo Horizonte - Minas Gerais
Postal Code: 30.310.580
E - mail: eduolive@medicina.ufmg.br

ABSTRACT

Hydronephrosis is the most common organ-specific fetal condition and still remains a diagnostic challenge. Biomarkers are potentially diagnostic tool that has recently become a focus of clinical research. Therefore, the aim of this cross-sectional study was to identify noninvasive biomarkers of clinically significant uropathies in patients with antenatal hydronephrosis. We have evaluated spot-urine levels of interleukin-6 (IL-6), transforming growth factor-β1 (TGF-β1) and tumoral necrosis factor-α (TNF-α) of 100 patients with intra-uterine hydronephrosis. Patients were divided into three groups: idiopathic renal pelvic dilatation (n=47), uropathies (n=35), and dysplastic kidneys (n=18). The most frequent uropathies were ureteropelvic junction obstruction (n=13) and vesicoureteral reflux (n=11). Dysplastic kidneys included multicystic dysplasia (n=14) and hypodysplasia (n=4). No significant differences of urinary TGF- β1, IL-6 and TNF-α levels were found in the comparison between these three groups. TGF-β1 levels have a trend to be higher in dysplastic kidney group as compared with idiopathic group (p=0.07). Of 100 patients, 29 presented a reduction in DMSA uptake at the first renal scintigraphy. Both absolute urinary concentration of TGF-β1 and TGF-β1 levels standardized to urinary creatinine presented significant elevation in patients with reduced DMSA uptake in comparison to those with normal DMSA ($p<0.05$ for both comparisons). On the other hand, urinary concentrations of IL6 and TNF-α did not differ in the comparison between these groups. In conclusion, although urinary cytokine measurements seemed not to be useful as screening test for clinically significant uropathies, increased concentrations of TGF-β1 pointed out to renal damage as indicated by reduced DMSA uptake.

Key words: biomarkers, cytokines, TGF-β1, renal damage, fetal hydronephrosis

INTRODUCTION

Abnormalities of the genitourinary tract may be suspected in as many as 1 among 100 pregnancies, depending on the ultrasonographic criteria[1-2]. Renal pelvis dilatation (RPD) is the most common organ-specific fetal condition detected at antenatal period and one of the most difficult diagnostic challenges [3-4]. An increasing number of renal anomalies are identified intrauterus in otherwise uncomplicated pregnancies. This antenatal screening leads to a clinical group of patients in whom post-natal follow-up is required. Normally, these patients remain entirely asymptomatic and the natural history of the disorder is, at best, poorly understood [5]. Moreover, the high frequency of detection of antenatal RPD leads to considerable parental anxiety and utilization of medical resources [6].

Biomarkers are potentially useful diagnostic tool that has recently become a focus of clinical research [7]. Regarding pediatric nephrourology setting, there have been various attempts to identify noninvasive diagnostic markers of congenital renal obstruction and vesicoureteral reflux (VUR) [8]. For instance, Furness et al have shown urinary tumor growth factor-β1 (TGF-β1) levels 4 fold elevated in children with upper urinary tract obstruction as compared to controls [9]. Galanakis et al.[10] have found elevated urinary Interleukin-8 (IL-8) concentration in infants with VUR and suggested a cutoff of 5 pg of IL-8/μmol of creatinine for diagnosing of this uropathy. Recently, Almodhen et al [7], have detected a cutoff of 17 pg/mmol creatinine bladder TGF-β1 levels collected in the first 3 months of life was 82% sensitive and 86% specific in predicting surgery in patients with unilateral prenatal hydronephrosis.

We have recently described the clinical course of a cohort of 192 infants with mild to severe RPD and with several uropathies [11-12]. As an attempt to identify noninvasive markers of clinically significant uropathies, in this cross-sectional study we evaluated urine

levels of interleukin-6 (IL-6), transforming growth factor-β1 (TGF-β1) and tumoral necrosis factor-α (TNF-α) in patients with antenatal hydronephrosis.

Patients and Methods

Study design. The present cross-sectional study used a coorte sample of children and adolescents with antenatal diagnosis of RPD, followed-up at the Pediatric Nephrology Unit of our institution from 1989 to 2008. The criterion for inclusion was presence of RPD equal to or greater than 5 mm in prenatal ultrasound performed after 28 weeks of gestation. Our interdisciplinary group for prenatally detected nephrouropathies was established in 1987 and has followed-up approximately 850 children according to a specific protocol that includes definition of urinary tract malformation, assessment of clinical course, laboratorial findings and urinary tract imaging alterations, institution of treatment protocols and indication of surgical approach based on clinical and imaging findings[13-14.]

Patients. The study group comprised 100 patients diagnosed with antenatal RPD who were prospectively followed at the Pediatric Nephrourology Unit (UFMG, Belo Horizonte, Brazil). RPD was measured based on the assessment of the anteroposterior diameter of the renal pelvis in prenatal ultrasound performed after 28 weeks of gestation. When this parameter was equal to or greater than 5 mm, the patient was followed-up at our outpatient clinic. Exclusion criteria were patients with cromossomopathies and with major malformations, patients with acute illness at the time of urine collection, such as urinary tract infection, and children with chronic kidney disease.

Ethical aspects. The Ethics Committee of the Federal University of Minas Gerais approved the study. Informed consent was obtained from parents of all included subjects. The research protocol did not interfere with any medical recommendations or prescriptions.

The follow-up of the patients was guaranteed even in cases of refusal to participate in the study.

Study protocol. After birth, all patients with prenatal RPD were submitted to a first postnatal ultrasound (US) scan at a median time of 14 days of life (IQ range, 9 – 21 days). Following the initial US, patients underwent clinical, laboratorial and urinary tract imaging evaluation according to a systematic protocol described in detail elsewhere [13-14]. Briefly, all infants were submitted to a voiding cystourethrogram (VCUG) within three months of life. When a postnatal US scan demonstrated RPD equal to or greater than 10 mm, renal scintigraphy (99tm-DMSA and 99tm-DTPA) was performed after the first month. Independently of the initial magnitude of RPD, renal scintigraphy was also performed in all patients with VUR or in patients with increasing RPD in subsequent US tests. The clinical approach consisted of complete physical examination, including evaluation of growth and blood pressure performed at 6-month intervals. Urine cultures were obtained during follow-up visits or during any febrile episodes. Plasma creatinine concentration was determined at baseline and yearly thereafter. Glomerular filtration rate (GFR) was estimated by the Schwartz formula [15]. Reference values and definitions of normal blood pressure were based on the Fourth Task Force Report [16]. For analysis, patients were divided into three groups: idiopathic renal pelvic dilatation (n = 47), uropathies (n = 35), and dysplastic kidneys (n = 18).

Urine sampling. A single urine sample was collected from 7.30 AM to 9.00 AM at the same day of the collection of routine exams. The urine was collected by bags in children without voiding control. After homogenization, urine samples were centrifuged at 4ºC for 20 minutes at 1300 g. Cell-free urine was aliquoted into 0.5 ml tubes and stored at -80ºC until measurements.

Cytokines measurements. Urinary levels of IL-6, TNF-α and TGF-β1 were measured by specific enzyme-linked immunoassay (ELISA) kits (R&D Systems, Minneapolis, MN), following the manufacturer's instructions, as described elsewhere [17]. Urine cytokine levels were expressed as absolute concentrations (pg/ml) as well as standardized to urine creatinine measured in the same spot urine (pg/mg cr). All samples were assayed in duplicate in a single assay to avoid interassay variation. Our intra-assay variation for the ELISA measurements was below 3%. For measurement of TGF-β1, we used a Quantikine kit (R&D Systems, Minneapolis, MN), and the samples were activated before the assay. The detection limits were 0.039 pg/ mL for IL-6, 0.106 pg/ mL for TNF-α and 6 pg/ mL for TGF- β1.

Clinical variables. The following variables were included in the analysis: gender, RPD laterality, fetal RPD, postnatal RPD, the presence of urinary tract malformation, occurrence of UTI and renal damage during follow-up. The maximum anteroposterior prenatal RPD measurement was used in the data analysis. For analysis purpouse, RPD was classified as mild (5 – 9.9 mm) and moderate/severe (≥ 10 mm). For patients with bilateral renal pelvis dilation, only the unit with the greater RPD was considered for analysis. UTI was defined as growth of at least 100,000 cfu/ml in urine obtained by bag or from a mid-stream sample, with fever (38.0oC or more) and urinary symptoms (in older children). Renal damage was identified by the presence of renal scars or by contraction of the whole renal unit detected in DMSA scan [19-20]. For analysis purpose, DMSA renal uptake was classified as normal (≥ 45%) and abnormal (<45%).

Statistical analysis. The values are expressed as medians or means and standard deviation, when appropriate. The Mann Whitney test was used to compare medians between two groups and Kruskal Wallis test for multiple medians comparisons. Spearman test was used

to test correlations. Dichotomous variables were compared by the two-sided chi-square test. Receiver-operating characteristic (ROC) curves were analyzed for the overall diagnostic accuracy TGF-β1, IL-6 and TNF-α levels in discriminating infants with versus without uropathies. Similarly, their overall diagnostic accuracy was also assessed for discriminating infants with versus without renal damage. The area under the curve (AUC) was interpreted as the probability that a randomly selected patient with uropathy had a larger TGF-β1, IL-6 and TNF-α levels than a randomly selected patient without uropathy. The level of significance was set at $p<0.05$.

RESULTS

A total of 100 patients were included in the analysis (66 boys). The main baseline clinical characteristics of both groups are summarized in Table 1. Patients were divided into three groups: idiopathic renal pelvic dilatation (n=47), uropathies (n=35), and dysplastic kidneys (n=18). The uropathies were ureteropelvic junction obstruction (n=13), vesicoureteral reflux (n=11), primary megaureter (n=6), and others (n=5). Dysplastic kidneys included multicystic dysplasia (n=14) and hypodysplasia (n=4). All patients were normotensive and had normal GFR at the time of urine collection. There were a significant higher proportion of females, bilateral alterations, severe RPD, and occurrence of UTI in infants with uropathies. DMSA uptake was also smaller in patients with urinary tract anomalies. Among the patients with uropathies, 17% were submitted to surgical treatment. No significant differences in the levels of TGF- β1, IL-6 and TNF-α were found in the comparison between patients with uropathies and those with idiopathic RPD or dysplastic kidneys (Table 1).

Table 1

Association of urinary TGF-β1, IL-6 and TNF-α levels with clinical features

The absolute urinary concentrations of TGF-β1, IL-6 and TNF-α in each studied group (idiopathic, uropathies, and dysplastic kidneys) are shown in Figure 1 (A-C). No significant differences among these groups were detected (Table 2). Considering a bivariate analysis, no differences were also found between idiopathic and uropathy groups comparison of urinary TGF-β1 (p=0.30), IL-6 (p=0.29), and TNF-α levels (p=0.39). The comparison between uropathy and dysplastic kidney groups also showed the absence of statistical differences: TGF-β1 (p=0.39), IL-6 (p=0.53), and TNF-α levels (p=0.85). However, despite not reaching statistical significance, TGF-β1 levels have a trend to be higher in dysplastic kidney group as compared with idiopathic group (p=0.07), while similar values for IL-6 (p=0.95) and TNF-α levels (p=0.45) were found in these groups

The same analysis by adopting cytokine levels standardized to urinary creatinine (pg/ mg cr) also revealed similar values for urinary TGF-β1/cr, IL-6/cr, and TNF-α/cr among the three studied groups (Table 2). In bivariate analysis, considering idiopathic and dysplastic kidney groups, no differences were detected in urinary TGF-β1/cr, (p=0.35), IL-6/cr, (p=0.60), and TNF-α/cr levels (p=0.49). No differences were also found for the comparison between idiopathic and uropathy groups (TGF-β1/cr, p=0.55; IL-6/cr, p=0.69; and TNF-α/cr, p=0.46) and between uropathy and dysplastic kidney groups (TGF-β1/cr, p=0.66; IL-6/cr, p=0.37; and TNF-α/cr, p=0.98).

In relation to the presence of urinary tract infection, no differences were detected in absolute urinary levels of TGF-β1 (p=0.65), IL-6 (p=0.57), and TNF-α (p=0.85) between patients who had urinary tract infection versus those without this complication. The same

result was obtained if the comparison used cytokine levels standardized to urine creatinine (TGF- β1/cr, p=0.88; IL-6/cr, p=0.57; and TNF-α/cr, p=0.94).

Considering the comparison between surgical treated patients and conservatively managed ones, no differences were observed for the absolute urinary cytokine levels (TGF-β1, p=0.80; IL-6, p=0.68; and TNF-α, p=0.36) as well as for the concentrations related to creatinine (TGF-β1/cr, p=0.85; IL-6/cr, p=0.78; and TNF-α/cr, p=0.94).

Table 2

Figure 1A-C

Association of urinary TGF-β1, IL-6 and TNF-α levels with RPD and renal damage

A total of 82 patients presented with RPD either in prenatal or in postnatal US scans. In prenatal US, 35 patients (42.7%) presented mild dilatation, 29 had moderate dilatation (35%) and 18 had severe dilatation (22%). Postnatal US showed mild dilatation in 44 infants (53.6%), moderate in 20 (24.5%), and severe in 18 infants (21.9%). For analysis, patients were divided into two groups (mild vs. moderate/severe dilatation). Regarding fetal RPD, absolute urinary cytokine concentrations and concentrations standardized to creatinine in each group (mild vs. moderate/severe dilatation) are shown in (Table 3). No differences were detected for all comparisons between mild and moderate/severe dilatation. Similarly, considering postnatal RPD, no significant differences were found in urinary cytokine levels (absolute and relative to creatinine) between mild and moderate/severe dilatation.

Table 3

Of 100 patients, 29 presented a reduction in DMSA uptake at the first renal scintigraphy. Both absolute urinary concentration of TGF-β1 and TGF-β1 levels standardized to urinary creatinine presented significant elevation in patients with reduced DMSA uptake in comparison to those with normal DMSA (Figure 2). On the other hand, urinary IL6 and TNF-α concentrations (absolute and relative to creatinine) did not differ in the comparison between these groups (table 4).

Table 4

Figure 2

Diagnostic performance of urinary TGF-β1, IL-6 and TNF-α levels

The overall diagnostic performance for detecting uropathies was assessed by using the absolute urinary levels of TGF-β1, IL-6 and TNF-α. The AUC was 0.59 (95% confidence intervals, CI = 0.48 - 0.70) for TGF-β1, 0.54 (95% CI, 43 – 66) for IL-6, and 0.56 (95%CI, 0.44 – 0.67) for TNF-α (Figure 3). The same analysis was performed by adopting cytokine levels standardized to urinary creatinine. Similarly, the AUC was 0.55 (95% CI = 0.43 - 0.66) for TGF-β1/cr, 0.50 (95% CI, 39 – 62) for IL-6/cr, and 0.45 (95% CI, 0.33 – 0.56) for TNF-α/cr. The overall diagnostic performance for detecting DMSA uptake reduction was also assessed by using both the absolute and relative levels of urinary TGF-β1. The AUC was 0.67 (95% CI, 0.56 - 0.79) for TGF-β1 and 0.63 (95% CI, 0.52 - 0.74) for TGF-β1/cr (Figure 4).

Figure 3

Figure 4

DISCUSSION

In a spite of the high frequency of fetal hydronephrosis[1], the proper approach of these patients remains a challenge to pediatric nephrologists and urologists [3]. Some studies have suggested a role for urinary cytokine measurements as a tool for the management of antenatal hydronephrosis [21, 25]. New biomarkers of nephro-uropathies that can improve the diagnostic capability or help determining the risk for renal damage are sorely needed [26]. In this regard, pro-inflammatory cytokines (IL-6 and TNF-α) and TGF-β1 emerge as putative biomarkers. While IL-6 and TNF-α mediate kidney inflammation, TGF-β1 takes part in the main pathways of renal fibrosis in patients with RPD [22-24]. TGF-β1 is highly involved in tubulointerstitial fibrosis by increasing matrix synthesis and collagen deposition and by promoting tubular apoptosis [21, 27, 28]. TNF-α plays a role in initiating tissue injury in obstructed kidneys. [23.] The elevation of local TNF-α levels stimulates the production of chemoattractants for monocytes, which, in turn, contributes for leukocyte infiltration in the obstructed kidney [21, 23, 24]. IL-6 induces B and T cells activation and differentiation during inflammation.[28.] Therefore, in this cross-sectional study, we hypothesized a possible role for spot-urine measurement of IL-6, TGF-β1 and TNF-α in indentifying clinically significant uropathies or renal parenchyma damage in patients with intra-uterine hydronephrosis. Our results showed that these biomarkers do not provide good diagnostic accuracy in discriminating patients with and without clinically significant uropathies. However, higher urinary levels of TGF-β1 were found in patients with renal parenchyma damage as revealed by reduced DMSA uptake.

To our best knowledge, there is very little information available concerning the role of IL-6, TGF-β1 and TNF-α in patients with fetal hydronephrosis. Regarding pediatric urological disease, most of the studies available in the literature are about ureteropelvic

junction obstruction (UPJO) [7, 9, 29-32] or vesicoureteral reflux (VUR) [10, 33-37]. For instance, a number of studies have shown higher TGF-β1 levels in infants who presented severe UPJO and needed surgical intervention. El-Sherbiny [30] et al have detected that the mean bladder urine TGF-β1 was 3-fold higher in 15 children with upper tract obstruction than in controls. Taha et al., in a study including 35 children with unilateral UPJO, have shown that the level of bladder TGF-β1 in patients before surgery was significantly higher than in healthy controls. A threshold of 190 pg of TGF-β1/ mg of creatinine gave a sensitivity of 100%, a specificity of 80%, a positive predictive value of 85.4%, negative predictive value of 100% and an overall accuracy of 90.8%. Recently, Almodhen et al.[7] in a longitudinal prospective study of 42 newborns with unilateral prenatal hydronephrosis have shown that bladder urine TGF-β1 obtained at the first 3 months of life was able to predict the need for surgery in newborns with prenatal hydronephrosis. Unfortunately, these studies did not evaluated the relationship between DMSA uptake and TGF-β1. However, one might speculate that if a criterion for pyeloplasty indication in these group of patients is a reduction in DMSA uptake, it is possible that the higher levels of TGF-β1 found in patients who underwent surgery would be related to renal parenchymal damage.

Likewise, children with VUR and renal scarring were found to have elevated levels of serum basic fibroblast growth factor (FGF), laminin, and TGF-β1 [36, 38] These mediators are probably involved in renal fibrosis pathways[36, 38]. A number of experimental and clinical studies have also revealed increased TGF-β1 expression in the cells of various renal structures in patients with reflux nephropathy [39, 40]. Solari et al. [41] have demonstrated a significant association between coding region 10 and the polymorphic variant -509 of the TGF-β1 gene in renal scarring associated with VUR. These authors concluded that genetic polymorphism involving the TGF-β1 gene may provide a genetic predisposition for the

development of renal scarring in patients with reflux [41]. More recently, Sabasiñska et al have found increased levels of TGF-β1 in patients with high-grade VUR [36]. The authors speculated a connection between this finding and the presence of renal parenchyma damage in severe VUR [36].

TGF-β1 is a peptide of low molecular mass and with pleiotropic actions. This cytokine stimulates the growth of cells of mesenchymal origin, while inhibits proliferation of epithelial and haemopoietic cells. TGF-β1 is highly involved in renal fibrosis through the accumulation of extracellular matrix via increased synthesis and decreased degradation of its components and via upregulation of integrins on cell surface, thus facilitating the deposition of matrix in the interstitial space [42, 43]. In this context, it is reasonable to believe that the elevation of the urinary concentration of this cytokine might reflect renal tissue fibrosis. In agreement to this hypothesis, we have found an association between reduced DMSA uptake and higher urinary levels of TGF-β1 that probably suggests the role of fibrogenic pathways in patients with renal parenchyma damage.

We are aware of the limitations associated with the cross-sectional design of our study. The main possible weakness was the use of a convenience sample, which makes homogeneity among the selected groups very difficult to obtain, since the type of uropathy, the previous surgical correction of some patients and the stage of renal disease at the time of collection may interfere with cytokine measurements. Nevertheless, some aspects of the study may increase the strength of our findings, such as the utilization of strictly defined inclusion and exclusion criteria and a well-established protocol for the measurements of cytokines with very low intra-assay variability [17].

In conclusion, although urinary cytokine measurements seemed not to be useful as screening test for clinically significant uropathies, increased concentrations of TGF-β1

pointed out to renal parenchyma damage as indicated by reduced DMSA uptake. Our observations may indicate a potential role of this cytokine in fibrogenesis process of congenital uropathies. Therefore, the measurement of urinary concentration of TGF-β1 could be combined with other useful diagnostic tests to confirm clinically significant upper urinary tract obstruction or reflux nephropathy in this population. The understanding of the mechanisms of TGF-β1 effects in urinary tract malformations may lead to innovative interventions for preventing parenchyma damage. Moreover, further prospective studies are needed to investigate the role of TGF-β1 in the follow up of these patients, which may be useful for evaluating success after corrective surgery or clinical management.

Acknowledgements. This study was partially supported by CNPq (Brazilian National Research Council) and FAPEMIG. LRM, FFS, JMO and FFC were recipients of CNPq fellowships. Dr. EA Oliveira, Dr. AC Simões e Silva and Dr MM Teixeira received a research grant from the Brazilian Research Council (CNPq).

Conflicts of interest: none

REFERENCES

1. Carr MC. Prenatal management of urogenital disorders. Urol Clin North Am. 2004 Aug;31(3):389-97, vii.

2. Morin L, Cendron M, Crombleholme TM, Garmel SH, Klauber GT, D'Alton ME. Minimal hydronephrosis in the fetus: clinical significance and implications for management. J Urol. 1996 Jun;155(6):2047-9.

3. Chudleigh T. Mild pyelectasis. Prenat Diagn. 2001 Nov;21(11):936-41.

4. Hothi DK, Wade AS, Gilbert R, Winyard PJ. Mild fetal renal pelvis dilatation: much ado about nothing? Clin J Am Soc Nephrol. 2009 Jan;4(1):168-77.

5. Barnacle AM, McHugh K. Dilemmas in the radiological investigation of the renal tract in children. Current Paediatrics. 2003;13:448-54.

6. Welch TR. The fetal kidney. J Pediatr. 2009 Jan;154(1):A3.

7. Almodhen F, Loutochin O, Capolicchio JP, Jednak R, El-Sherbiny M. The role of bladder urine transforming growth factor-beta1 concentrations in diagnosis and management of unilateral prenatal hydronephrosis. J Urol. 2009 Jul;182(1):292-8; discussion 8.

8. Lee RS. Biomarkers for pediatric urological disease. Curr Opin Urol. 2009 Jul;19(4):397-401.

9. Furness PD, 3rd, Maizels M, Han SW, Cohn RA, Cheng EY. Elevated bladder urine concentration of transforming growth factor-beta1 correlates with upper urinary tract obstruction in children. J Urol. 1999 Sep;162(3 Pt 2):1033-6.

10. Galanakis E, Bitsori M, Dimitriou H, Giannakopoulou C, Karkavitsas NS, Kalmanti M. Urine Interleukin-8 as a Marker of Vesicoureteral Reflux in Infants. Pediatrics. 2006 Apr 3.

11. Coelho GM, Bouzada MC, Pereira AK, Figueiredo BF, Leite MR, Oliveira DS, et al. Outcome of isolated antenatal hydronephrosis: a prospective cohort study. Pediatr Nephrol. 2007 Oct;22(10):1727-34.

12. Coelho GM, Bouzada MC, Lemos GS, Pereira AK, Lima BP, Oliveira EA. Risk factors for urinary tract infection in children with prenatal renal pelvic dilatation. J Urol. 2008 Jan;179(1):284-9.

13. Bouzada MC, Oliveira EA, Pereira AK, Leite HV, Rodrigues AM, Fagundes LA, et al. Diagnostic accuracy of fetal renal pelvis anteroposterior diameter as a predictor of uropathy: a prospective study. Ultrasound Obstet Gynecol. 2004 Dec;24(7):745-9.

14. Bouzada MC, Oliveira EA, Pereira AK, Leite HV, Rodrigues AM, Fagundes LA, et al. Diagnostic accuracy of postnatal renal pelvic diameter as a predictor of uropathy: a prospective study. Pediatr Radiol. 2004 Oct;34(10):798-804.

15. Schwartz GJ, Haycock GB, Edelmann CM, Spitzer A. A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. Pediatrics. 1976;58:259-63.

16. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. *Pediatrics.* 2004;114(suppl) :555 –576

17. Souto MFO, Teixeira AL, Russo RC, Penido MGMG, Silveira KD, Teixeira MM, Simões e Silva AC. Immune mediators in idiopathic nephrotic syndrome: Evidence for a relationship between interleukin 8 and proteinuria. Pediatr Res. 2008;64(6):637-42.

18. Fernbach SK, Maizels M, Conway JJ. Ultrasound grading of hydronephrosis: introduction to the system used by the Society for Fetal Urology. Pediatr Radiol. 1993;23(6):478-80.

19. Caione P, Ciofetta G, Collura G, Morano S, Capozza N. Renal damage in vesico-ureteric reflux. BJU Int. 2004 Mar;93(4):591-5.

20. Goldraich NP, Ramos OL, Goldraich IH. Urography versus DMSA scan in children with vesicoureteric reflux. Pediatr Nephrol. 1989;3(1):1-5.

21. Klahr S. Obstructive nephropathy. Kidney International. 1998;54:286-300.

22. Zhou, Y, Takahashi G, Shinagawa T, Okuhara T, Yonamine K, Aida, Y, Tadokoro M. Increased transforming growth factor-β1 and tubulointersticial fibrosis in rats with congenital hydronephrosis. International Journal of Urology. 2002; 9:491-500.

23. Misseri R, Meldrum DR, Daugher P, Hile K, Rink RC, Meldrum KK. Unilateral ureteral obstruction induces renal tubular cell production of necrosis factor-α independent of inflammatory cell infiltration. The Journal of Urology. 2004; 172:1595-1599.

24. Klahr S, Morrisey J. Obstructive nephropathy and renal fibrosis. Am J of Physiol Renal Physiol. 2002; 283:F861-F875.

25. Seremites GM, Maizels M. TFG-β mRNA expression in the renal pelvis after experimental and clinical ureteropelvic junction obstruction. The Journal of Urology.1996 July; 156:261-266.

26. Lee RS. Biomarkers for pediatric urological diseases. Current Opinion in Urology. 2009; 19:397-401.

27. Miyajima A, Chen J, Lawrence C, Ledbetter S, Soslow RA, Stern J, Jha S, Pigato J, Matthew LL, Poppas DP, Vaughan Jr ED, Felsen D. Antibody to transform growth factor-β amiliorates tubular apoptosis in unilateral ureteral obstruction. Kidney International. 2000. 58: 2301-2313.

28. Borish LC, Steinke JW. Cytokines and chemokines. J Allergy Clin Immunol. 2000; 111: S460-475.

29. Palmer LS, Maizels M, Kaplan WE, Firlit CF, Cheng EY. Urine levels of transform growth factor-beta 1 in children with ureteropelvic junction obstruction. Pediatric Urology. 1997; 50(5): 769-773.

30. El-Sherbiny MT, Mousa OM, Shokier AA, Ghoneim MA. Role of urinary transforming growth factor-β1 concentration in the diagnosis of upper urinary tract obstruction in children. The Journal of Urology. 2002; 168: 1798-1800.

31. Taha MA, Shokeir AA, Osman HG, El-Aziz AEAFA, Farahat SE. Pelvi-ureteric obstruction in children: the role of urinary transform growth factor-β1 and epidermal growth factor. Pediatric Urology. 2007; 99:899-903.

32. Grandaliano G, Gesualdo L, Bartoli F, Ranieri E, Monno Rafaela, Leggio A, Paradies G, Caldarulo E, Infante B, Schena FP. MCP-1 and EGF renal expression and urine excretion in human congenital obstructive nephropathy. Kidney International. 2000; 58:182-192.

33. Haraoka M, Senoh K, Ogata N, Furukawa M, Matsumoto T, Kumazawa J. Elevated interleukin-8 levels in the urine of children with renal scarring and/or vesicoureteral reflux. The Journal of Urology. 1996; 155:678-680.

34. Ninan GK, Juntley RS, Eremin O. Urinary Cytokines as markers of reflux nephropathy. The Journal of Urology. 1999 November; 162:1739-1742.

35. Wang J, Konda R, Sato H, Sakai K, Ito S, Orikasa S. Clinical significance of urinary interleukin-6 in children with reflux nephropathy. The Journal of Urology. 2001 January; 165:210-214.

36. Sabasiñska A, Zoch-Zwierz W, Wasilewska A, Porowski T. Laminin and Transform growth factor beta-1 in children with vesicoureteral reflux. Pediatric Nephrology. 2008; 23:769-774.

37. Gokce I, Alpay H, Biyikli, Unluguzel G, Dede F, Topuzoglu A. Urinary levels of interleukin-6 and interleukin-8 in patients with vesicoureteral reflux and renal parenchymal scar. Pediatric Nephrology.2010.

38. Kobayashi H, Miyakita H, Yamataka A, et al. Serum basic fibroblast growth factor as a marker of reflux nephropathy. J Pediatr Surg 2004; 39:1853–1855.

39. Maruyama T, Hayashi Y, Nakane A, Sasaki S, Kohri K. Intermittent pressure-loading increases transforming growth factor-beta-1 secretion from renal tubular epithelial cells: in vitro vesicoureteral reflux model. Urol Int. 2005; 75:150–158; 28.

40. Eddy AA. Molecular basis of renal fibrosis. Pediatr Nephrol.2000; 15:290–301.

41. Solari V, Owen D, Puri P (2005) Association of transforming growth factor-beta1 gene polymorphism with reflux nephropathy. J Urol. 2005; 174:1609–1611.

42. Border WA, Noble NA. Transforming growth factor beta in tissue fibrosis. N Engl J Med. 1994; 331:1286–1292.

43. Sales VL, Engelmayr GC Jr, Mettler BA, Johnson JA Jr, Sacks MS, Mayer JE Jr. Transforming growth factor-beta1 modulates extracellular matrix production, proliferation, and apoptosis of endothelial progenitor cells in tissue-engineering scaffolds. Circulation. 2006; 4:I193–I199.

Table 1 – Patient demographics and baseline characteristics (n = 100)

	Uropathies (%) N = 53	Idiopathic RPD (%) N = 47	P
Gender			
Male	29 (44.0)	37 (56.0)	0.01
Female	24 (70.6)	10 (29.4)	
RPD Laterality			
Unilateral	23 (38.3)	37 (61.7)	0.03
Bilateral	24 (60.0)	16 (40.0)	
Fetal RPD			
Mild	4 (11,4)	31 (88.6)	<0.001
Moderate	17 (57.1)	12 (42.9)	
Severe	14 (78.9)	4 (21.1)	
Postnatal RPD			
Absent/Mild	9 (15.0)	35 (85.0)	<0.001
Moderate	11 (55.0)	9 (45.0)	
Severe	15 (81.8)	3 (18.2)	
DMSA uptake Interquartile range	41.5% (0.0 – 47.4%)	47% (46.6 – 48.1)	0.002
Median of urinary TGF-β1 Interquartile range	118.0 0.0 – 413.5	42.0 0.0 – 280	0.11
Median of urinary IL-6 Interquartile range	0.257 0.0 – 0.842	0.0630 0.0 – 0.597	0.42
Median of urinary TNF-α Interquartile range	2.115 0.899 – 3.09	1.350 0.816 – 2.889	0.32
UTI (during follow-up)			
Absent	36 (44.4)	45 (55.6)	<0.001
Present	17 (89.5)	2 (10.5)	

RPD - renal pelvis dilation; TGF-β1 - transforming growth factor beta-1; IL-6 - interleukin 6; TNF-α - tumor necrosis factor alfa; UTI – urinary tract infection; GFR – glomerular filtration rate.

Table 2 – Median and interquartile range of absolute and standardized to creatinine cytokine levels in patients with idiopathic hydronephrosis (n=47), uropathies (n=35) and dysplastic kidneys (n=18)

	Groups	Percentiles			
		25	50	75	P
TGF-β1	Idiopathic	0.00000	42.00000	280.00000	
	Uropathies	0.00000	63.00000	321.00000	0.19
	Dysplastic kidneys	3.00000	188.00000	666.00000	
IL-6	Idiopathic	0.0000	0.0630	0.5970	
	Uropathies	0.0000	0.2570	0.6940	0.57
	Dysplastic kidneys	0.0000	0.0695	1.0610	
TNF-α	Idiopathic	0.81600	1.3500	2.8890	
	Uropathies	1.09600	2.1150	3.2280	0.60
	Dysplastic kidneys	0.64900	2.0220	3.0373	
TGF-β1/cr	Idiopathic	0.00000	62,2007	758.56333	
	Uropathies	0.00000	92.9638	722.7138	0.62
	Dysplastic kidneys	8.86020	145.4943	763.5154	
IL-6/cr	Idiopathic	0.0000	0.15000	3.00000	
	Uropathies	0.0000	0.46000	3.00000	0.69
	Dysplastic kidneys	0.0000	0.18500	0.81750	
TNF-α/cr	Idiopathic	1.1300	3.0000	10.0000	
	Uropathies	0.9880	2.0000	7.8660	0.68
	Dysplastic kidneys	0.3270	2.7200	5.5000	

TGF-β1 - transforming growth factor beta-1; IL-6 - interleukin 6; TNF-α - tumor necrosis factor alfa; TGF-β1/cr - transforming growth factor beta-1 standardized to urinary creatinine; IL-6/cr - interleukin 6 standardized to urinary creatinine; TNF-α/cr - tumor necrosis factor alfa standardized to urinary creatinine.

Table 3 – Median and interquartile range of absolute and standardized to creatinine urinary cytokine levels according to the severity of fetal renal pelvis dilation (RPD)

	Fetal RPD	Percentiles			
		25	50	75	P
TGF-β1	mild	0.00000	50.00000	311.25000	0.573
	moderate/severe	0.00000	51.50000	447.50000	
TFG-β1/cr	mild	0.0000	77.5823	706.9745	0.772
	moderate/severe	0.0000	180.0500	900.4683	
IL-6	mild	0.0000	0.2300	0.6940	0.255
	moderate/severe	0.0000	0.1205	0.7695	
IL6/Cr	mild	0.0000	0.3950	3.0000	0.508
	moderate/severe	0.0000	0.0700	2.3250	
TNF-α	mild	0.8638	1.7155	3.2430	0.561
	moderate/severe	0.3150	1.2625	2.3478	
TNF-α/cr	mild	1.3075	2.7000	7.8495	0.234
	moderate/severe	0.3000	1.6880	9.2077	

TGF-β1 - transforming growth factor beta-1; IL-6 - interleukin 6; TNF-α - tumor necrosis factor alfa; TGF-β1/cr - transforming growth factor beta-1 standardized to urinary creatinine; IL-6/cr - interleukin 6 standardized to urinary creatinine; TNF-α/cr - tumor necrosis factor alfa standardized to urinary creatinine.

Table 4 – Median and interquartile range of absolute and standardized to creatinine urinary cytokine levels according DMSA renal uptake

		Percentiles			
	DMSA	25	50	75	P
TGF-β1	abnormal	22.00000	233.00000	836.00000	0.005
	normal	0.00000	40.00000	245.00000	
IL-6	abnormal	0.0000	0.1990	1.1180	0.68
	normal	0.0000	0.1670	0.6400	
TNF-α	abnormal	1.2530	2.1190	2.9605	0.26
	normal	0.8080	1.5720	3.2280	
TGF-β1/cr	abnormal	36.2680	300.0000	921.4671	0.036
	normal	0.0000	34.8119	659.7564	
IL-6/Cr	abnormal	0.0000	0.3334	1.6900	0.99
	normal	0.0000	0.2000	3.0000	
TNF-α/cr	abnormal	1.4646	2.3300	6.3500	0.93
	normal	0.9300	2.3800	7.8660	

TGF-β1 - transforming growth factor beta-1; IL-6 - interleukin 6; TNF-α - tumor necrosis factor alfa; TGF-β1/cr - transforming growth factor beta-1 standardized to urinary creatinine; IL-6/cr - interleukin 6 standardized to urinary creatinine; TNF-α/cr - tumor necrosis factor alfa standardized to urinary creatinine.

Figure legends

Figure 1 - Absolute levels of urinary cytokines in patients with idiopathic renal pelvis dilation, uropathies and dysplasic kidneys. Panel A – Urinary levels of transforming growth factor beta-1 (TGF-β1). Panel B – Urinary levels of interleukin 6 (IL-6). Panel C – Urinary levels of tumor necrosis factor alfa (TNF-α). $P > 0.05$ for all comparisons.

Figure 2 - Absolute levels of urinary transforming growth factor beta-1 (TGF-β1) and TGF-β1 levels standardized to urinary creatinine in patients with reduced DMSA uptake and with normal DMSA.

Figure 3 – Receiver operating curve (ROC) for detecting uropathies obtained with the measurement of absolute levels of urinary interleukin 6 (IL-6), tumor necrosis factor alfa (TNF-α), and transforming growth factor beta-1 (TGF-β1)

Figure 4 - Receiver operating curve for detecting DMSA uptake reduction obtained with the measurement of absolute levels of urinary transforming growth factor beta-1 (TGF-β1) and of TGF-β1 levels standardized to urinary creatinine.

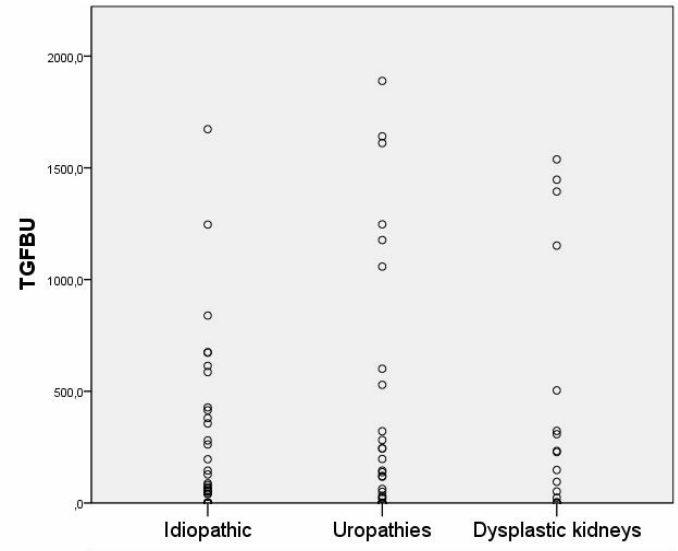
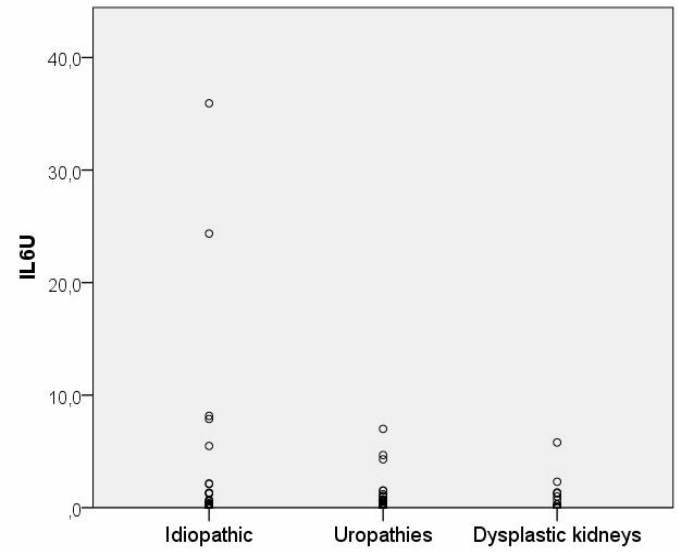
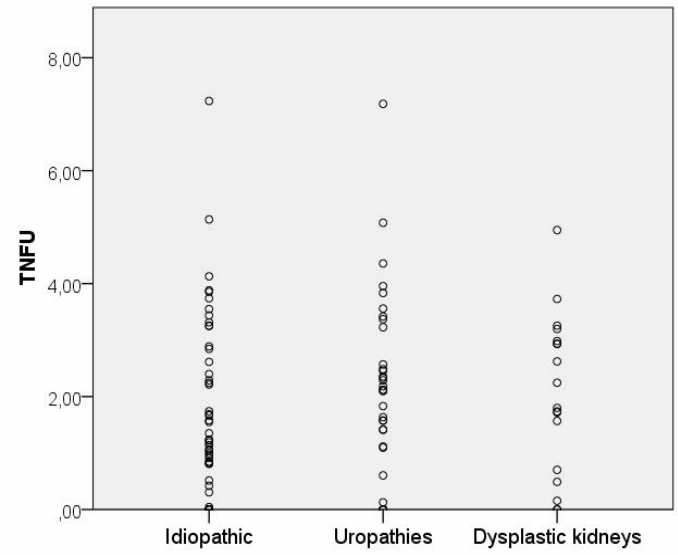

TGFBU
2000,0
1500,0
1000,0
500,0
,0
Idiopathic
Uropathies
Dysplastic kidneys

Figure 1

A

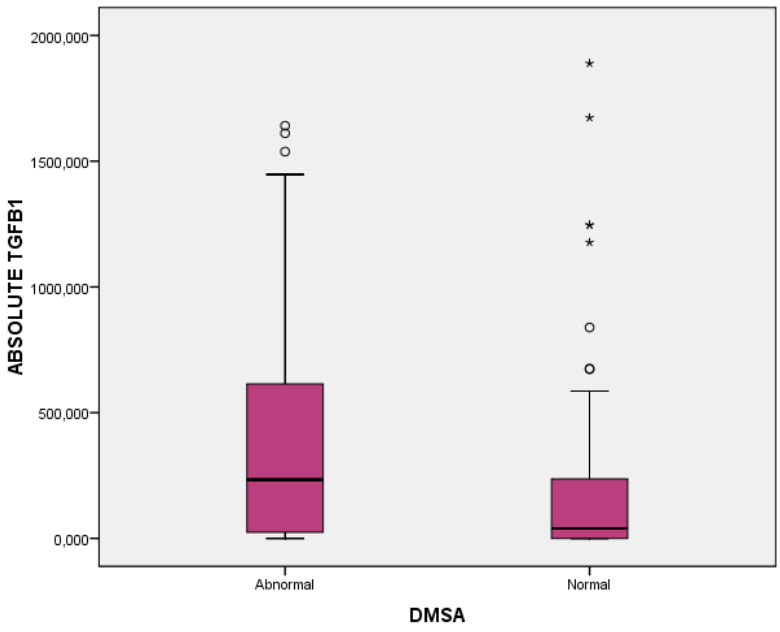

IL6U
40,0
30,0
20,0
10,0
,0
Idiopathic
Uropathies
Dysplastic kidneys

B


TNFU
8,00
6,00
4,00
2,00
,00
Idiopathic
Uropathies
Dysplastic kidneys

C

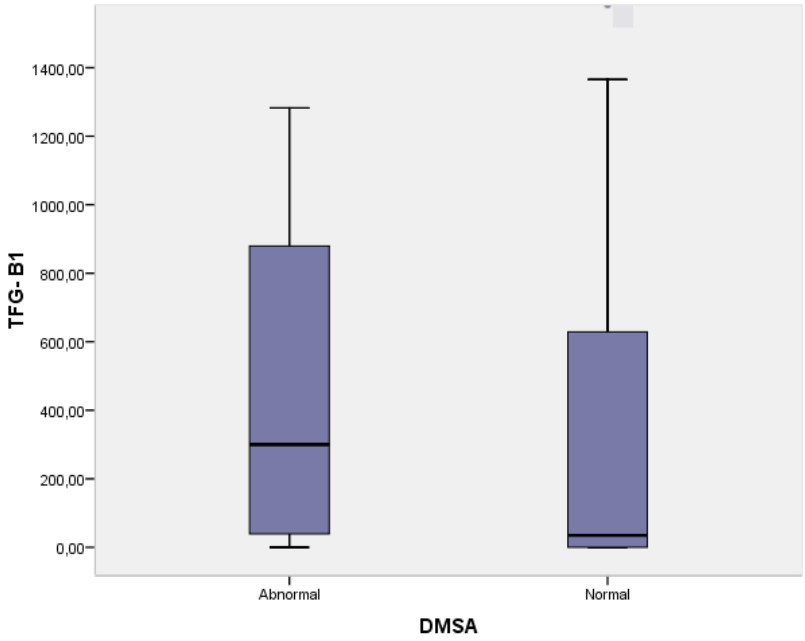
Figure 2



A

Absolute levels of urinary TGF-β1

* p < 0,05 for the comparison of absolute levels in patients with normal versus abnormal DMSA uptake



B

Urinary TGF-β1 standardized to urinary creatinine

*P<0.05 for the comparison of relative urinary TGF-β1 levels in patients with normal versus abnormal DMSA uptake

Figure 3

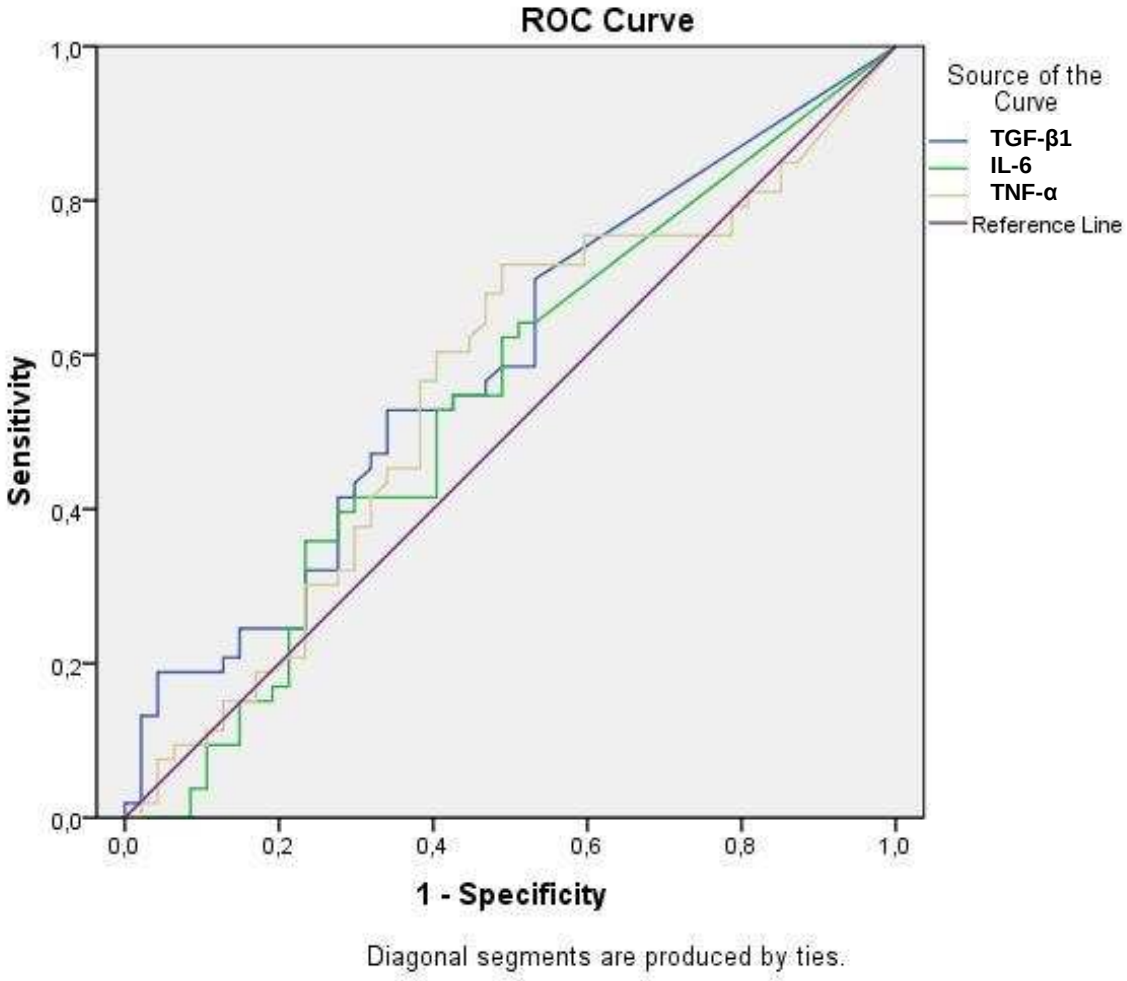
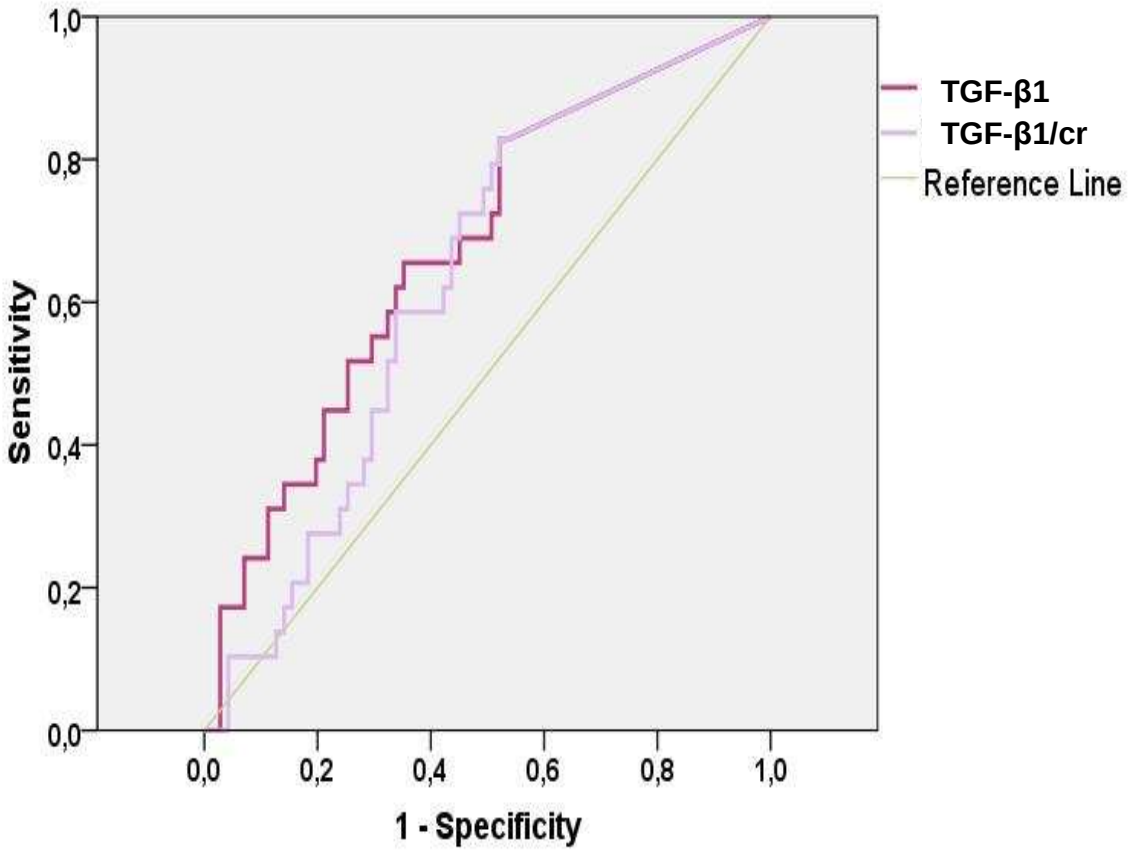

ROC Curve
Sensitivity
1 - Specificity
0,0
0,2
0,4
0,6
0,8
1,0
Source of the Curve
TGF-β1
IL-6
TNF-α
Reference Line
Diagonal segments are produced by ties.

Figure 4


1,0
0,8
0,6
0,4
0,2
0,0
Sensitivity
0,0
0,2
0,4
0,6
0,8
1,0
1 - Specificity
TGF-β1
TGF-β1/cr
Reference Line

CONCLUSÕES

O presente estudo transversal permitiu concluir que:

- Os níveis urinários de IL-6, TNF-α, TGF-β não foram biomarcadores com boa acurácia diagnóstica em diferenciar uropatias clinicamente significativas das não significativas.
- Os níveis urinários de TGF-β1 estão significativamente elevados em pacientes com diminuição da captação ao DMSA, sendo p =0,005 para os níveis absolutos e p=0,036 para a relação com a creatinina urinária.
- O desempenho diagnóstico global dos níveis urinários de IL-6, TNF-α, TGF-β1 foi respectivamente de 0.59 (95%IC = 0.48 - 0.70), 0.54 (95% CI, 43 – 66) e 0.56 (95%CI, 0.44 – 0.67).
- O desempenho diagnóstico para identificar diminuição da captação ao DMSA níveis urinários do TGF-β1 foi de 0.67 (95% CI, 0.56 - 0.79) e 0.63 (95% CI, 0.52 - 0.74) para TGF-β1/cr.

7. ANEXO

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Estamos desenvolvendo a pesquisa intitulada "ESTUDO PROSPECTIVO DAS UROPATIAS DIAGNOSTICADAS POR ULTRA-SONOGRAFIA FETAL: O PAPEL DAS CITOCINAS" no Hospital das Clinicas da UFMG. Estudaremos substâncias do sangue e da urina chamada pelos médicos de citocinas que podem participar da piora do funcionamento do rim que pode ocorrer em crianças com doenças dos rins e das vias urinárias. Já se sabe que essas substâncias participam de uma série de doenças dos rins e do coração e também devem participar do problema renal que surge nos pacientes com anomalias dos rins.

Além disso, já existem alguns remédios capazes de mudar a quantidade de citocinas que aparecem na urina e no sangue, como, por exemplo, o captopril, o enalapril, o losartan, o valsartan e alguns outros remédios usados normalmente para tratamento de pressão alta. Alguns estudos acreditam que esses remédios podem melhorar alguns pacientes com doença renal porque diminuem essas substâncias que queremos estudar.

O seu filho ou filha esta sendo convidado a participar deste estudo por ter sido identificado ainda durante a gestação que o mesmo apresentava alguma malformação urinária.

Este estudo quer, então, saber a quantidade dessas substâncias que está no sangue e na urina das crianças com malformações dos rins e das vias urinárias, tentando ver se isso tem relação com a forma da doença (se é mais branda ou mais forte), a resposta ao tratamento e a piora do funcionamento dos rins.

A dosagem das citocinas na urina será feita coletando urina por amostra única. Estamos garantindo que a realização destes exames só será feita se você concordar. Você pode se recusar a autorizar a participação de seu filho (a) no estudo ou mesmo retirar o seu consentimento a qualquer momento seu que isto altere o tratamento de seu filho (a) no Hospital das Clínicas.

Garantimos ainda que a identidade de seu filho (a) não será revelada em nenhum momento do estudo ou durante a publicação dos resultados em revistas científicas. Os

resultados desse estudo somente serão utilizados para aumentar os conhecimentos da medicina no estudo destas doenças. Os resultados estarão guardados com o pesquisador e lhe serão entregues se você desejar.

Eu, _____, mãe, (ou pai ou responsável) pelo paciente_____entendi tudo que foi explicado sobre a pesquisa e concordo que meu filho (ou minha filha ou outro grau de parentesco) participe do estudo sobre a quantidade dos citocinas no sangue e na urina. Este estudo será feito pelos Drs Mariana Affonso Vasconcelos, Sérgio Veloso Pinheiro, Ana Cristina Simões e Silva, Eduardo Araújo Oliveira, e Maria Cândida Ferrarez Bouzada Viana do Hospital das Clínicas da UFMG. Dou meu consentimento para que seja coletado sangue e urina de meu filho (minha filha ou outro grau de parentesco) para medir as quantidades de citocinas. Confirmo que meu filho (minha filha ou outro grau de parentesco) foi selecionado de forma voluntária para participar dessa pesquisa. Eu assinei e recebi uma cópia dessa autorização.

Data e local:_____

Assinatura do paciente:_____

Assinatura do responsável:_____

Grau de parentesco do responsável:_____

Assinatura do pesquisador:_____

TELEFONE DOS PESQUISADORES: (31)32489445
TELEFONE DO COMITÊ DE ÉTICA: (31)3499-4592
End. COEP: Av Antônio Carlos, 6627
Unidade Administrativa II, 2^0 andar
Campus Pampulha
Belo Horizonte MG – Brasil
31270-901