Int J Clin Exp Med 2015;8(5):7861-7866
www.ijcem.com /ISSN:1940-5901/IJCEM0006215

Original Article

Serum galactose-deficient IgA1 levels in children with IgA nephropathy

Mengjie Jiang[1*], Xiaoyun Jiang[1*], Liping Rong[1], Yuanyuan Xu[1], Lizhi Chen[1], Zeting Qiu[2], Ying Mo[1]

[1]*Department of Pediatrics, The First Affiliated Hospital, Sun Yat-Sen University, Guangzhou, Guangdong, China;* [2]*Department of Clinic Medicine, Sun Yat-Sen University, Guangzhou, Guangdong, China.* [*]*Equal contributors.*

Received January 21, 2015; Accepted May 8, 2015; Epub May 15, 2015; Published May 30, 2015

Abstract: Immunoglobulin A nephropathy (IgAN) is an immunopathologic diagnosis based on a renal biopsy, it is characterized by deposits of IgA-containing immune complexes in the mesangium. Adults with IgAN have a galactose-deficient IgA1 in the circulation and glomerular deposition. There are few studies on the glycosylation of serum IgA1 in children with IgAN. To measure the serum levels of galactose-deficient IgA1 in pediatric patients with IgAN, 72 biopsy-proven IgAN children were divided into 3 groups based on the clinical features: isolated hematuria group (24 patients), hematuria and proteinuria group (22 patients), and nephritic syndrome group (26 patients). They were also divided into 3 groups according to pathologic grading: grade I + II group (25 patients), grade III group (33 patients) and grade IV + V group (14 patients). 30 healthy children were recruited as a control group. We used vicia villosa lectin binding enzyme-linked immunosorbent assay to measure the serum levels of galactose-deficient IgA1 in all groups and controls. Serum levels of galactose-deficient IgA1 in children with IgAN were higher than controls ($P < 0.01$). There were no significant differences in serum levels of galactose-deficient IgA1 among the different clinical and pathologic grading groups. The values of the area under the curve for galactose-deficient IgA1 levels were 0.976 (95% CI, 0.953-1.000). The cutoff point for galactose-deficient IgA1 levels was 0.125, with a sensitivity of 87.5% and a specificity of 83.3%, with a positive predictive value of 92.6% and a negative predictive value of 73.5% ($P < 0.01$). Children with IgAN presented serum galactose-deficient IgA1, which has shown no relationship with the clinical manifestations and pathologic grading of the disease. Detection of serum galactose-deficient IgA1 levels by vicia villosa lectin binding enzyme-linked immunosorbent assay has a certain clinical value in diagnosis of children with IgAN.

Keywords: Glomerulonephritis, IgA, galactose-deficient IgA1, *Vicia villosa* Lectin

Introduction

Immunoglobulin A nephropathy (IgAN) is one of the most common forms of primary glomerulonephritis in children [1, 2]. There are about 11.18% children with IgAN in the pediatric renal biopsy cases in China [3] and 30-40% in Japan and Korea [4]. The pathological process is characterized by immune complex deposition containing IgA as the major immunoglobulin in the mesangium and/or in the capillary loops. The diagnosis is mainly based on renal biopsy. To avoid this invasive procedure, particularly in children, we seek to develop a noninvasive test to support the diagnosis of IgAN. Studies of IgAN in adults [5, 6] revealed that IgA in the circulation and glomerular deposition is IgA1 with under-glycosylation of the hinge region, presenting under-glycosylation of O-linked N-acetylgalactosamine (GalNc) residues as well as increased GalNc, which was defined as under- glycosylation of IgA1. Currently, few studies are available on the glycosylation of serum IgA1 in children with IgAN. Recently, vicia villosa lectin (VVL) binding enzyme-linked immunosorbent assay (ELISA) has been used to detect galactose-deficient IgA1. The VVL binding capacity to GalNc in the hinge region of serum IgA1 (VVL-IgA1) was used to indirectly express the levels of galactose-deficient IgA1 (increased capacity means low level of galactose-deficient IgA1). In the present study, we use VVL binding ELISA to determine whether serum levels of galactose-deficient IgA1 differ between pediatric patients with IgAN and healthy controls.

Materials and methods

Study population

IgAN group: 72 children with IgAN, hospitalized in the pediatric renal center of the first affiliated hospital of Sun Yat-sen University, were included in the study. The diagnosis of IgAN was based on a renal biopsy. Through light microscopy, immunofluorescence and electron density in ultra-structure, IgA was the dominant or the codominant immunoglobulin in a predominantly mesangial distribution. Of these 72 patients, 57 were males and 15 were females. All other secondary IgAN cases were excluded. The median age at biopsy was 9.3 years (range 4.1-14.1 years). The duration of disease from onset to renal biopsy was between 6 days and 77 months with a median duration of 3.5 months. None of the patients had infections, received corticosteroids or immunosuppressive drugs for two weeks before the serum specimens were collected. All were during the active stage of IgAN, demonstrating hematuria and/or proteinuria, whereas the clinical type of nephrotic syndrome was defined as the presenting different degrees of edema, 3-4 plus of urine protein from urine analysis, the urine protein quantitation of 24 hours ≥ 0.05 g/kg and serum Albumin < 20 g/L. In accordance to the guidelines [7] of 2010 from the Subspecialty Group of Nephrology, Society of Pediatrics, and Chinese Medical Association; the IgAN children were divided into 3 groups according to their clinical features: isolated hematuria group (24 patients; 14 cases of gross hematuria and 10 cases of microscopic hematuria), hematuria and proteinuria group (22 patients) and nephritic syndrome group (26 patients). In line with the pathological grading of the WHO standards in 1982 [8], some different pathologic grading patients were combined and all together were further divided into three groups according to their pathologic grading: grade I + II group (25 patients containing 2 cases of grade I), grade III group (33 patients) and grade IV + V group (14 patients containing one case of grade V).

Control group: 30 healthy children of 21 males and 9 females, from our outpatient clinic in the same period, were included. They had no respiratory or other system infections for the past month. The median age at biopsy was 9.7 years (range 3.8-12.7 years). Compared with the IgAN group, there was no significant difference in age and gender between the two groups.

Parents and guardians were informed of the objectives of the study prior to enrolling the children in the study and written consents were obtained.

Laboratory investigation

Blood samples collection: 4 ml fasting venous blood sample was collected from children with IgAN the morning before the renal biopsy, followed by centrifugation (3500 rpm, 10 min) to separate the serum, the separated plasma was stored at -70°C until assay avoiding thawing and refreezing. In the control group, 4 ml fasting venous blood sample was collected in the early morning while having the health examination followed by the serum separation and stored as mentioned before.

Determination of levels of serum IgA: Using immunoturbidimetry, the kits for IgA determination and the immune turbidimetric instrument were purchased from Dade Behring, Inc (Westwood, MA, USA).

Determination of levels of serum IgA1: Using immunoturbidimetry, the kits for IgA1 determination and the Minineph Laser scattering protein turbidimetric instrument were purchased from The Binding Site Group Ltd, (Birmingham, UK).

Determination of levels of serum galactose-deficient IgA1: Using VVL binding ELISA. VVL were purchased from Vector Labs (Burlingame, CA, USA). Horseradish peroxidase (HRP)-conjugated rat anti-human IgA1 (specific α1 hinge region) monoclonal antibodies were purchased from Southern Biotech (Birmingham, AL, USA).

Method: VVL diluted to 1 mg/L in TBS (Tris-HCl buffer, PH 8.0) was coated to the enzyme label plates and stored at 4°C overnight. Taking out the reaction board, the liquid in each plate was removed and then the plates were washed three times with 0.01 M phosphate buffered saline containing 0.1% Tween 20 (PBS-T). The plates were then blocked with 10 g/L (1%) BSA at 37°C for 2 hours (200 μl/plate). The plates were then washed with PBS-T to cleanse the blocking liquid. The serum samples were then added to the plates (50 μl/plate) along with 1% BSA added to the blank control plates. After 1 hour incubation and three times washing with

Table 1. Basic data and levels of serum IgA, IgA1 and VVL-IgA1 for study subjects

Group	N	Gender Ratio (M/F)	Median age at study (years)	Serum IgA level (g/L) median (range)	Serum IgA level > normal level mean + 2SD (%)	Serum IgA1 level (g/L) median (range)	Serum VVL-IgA1 level (A450 nm) median (range)	Subjects with Serum VVL-IgA1 level > 0.125 (%)
IgAN	72	3.8	9.3	1.90 (0.90-3.80)	51.4 (37/72)	1.67 (0.70-3.15)	0.20 (0.12-0.35)	87.5 (63/72)
Controls	30	2.3	9.7	1.28 (0.70-1.85)	10.0 (3/30)	0.91 (0.44-1.65)	0.03 (0.02-0.15)	16.7 (5/30)
H/χ^2		0.989	-0.485	-5.972	15.218	-5.848	-7.596	47.813
p		0.320	0.628	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

VVL-IgA1 VVL binding capacity to GalNc in hinge region of serum IgA1, indirectly express the levels of galactose-deficient IgA1.

Table 2. Diagnostic value of serum IgA and VVL-IgA1 assays

	IgAN		Controls		Sensitivity	Specificity	Positive predictive	Negative predictive
	Yes	No	Yes	No				
IgA > normal level mean + 2SD	37	35	3	27	51.4	90.0	92.5	43.5
VVL-IgA1 > 0.125	63	9	5	25	87.5	83.3	92.6	73.5

PBS-T, HRP-conjugated rat anti-human IgA1 monoclonal antibodies (0.5 mg/ml) were diluted with antibody diluents to 1:1000, and then the enzyme-labeled antibodies were added to microplate (50 μl/plate) while the blank control did not receive any for 1 hour incubation at room temperature. After the incubation, the plates were washed three times with PBS-T and then the freshly prepared substrate was added (200 μl/plate) followed by color developing in a darkroom at room temperature for 30 mins. Finally the enzyme reaction was stopped with 125 g/L (12.5%) Sulfuric acid (50 μl/plate). The absorbance value (A) at 450 nm was recorded in an enzyme-linked immunosorbent assay reader. The higher the absorbance value expressed, the more GalNAc exposed in IgA1 molecules, which means higher level of serum galactose-deficient IgA1.

Statistical analysis

Wilcoxon rank sum test was used to compare subject and control groups for age, IgA, IgA1 and galactose-deficient IgA1 levels. Chi-square test was used to compare categorical data. Kruskal-Wallis test was used to determine significance of serum levels of IgA, IgA1 and galactose-deficient IgA1 among subject and control groups. Spearman correlation coefficient was used to assess the relationship between serum IgA and IgA1 levels. Receiver operating curve (ROC) analysis was used to determine optimal cutoff scores for galactose-deficient IgA1 in relation to the area under the curve (AUC), sensitivity, and specificity. $P \leq 0.05$ was considered to be significant.

Results

Since erum IgA levels vary in different ages in childhood, and the sample of the control group was small, the normal upper limit of serum IgA levels was considered to be two standard deviations (SD) above the mean value of each age period of healthy children; (3-7 years: 1.00 g/L, 7-12 years: 1.71 g/L, 12-14 years: 1.92 g/L) [9]. Serum IgA levels of IgAN group were significantly different from the controls (**Table 1**). Serum IgA levels were significantly increased in 37 of the 72 patients with IgAN. In contrast, only 3 of the 30 children in controls showed significantly increased serum IgA levels ($P < 0.01$). These data indicated a sensitivity of 51.4% and a specificity of 90.0% with a positive predictive value of 92.5% and a negative predictive value of 43.5% (**Table 2**).

Serum IgA1 levels in the IgAN group were positively correlated with serum IgA levels ($r = 0.966$, $P < 0.01$) (**Figure 1**). Median serum IgA1 level differed significantly between IgAN group and control group ($P < 0.01$, **Table 1**).

Median value of VVL-IgA1 in IgAN group was 0.20, significantly differed from 0.03 in control group ($P < 0.01$, **Table 1**). The ROC curve for VVL-IgA1 levels was shown in **Figure 2**. The AUC values for VVL-IgA1 levels were 0.976 (95% CI, 0.953-1.000). The cutoff point for VVL-IgA1 levels was 0.125, with a sensitivity of 87.5% and a specificity of 83.3% with a positive predictive value of 92.6% and a negative predictive value of 73.5% ($P < 0.01$, **Table 2**).

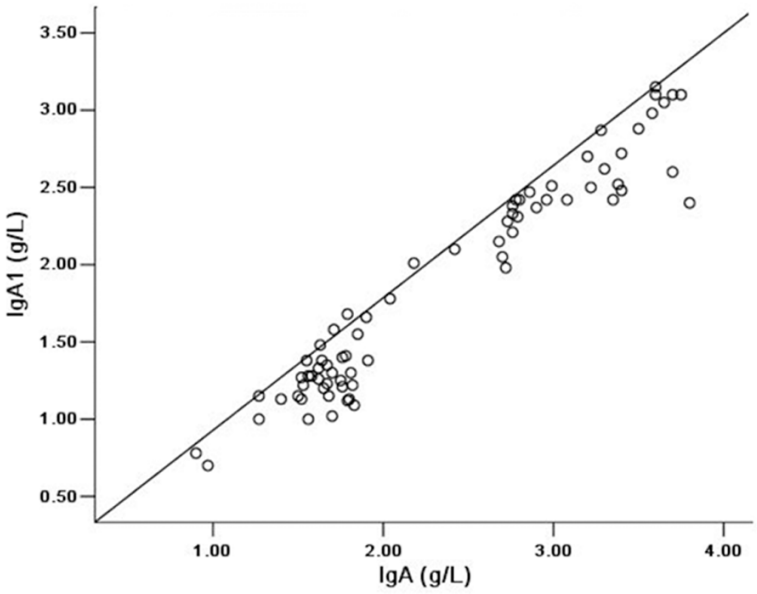

Figure 1. Scatter Plot of Correlation between Serum levels of IgA and IgA1 in IgAN patients. (r = 0.966, P < 0.01).

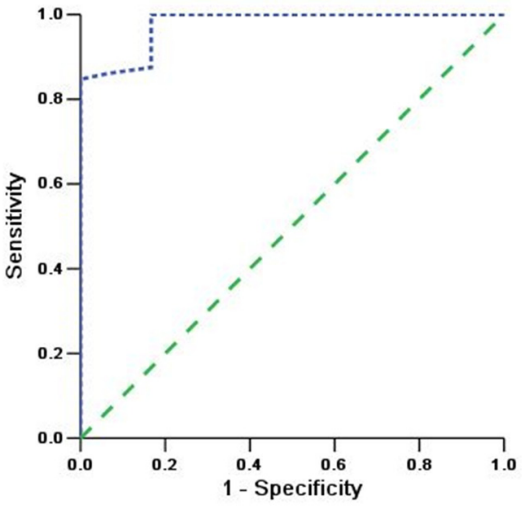

Figure 2. Serum levels of VVL-IgA1 in IgAN patients and healthy controls. ROC curve for VVL-IgA1 levels. Area under the curve is 0.976. VVL-IgA1 level 0.125; sensitivity 87.5, specificity 83.3.

The median serum VVL-IgA1 levels of IgAN patients with different clinical types were significantly higher than that of controls. The highest serum VVL-IgA1 levels were in patients manifested as isolated hematuria, followed by hematuria and proteinuria group and nephritic syndrome group. However, there was no significant difference among those three groups (**Table 3**). In addition, there was no significant difference among various pathological groups (**Table 4**).

Discussion

In the present study, we found that the children with IgAN had a significantly higher serum level of galactose-deficient IgA1 than that of healthy controls, through using VVL binding ELISA for measurement. Although 51.4% of children with IgAN had increased total serum IgA levels, serum levels of galactose-deficient IgA1 showed a higher sensitivity and equal specificity for differentiating the IgAN patients from the healthy controls. In addition, the high serum levels of total IgA and IgA1 do not necessarily lead to IgAN; for example, patients of IgA myeloma or HIV with obviously increased serum level of IgA rarely develop IgAN. Herein, the increased levels of serum IgA can be used as a reference for IgAN diagnosis rather than just evidence.

Recently, many studies have focused on the aberrantly glycosylated IgA1 in the pathogenesis of IgAN. Several studies [10-13] showed that children with IgAN have the hinge region of IgA1 aberrantly glycosylated both in circulation and mesangial deposition. It presents reduced galactosylation of O-linked N-acetylgalactosamine (GalNc) residues as well as increased GalNc. The galactose-deficient IgA1 molecules develop new antigenic determinants, inducing the production of antibodies, which could form immune complexes and deposits in the glomerular mesangium with subsequent damage of glomeruli. Meanwhile, galactose-deficient IgA1 molecules are prone to self-aggregation forming macromolecular complexes, getting rid of the clearance of hepatocyte receptors with a result of increased serum levels of galactose-deficient IgA1. This finding may have indicated that it was the galactose-deficient IgA1 that plays an important role in the pathogenesis of IgAN, as Tanaka M [14] has previously reported.

Table 3. Levels of serum IgA, IgA1 and VVL-IgA1 for various clinical feature groups

Group	N	Serum IgA level (g/L) median (range)	Serum IgA level > normal level mean + 2SD (%)	Serum IgA1 level median (range) (g/L)	Serum VVL-IgA1 level (A450 nm) median (range)	Subjects with Serum VVL-IgA1 level > 0.125 (%)
IH	24	2.76 (1.55-3.58)*	48.1 (13/24)*	2.27 (1.00-2.98)*	0.22 (0.12-0.28)*	95.8 (23/24)*
HP	22	2.00 (0.90-3.80)*	54.5 (12/22)*	1.85 (0.78-3.15)*	0.20 (0.12-0.31)*	86.4 (19/21)*
NS	26	1.81 (0.97-3.75)*	46.2 (12/26)*	1.29 (0.70-3.10)*	0.20 (0.12-0.35)*	80.8 (21/26)*
Controls	30	1.28 (0.70-1.85)	10.0 (3/30)	0.91 (0.44-1.65)	0.03 (0.02-0.15)	16.7 (5/30)
H		36.762	15.687	36.181	58.276	49.105
P		< 0.001	0.001	< 0.001	< 0.001	< 0.001

IH Isolated Hematuria, *HP* Hematuria and proteinuria, *NS* Nephritic syndrome; *There was significant difference. when compared with the control group ($P < 0.01$); No significant difference was found among IH, HP and NS group ($P > 0.05$).

Table 4. Levels of serum IgA, IgA1 and VVL-IgA1 for various pathological groups

Group	N	Serum IgA level (g/L) median (range)	Serum IgA level > normal level mean + 2SD (%)	Serum IgA1 level median (range) (g/L)	Serum VVL-IgA1 level (A450 nm) median (range)	Subjects with Serum VVL-IgA1 level > 0.125 (%)
Grade I + II	25	2.76 (1.27-3.75)	68.0 (17/25)	2.28 (1.00-3.10)	0.20 (0.12-0.35)	92.0 (23/25)
Grade III	33	1.83 (0.90-3.80)	45.6 (15/33)	1.41 (0.70-3.15)	0.24 (0.12-0.33)	84.9 (28/33)
Grade IV + V	14	1.73 (1.27-3.40)	35.7 (5/14)	1.43 (1.00-2.72)	0.20 (0.12-0.28)	85.7 (12/14)
Controls	30	1.28 (0.70-1.85)*	10.0 (3/30)*	0.91 (0.44-1.65)*	0.03 (0.02-0.15)*	16.7 (5/30)*
H		38.542	20.043	36.198	58.149	48.165
P		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

*There was significant difference when compared with the control group ($P < 0.01$); No significant difference was found among grade I + II group, grade III group and grade IV + V group, ($P > 0.05$).

It is difficult to distinguish between the structure of the galactose-deficient IgA1 and the normal IgA1 through conventional antigen-antibody methods. Based on the fact that lectin specifically binds to a sugar group, the VVL binding capacity to GalNc in hinge region of IgA1 can be used to indirectly express levels of galactose-deficient IgA1 [15, 16]. Consequently, we used a sensitive and quantitative VVL binding ELISA for measurement of galactose-deficient IgA1. Several studies [15, 17] have demonstrated increased serum levels of galactose-deficient IgA1 in patients with IgAN and Henoch-Schönlein purpura nephritis by using Helix aspera assay and Glycine max lectin, however, using VVL binding ELISA for measurement of the serum galactose-deficient IgA1 in pediatric patients with IgAN was rarely reported.

In the present study, we have demonstrated that the serum level of galactose-deficient IgA1 was increased in 87.5% of patients and as a diagnostic maker, its specificity and positive predictive value were 83.3% and 92.6% respectively, presenting higher effectiveness (high sensitivity and specificity) and good application (high positive predictive value and negative predictive value). Therefore, using VVL binding ELISA for testing serum galactose-deficient IgA1 has the potential to develop into a non-invasive diagnostic assay for IgAN with a certain clinical value, because currently, the established diagnosis relies only on renal biopsy by immunofluorescence examinations. As Hogg RJ[2] reported, currently, there are no non-invasive techniques that can establish the diagnosis of IgAN. Thus, highly sensitive and specific laboratory diagnostic methods and serum blood markers are urgently required.

Furthermore, the clinical and pathological manifestations of IgAN were of diversity and heterogeneity. The study in adults with IgAN revealed the galactose-deficient IgA1 was closely related to the kidney pathological manifestation and likely to affect the natural history and clinical manifestation of IgAN [18]. In the present study, there was no significant difference in the serum levels of galactose-deficient IgA1 when compared in various clinical and pathological types. This finding indicated that the serum levels of galactose-deficient IgA1 have no relation

with clinical manifestations or changes of renal pathology. Thus it can't reflect the severity of the clinical manifestations as well as the pathology, same conclusion was previously reported by Lau KK [17] in children with IgAN. Investigation of a larger sample and various detection methods are needed for further exploration to further study whether there are differences between adults and children.

Acknowledgements

This work was supported by the medical science and technology development, project of the national health commission (W2012RQ03), medical scientific research fund of Guangdong province (NO. s2012010009335), Baili scientific research fund for pediatrics of China (NO. HT1203010), and the national innovation training program project for college students (NO. 201310558086).

Disclosure of conflict of interest

None.

Address correspondence to: Ying Mo, Department of Pediatrics, The First Affiliated Hospital, Sun Yat-Sen University, Guangzhou, Guangdong, China. E-mail: 13711474082@163.com

References

[1] Coppo R. Pediatric IgA nephropathy: clinical and therapeutic perspectives. Semin Nephrol 2008; 28: 18-26.

[2] Hogg RJ. Idiopathic immunoglobulin A nephropathy in children and adolescents. Pediatr Nephrol 2010; 25: 823-829.

[3] Jiang XY. Clinical and pathological manifestations of Chinese childhood patients with primary IgA nephropathy: a national collaborative study of 33 hospitals. Zhonghua Er Ke Za Zhi 2007; 45: 272-278.

[4] Park YH, Choi JY, Chung HS, Koo JW, Kim SY, Namgoong MK, Park YS, Yoo KH, Lee KY, Lee DY, Lee SJ, Lee JE, Chung WY, Hah TS, Cheong HI, Choi Y and Lee KS. Hematuria and proteinuria in a mass school urine screening test. Pediatr Nephrol 2005; 20: 1126-1130.

[5] Narita I and Gejyo F. Pathogenetic significance of aberrant glycosylation of IgA1 in IgA nephropathy. Clin Exp Nephrol 2008; 12: 332-338.

[6] Barratt J, Smith AC and Feehally J. The pathogenic role of IgA1 O-linked glycosylation in the pathogenesis of IgA nephropathy. Nephrology (Carlton) 2007; 12: 275-284.

[7] The Subspecialty Group of Nephrology, Society of Pediatrics, Chinese Medical Association. Evidence-based guidelines on diagnosis and treatment of childhood common renal diseases (IV): IgA nephropathy. Zhonghua Er Ke Za Zhi 2010; 48: 355-337.

[8] Lee SM, Rao VM, Franklin WA, Schiffer MS, Aronson AJ, Spargo BH and Katz AI. IgA nephropathy: morphologic predictors of progressive renal disease. Hum Pathol 1982; 13: 314-322.

[9] Wang WP. Pediatrics. 7th edition. Beijing: People's Medical Publishing House; 2013. pp. 168.

[10] Xu LX, Yan Y, Zhang JJ, Zhang Y and Zhao MH. The glycans deficiencies of macromolecular IgA1 is a contributory factor of variable pathological phenotypes of IgA nephropathy. Clin Exp Immunol 2005; 142: 569-575.

[11] Gharavi AG, Moldoveanu Z, Wyatt RJ, Barker CV, Woodford SY, Lifton RP, Mestecky J, Novak J and Julian BA. Aberrant IgA1 glycosylation is inherited in familial and sporadic IgA nephropathy. J Am Soc Nephrol 2008; 19: 1008-1014.

[12] Mestecky J, Tomana M, Moldoveanu Z, Julian BA, Suzuki H, Matousovic K, Renfrow MB, Novak L, Wyatt RJ and Novak J. Role of aberrant glycosylation of IgA1 molecules in the pathogenesis of IgA nephropathy. Kidney Blood Press Res 2008; 31: 29-37.

[13] Hiki Y, Kokubo T, Iwase H, Masaki Y, Sano T, Tanaka A, Toma K, Hotta K and Kobayashi Y. Underglycosylation of IgA1 hinge plays a certain role for its glomerular deposition in IgA nephropathy. J Am Soc Nephrol 1999; 10: 760-769.

[14] Tanaka M, Seki G, Someya T, Nagata M and Fujita T. Aberrantly glycosylated IgA1 as a factor in the pathogenesis of IgA nephropathy. Clin Dev Immunol 2011; 470803.

[15] Moldoveanu Z, Wyatt RJ, Lee JY, Tomana M, Julian BA, Mestecky J, Huang WQ, Anreddy SR, Hall S, Hastings MC, Lau KK, Cook WJ and Novak J. Patients with IgA nephropathy have increased serum galactose-deficient IgA1 levels. Kidney Int 2007; 71: 1148-1154.

[16] Zhang JJ, Xu LX, Zhang Y and Zhao MH. Binding capacity of in vitro deglycosylated IgA1 to human mesangial cells. Clin Immunol 2006, 119: 103-109.

[17] Lau KK, Wyatt RJ, Moldoveanu Z, Tomana M, Julian BA, Hogg RJ, Lee JY, Huang WQ, Mestecky J and Novak J. Serum levels of galactose-deficient IgA in children with IgA nephropathy and Henoch-Schonlein purpura. Pediatr Nephrol 2007; 22: 2067-2072.

[18] Coppo R and Amore A. Aberrant glycosylation in IgA nephropathy (IgAN). Kidney Int 2004; 65: 1544-1547.