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The interstitial expression of alpha-smooth muscle actin in glomerulonephritis is associated with renal function

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Background: In a healthy kidney, contractile protein alpha-smooth muscle actin (ASMA) is immunohistochemically strongly expressed only in the blood vessels, while in pathological conditions it can be visualized in glomerular mesangial cells and interstitial myofibroblasts. The aim of this study was to explore the possible correlation between expression of ASMA in glomerulonephritis (GN) and indicators of renal function.

Material/Methods: We analyzed expression of ASMA in percutaneous renal biopsy of 142 adult and pediatric patients with GN and its correlation with blood pressure, serum creatinine, creatinine clearance and 24-hour urine protein at the time of biopsy. Immunoexpression of ASMA was analyzed quantitatively using computer-assisted morphometric analysis. Relative surface of ASMA expression in all glomeruli and interstitium was calculated for each patient.

Results: In adults and children, greater expression of ASMA in interstitium was associated with higher serum creatinine and reduced creatinine clearance. Conversely, greater ASMA expression in glomeruli was associated with normal or decreased serum creatinine in adults and increased creatinine clearance in children. In children, correlation was found between high blood pressure and ASMA expression in interstitium.

Conclusions: We confirmed that interstitial expression of ASMA is associated with reduced renal function at time of biopsy. The connection of ASMA expression in glomeruli with lower serum creatinine and normal or increased creatinine clearance suggests a favorable role of this phenotypic change in glomerular filtration rate; further investigation is needed.

key words: **alpha-smooth muscle actin • glomerulonephritis • renal function**

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Background

In the normal kidney, immunohistochemical expression of contractile protein alpha-smooth muscle actin (ASMA) is limited to the vascular smooth muscle cells. In pathological conditions, the expression of ASMA is found in the glomerular mesangial cells and the interstitial myofibroblasts [1]. The first experimental publication on this subject, by Johnson et al, involved a series of clinical studies on the role of ASMA in diffuse renal diseases [2]. In 1992 Alpers and colleagues found higher expression of ASMA in proliferative types of glomerulonephritis GN [3]. In IgA nephropathy, ASMA expression in interstitium is related to damaged renal function and progression of disease [4,5]. The expression of ASMA in interstitial myofibroblasts helps to differentiate between minimal change disease and the early phase of idiopathic membranous GN [6]. In patients with IgA nephropathy, higher mesangial expression of ASMA predicts a progressive decline in renal function [7]. In focal segmental glomerulosclerosis, increased expression of ASMA in interstitium was associated with greater proteinuria [8]. Expression of ASMA is a useful predictive marker in lupus nephritis [9]. Today, interest in the ASMA is increasing due to the fact that the ASMA in cytoplasm of the podocytes is associated with nephrin and thus is indirectly involved in the glomerular filtration barrier [10]. ASMA is analyzed in various types of GN together with markers such as metalloproteinase and nestin in investigation of tissue remodulation and epithelial-mesenchymal transition [11,12].

To our best knowledge, this is the first morphometrical study of the correlation of ASMA expression in GN and renal function at the time of biopsy.

Material and Methods

Patients

This retrospective study analyzed 142 patients – 82 adults and 60 children – who underwent percutaneous renal biopsy in the Department of Internal Medicine and Department of Pediatrics, University Hospital, Split, Croatia in the period from 1994 to 2007. Age range was 1–18 years for children and 19–73 years for adults. Patients' data were collected from the hospital records. Due to the small numbers of each type of GN in the sample, pathological diagnoses were classified into 2 categories – proliferative glomerulonephritis (PG) in 98 cases and non-proliferative glomerulonephritis (NPG) in 44 cases. In the PG category were: 29 IgA nephropathy, 24 mesangioproliferative GN, 11 Henoch-Schöenlein purpura, 9 focal segmental GN, 6 rapidly progressive GN, 6 endoproliferative GN and 6 lupus nephritis, 4 membranoproliferative GN and 1 hemolytic uremic syndrome, 1 Alport syndrome and 1 Churg-Strauss syndrome. In the NPG category were: 17 membranous GN, 15 focal segmental glomerulosclerosis, 5 minimal change disease, 2 fibrillar GN, 1 thin basement membrane disease, C1q nephropathy, IgM nephropathy, amyloidosis and hereditary nephropathy.

Laboratory parameters

Absolute values of blood pressure (BP), serum creatinine (SC), creatinine clearance (CCr) and 24-hour urine protein, measured within 7 days before the biopsy, were collected from the hospital records. For adults, absolute values were categorized according to the Common Terminology Criteria for Adverse Events (CTCAE) [13]. For children, BP was determined by body size and age using Center for Disease Control and Prevention growth charts and data from the National Health and Nutrition Examination Survey [14,15]. Serum creatinine was categorized according to the Pediatric reference ranges and divided into 3 categories: normal, high and low [16]. CCr was determined from SC, the patient's height and proportionality constant using the Schwartz method [17]. Pediatric CCr was standardized using correction for body surface area and CTCAE terminology criteria. The 24-hour urine protein was corrected for body surface area and categorized according to 95% confidence limits [18].

Immunohistochemistry

ASMA expression was analyzed by indirect immunohistochemistry (EnVision/HRP system (Dako, Denmark) using mouse monoclonal anti-alpha smooth muscle antibody (ASMA/HRP DAKO, Denmark). Paraffin-embedded tissue sections of renal biopsies were deparaffinized in xylol and rehydrated in alcohol gradient. Endogenous peroxidase was inhibited using 3% H_2O_2 solution in methanol for 10 min. Tissue sections were incubated with primary ASMA antibody (dilution 1:50) for 60 min and peroxidase-labeled secondary antibody for 20 min, followed by 10 min incubation with diaminobenzidine substrate-chromogen solution (DAKO, Denmark). Hematoxylin counter-staining was done; slides were dehydrated in alcohol gradient, cleared in xylol and mounted with Canada balsam. Internal positive control was ASMA expression in the tunica media of renal arteries. Negative control was section of renal tissue without application of primary antibody. Positive control samples were normal renal tissues of 5 adult patients who underwent nephrectomy for renal cancer.

Morphometric analysis

Computer-assisted morphometric image analysis was used to measure glomerular and interstitial ASMA expression using IBM computer and digital camera (Olympus 4.1 Zoom) connected with Olympus BX41 microscope (Olympus, Japan). A computer mouse was used to trace the perimeter of the area of interest on a computer monitor in successive sections, using "Analysis" software (Analysis Soft Imaging System, USA) [19]. Each case was analyzed morphometrically as follows. All foci of ASMA expression in the interstitium (including atrophic tubules), as well as the perimeter of histological slide, were measured in μm^2 at 100x magnification; their ratio was calculated as a percentage of ASMA expression in interstitium. Area of ASMA expression in each glomerulus was measured in μm^2 at 400× magnification and added together for all glomeruli (Figure 1A). A cross-section area of all glomeruli was measured at 100× magnification. Their ratio was calculated as a percentage of ASMA expression in glomeruli (Figure 1B). In 5 case controls, renal cortex in the highest distance from the tumor was randomly chosen. The area measuring 0.14 mm^2 was defined, and glomeruli and interstitium were analyzed in the same manner. In the control kidneys, percentage of ASMA expression in interstitium and glomeruli was 5.6±2.5 and 10.8±3.3, respectively.

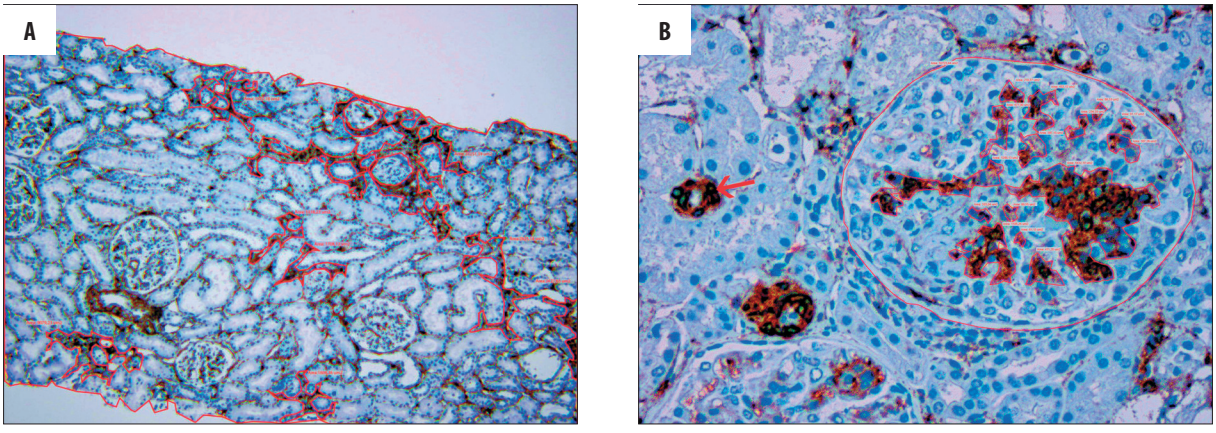

Figure 1. (**A**) Morphometric analysis of ASMA expression in interstitium and (**B**) glomeruli; arteriolar tunica media as positive control (arrow) (ASMA/HRP 100× and 400×).

Table 1. Expression of ASMA in proliferative and non-proliferative glomerulonephritis.

Patients	Category	Analyzed area	ASMA expression% (M±SD)	p
Children	NPG	Glomeruli	12.1±6.8	0.284
	PG		16.2±11.2	
	NPG	Interstitium	4.4±3.6	0.447
	PG		4.2±5.5	
Adults	NPG	Glomeruli	13.4±7.8	0.510
	PG		12.4±8.9	
	NPG	Interstitium	7.1±8.2	0.093
	PG		14.5±21.6	

NPG – non proliferative glomerulonephritis; PG – proliferative glomerulonephritis; ASMA – alpha-smooth muscle actin.

Statistical analysis

Separate statistical analyses were done for children and adult patients. Nonparametric Spearman's correlation, Mann-Whitney test, and analysis of variance (ANOVA, Kruskal-Wallis) were made using GraphPad Prism statistical software (GraphPad software, Inc. San Diego, CA, USA). Data were expressed as mean ± standard deviation (SD). Statistical significance was set at $p<0.05$.

Results

Renal ASMA expression

The expression of ASMA in glomeruli and interstitium was not significantly different between PG and NPG categories of GN in both children and adults (Table 1).

Blood pressure

The children with high blood pressure had greater expression of ASMA in interstitium compared to children with low blood pressure (10.7±9.4% *vs.* 3.7±4.3%, p=0.014) (Figure 2). The difference in expression of ASMA in glomeruli was not statistically significant between children with high and low blood pressure (7.9±4.4% *vs.* 15.4±10.6%, p=0.051). In adults, expression of ASMA in interstitium and in glomeruli were not significantly different between patients with high and normal blood pressure (10.7±14.9% *vs.* 9.9±12.6%, p=0.692) and (13.6±9.5% *vs.* 12.9±7.8%, p=1.00), respectively.

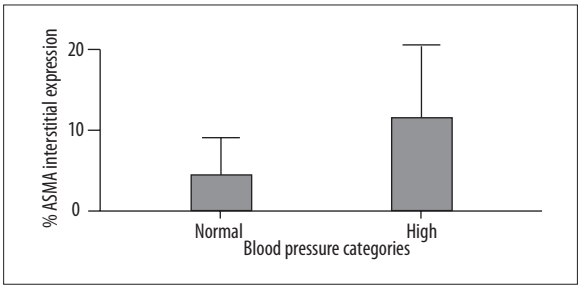

Figure 2. The expression of ASMA in interstitium of children with normal (N) and high (H) blood pressure.

Serum creatinine

In children, positive correlation was found between ASMA expression in interstitium and absolute value of SC (r=0.45, p=0.002) (Figure 3A). In categorized SC, grade III had higher expression of ASMA (p=0.0343). The negative correlation

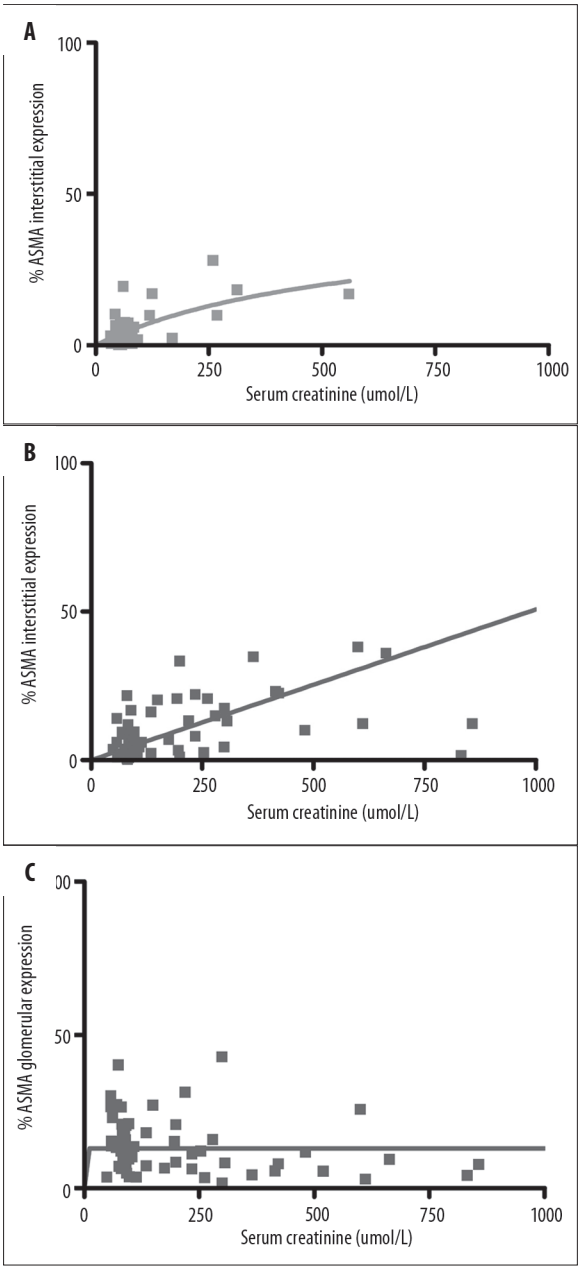

Figure 3. Correlation of serum creatinine and ASMA expression in interstitium of children (**A**) and adults (**B**), as well as in glomeruli of adults (**C**) with glomerulonephritis.

of SC and expression of ASMA in glomeruli was not significant (r=–0.154, p=0.291).

In adults, expression of ASMA in interstitium was correlated to SC (r=0.528, p<0.001) (Figure 3B). The significance was confirmed in categorized SC, where grade III had higher expression of ASMA in interstitium (p=0.0009). Significant negative correlation was found between expression of ASMA in glomeruli and SC (r=–0.395, p=0.002) (Figure 3C).

Creatinine clearance

In children, absolute values of CCr negatively correlated to expression of ASMA in interstitium (r=–0.375, p=0.009)

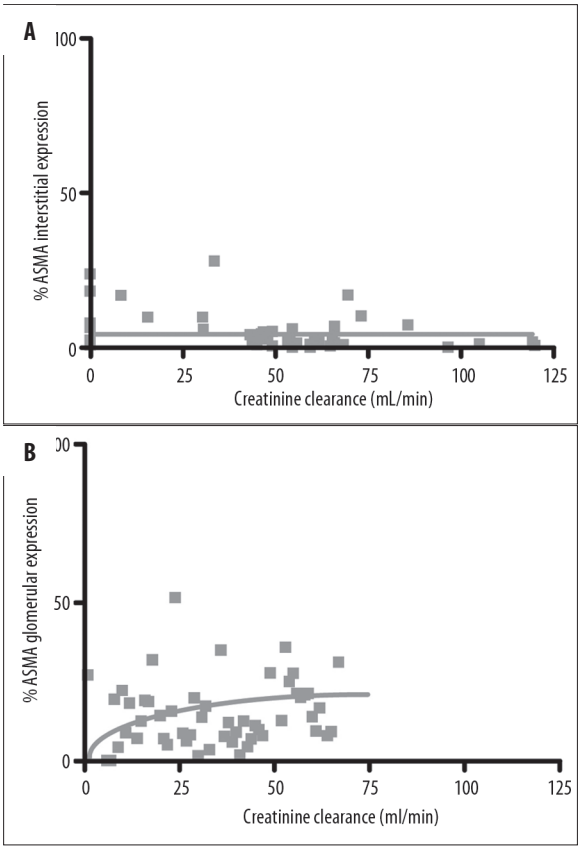

Figure 4. Correlation of absolute values of creatinine clearance and ASMA expression in interstitium (**A**) and glomeruli (**B**) of children with glomerulonephritis.

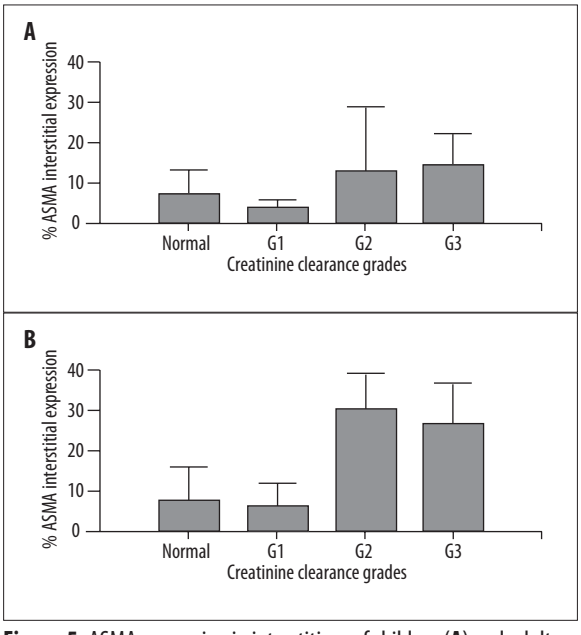

Figure 5. ASMA expression in interstitium of children (**A**) and adults (**B**) with different grades of creatinine clearance.

and positively to expression of ASMA in glomeruli (r=1.00, p<0.001) (Figure 4A,B). In adults, there were no significant correlations between ASMA expression in glomeruli

and CCr (r=0.058, p=0,643). Children and adults with CCr grade II and III had higher expression of ASMA in interstitium (p=0.0152 and p=0.0007, respectively) (Figures 5A,B).

24-hour urine protein

No significant association between renal expression of ASMA and 24-hour urine protein was found, regardless of the patient's age.

Discussion

Studies of ASMA expression in renal parenchymal diseases began more than 2 decades ago, when it was noticed that a) damaged glomerular mesangial cells change their immunophenotype expressing ASMA and b) ASMA-positive myofibroblasts start interstitial fibrosis [2].

A number of papers were published about ASMA expression in different types of human GN, its connection to proliferation markers and prognostic impact. Most authors agree that ASMA expression in glomeruli was higher in proliferative GN and increases as the disease worsens, which makes ASMA expression a potential clinical prognostic factor [3,20,21]. Some authors determined the relationship between IgA nephropathy, lupus nephritis and other types of GN with expression of ASMA [9,20,22,23]. Kim in 2001 found correlation between proliferation marker Ki-67 and ASMA in different types of GN [24].

In this study we focused on the connection of ASMA expression with impaired renal function in glomerulonephritis, measured at the time of biopsy. We did not find correlation with a special type of GN nor proliferation, possibly because of heterogeneity of the sample and the arbitrary method of categorization into proliferative and non-proliferative GN.

Increased expression of ASMA in the interstitium can be found in different diseases such as proliferative GN, diabetic nephropathy and renal transplant rejection. The ASMA has prognostic value due to the association with interstitial fibrosis, urine protein and SC [8,25–28]. ASMA-positive myofibroblasts are responsible for the increased amount of extracellular matrix and renal fibrosis [29]. This study also found higher expression of ASMA in interstitium of all patients with higher SC. Greater expression of interstitial ASMA in children and adults was associated with higher grades of CCr and lower absolute values of CCr. We confirm that elevated SC or lower values of CCr are associated with higher expression of ASMA in interstitium. Several studies have attempted to predict the development of progressive renal failure, measuring histomorphometric changes in the tubulointerstitial compartment. The best correlating parameters of interstitial fibrosis with renal function are the ratio of the accumulation of TGF-beta-1 and its antagonist decorin, interstitial expression of ASMA, and accumulation of interstitial collagen [30]. According to Jiang et al., who analyzed ASMA production in peritoneal fibroblasts stimulated by TNF-beta-1, hepatocyte growth factor could be important in blocking postoperative peritoneal adhesion [31].

Rastaldi et al. analyzed 133 biopsies of various human renal diseases, and found tubular epithelial cells with mesenchymal phenotype (vimentin and ASMA positive) whose numbers have been associated with the level of SC and degree of interstitial damage [32].

Utsonomiya et al. analyzed 27 patients with IgA nephropathy who had normal CCr at the time of biopsy, and found that expression of ASMA in mesangium predicts a progressive decline in renal function [7]. In our study, a negative connection of ASMA expression in glomeruli and SC at the time of biopsy was found, statistically significant in adults and non-significant in children, but with the same trend. It is well known that ASMA expression in the mesangial cell indicates its change to myofibroblastic immunophenotype. Our presumption is that ASMA-expressing mesangial cells are capable of higher contraction activity; this leads to glomerular hyperfiltration as an early adaptive mechanism to glomerular damage. We did not find direct confirmation of this data in published human studies. In a recent experimental study of membranous glomerulonephritis in rats, osteopontin and ASMA are expressed together in myofibroblasts in the crescents of damaged glomeruli [33]. In children, the absolute values of CCr are positively associated with ASMA expression in glomeruli. A possible explanation that children have a better CCr is due to phenotypic modulation of human mesangial cells to more contractile cells, which makes the glomerular function transitory normal or even increased.

We analyzed association of expression of ASMA and hypertension, because it is known that glomerular mesangial cells during hypertensive damage express myofibroblast phenotype and expression of ASMA increases [4]. The only significant association that we found was between expression of ASMA in interstitium and increased blood pressure in children.

We did not find significant correlation between expression of ASMA in glomeruli nor interstitium with 24-hour urine protein in our patients. This result confirms previous findings that urine protein is primary linked to the changes in permeability of glomerular filtration barrier [34]. Nevertheless, according to some studies ASMA expression in interstitium is linked to the degree of proteinuria and SC, and higher interstitial ASMA expression is an indicator of poor prognosis [25].

Conclusions

ASMA expression in interstitium is associated with SC and CCr and consecutively with decrease of renal function. ASMA expression in glomeruli is associated with lower values of SC in adults, as well as with normal or higher CCr in children. This correlation suggests that myofibroblastic phenotypic modulation of glomerular cells has a favorable impact on filtration. When calculated with precise computer-assisted quantitative morphometric technology, renal expression of ASMA may contribute to understanding of pathophysiological mechanisms of GN. The disadvantage of this study is its small sample size with different types of GN; the next survey should include a much narrower cohort of patients with a specific type of GN, as well as better phenotypic identification of ASMA-positive glomerular cells.

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REFERENCES:

1. Alpers CE: Potential future directions for renal biopsy. In: Silva FG, D'Agati VD, Nadasdy T, editors. Renal biopsy interpretation. New York: Churchill Livingstone; 1997; 403–21
2. Johnson RJ, Iida H, Alpers CE et al: Expression of smooth muscle cell phenotype by rat mesangial cells in immune complex nephritis. Alpha-smooth muscle actin is a marker of mesangial cell proliferation. J Clin Invest, 1991; 87: 847–58
3. Alpers CE, Hudkins KL, Gown AM, Johnson RJ: Enhanced expression of "muscle-specific" actin in glomerulonephritis. Kidney Int, 1992; 41: 1134–42
4. Kimura K, Suzuki N, Ohba S et al: Hypertensive glomerular damage as revealed by the expression of alpha-smooth muscle actin and non-muscle myosin. Kidney Int Suppl, 1996; 55: S169–72
5. Goumenos DS, Brown CB, Shortland J, el Nahas AM: Myofibroblasts, predictors of progression of mesangial IgA nephropathy? Nephrol Dial Transplant, 1994; 9: 1418–25
6. Danilewicz M, Wagrowska-Danilewicz M, Antoszczyk L: A quantitative study of the interstitial expression of alpha-smooth muscle actin (alpha-SMA) in idiopathic membranous glomerulonephritis and minimal change disease in adults. Pol J Pathol, 2000; 51: 37–43
7. Utsunomiya Y, Kawamura T, Abe A et al: Significance of mesangial expression of alpha-smooth muscle actin in the progression of IgA nephropathy. Am J Kidney Dis, 1999; 34: 902–10
8. Geleilete TJ, Costa RS, Dantas M, Coimbra TM: Alpha-smooth muscle actin and proliferating cell nuclear antigen expression in focal segmental glomerulosclerosis: functional and structural parameters of renal disease progression. Braz J Med Biol Res, 2001; 34: 985–91
9. Makni K, Jarraya F, Khabir A et al: Renal alpha-smooth muscle actin: a new prognostic factor for lupus nephritis. Nephrology (Carlton), 2009; 14: 499–505
10. Barisoni L, Mundel P: Podocyte biology and the emerging understanding of podocyte diseases. Am J Nephrol, 2003; 23: 353–60
11. Daniel C, Albrecht H, Ludke A, Hugo C: Nestin expression in repopulating mesangial cells promotes their proliferation. Lab Invest, 2008; 88: 387–97
12. Danilewicz M, Wagrowska-Danilewicz M: Differential glomerular immunoexpression of matrix metalloproteinases MMP-2 and MMP-9 in idiopathic IgA nephropathy and Schoenlein-Henoch nephritis. Folia Histochem Cytobiol, 2010; 48: 63–67
13. CTCAE. Common Terminology Criteria for Adverse Events. Available at: *http://ctep.cancer.gov/forms/CTCAEv3/pdf*
14. CDC. Growth Charts. Center for Disease Control and Prevention. Available at: *http://www.cdc.gov/growthcharts/*. 2000
15. Taylor EN, Forman JP, Farwell WR: Serum anion gap and blood pressure in the national health and nutrition examination survey. Hypertension, 2007; 50: 320–24
16. Soldin SJ, Brugnara C, Wong EC: Pediatric reference ranges. 4th edition. Washington, SAD: AACC Press; 2003
17. Schwartz GJ, Furth SL: Glomerular filtration rate measurement and estimation in chronic kidney disease. Pediatr Nephrol, 2007; 22: 1839–48
18. Loghman-Adham M: Evaluating proteinuria in children. American Academy of Physicians. Available at: *www.aafp.org*. 1998
19. Soft Imaging System GmbH. User's guide analysis. Munster. 2001; 64
20. Kawasaki Y, Suzuki J, Sakai N et al: Predicting the prognosis of renal dysfunction by renal expression of alpha-smooth muscle actin in children with MPGN type 1. Am J Kidney Dis, 2003; 42: 1131–38
21. Tamimi NA, Stevens PE, O'Donnell PL et al: Expression of cytoskeletal proteins differentiates between progressors and non-progressors in treated idiopathic membranous nephropathy. Exp Nephrol, 1998; 6: 217–25
22. Pastorello MT, Costa RS, Ravinal RC et al: Alpha-SM actin expression as prognostic indicator in IgA nephropathy (Berger's disease). Dis Markers, 2005; 21: 21–27
23. Shoji T, Nakanishi I, Suzuki A et al: Early treatment with corticosteroids ameliorates proteinuria, proliferative lesions, and mesangial phenotypic modulation in adult diffuse proliferative IgA nephropathy. Am J Kidney Dis, 2000; 35: 194–201
24. Kim O: Immunohistochemical study of the expression of alpha-smooth muscle actin and the proliferation marker Ki-67 of glomerulonephritis. J Korean Med Sci, 2001; 16: 455–61
25. Chebotareva NV, Bobkova IN, Varshavskii VA et al: [The role of smooth muscle alpha-actin in development of renal fibrosis in patients with chronic glomerulonephritis]. Ter Arkh, 2006; 78: 17–21
26. Ranieri E, Gesualdo L, Grandaliano G et al: The role of alpha-smooth muscle actin and platelet-derived growth factor-beta receptor in the progression of renal damage in human IgA nephropathy. J Nephrol, 2001; 14: 253–62
27. Yonemoto S, Machiguchi T, Nomura K et al: Correlations of tissue macrophages and cytoskeletal protein expression with renal fibrosis in patients with diabetes mellitus. Clin Exp Nephrol, 2006; 10: 186–92
28. Boersema M, Rienstra H, van den Heuvel M et al: Donor and recipient contribution to transplant vasculopathy in chronic renal transplant dysfunction. Transplantation, 2009; 88: 1386–92
29. Rastaldi MP: Epithelial-mesenchymal transition and its implications for the development of renal tubulointerstitial fibrosis. J Nephrol, 2006; 19: 407–12
30. De Heer E, Sijpkens YW, Verkade M et al: Morphometry of interstitial fibrosis. Nephrol Dial Transplant, 2000; 15(Suppl.6): 72–73
31. Jiang D, Xu C, Li Z et al: Protective action of hepatocyte growth factor on transforming growth factor beta-1-induced alpha-smooth muscle actin and extracellular matrix in cultured human peritoneal fibroblasts. Med Sci Monit, 2010; 16(8): BR250–54
32. Rastaldi MP, Ferrario F, Giardino L et al: Epithelial-mesenchymal transition of tubular epithelial cells in human renal biopsies. Kidney Int, 2002; 62: 137–46
33. Merszei J, Wu J, Torres L et al: Osteopontin overproduction is associated with progression of glomerular fibrosis in a rat model of anti-glomerular basement membrane glomerulonephritis. Am J Nephrol, 2010; 32: 262–71
34. Singh A, Satchell SC: Microalbuminuria: causes and implications. Pediatr Nephrol, 2011; 26: 1957–65