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The glycans deficiencies of macromolecular IgA1 is a contributory factor of variable pathological phenotypes of IgA nephropathy

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Summary

Recent evidence has suggested that IgA1-containing macromolecules and the glycosylation of IgA1 in sera from patients with IgAN might involve the pathogenesis of IgAN. However, whether the different histological phenotypes can be attributed or not to the aberrant glycosylation of macromolecular IgA1 has not yet been elucidated. The aim of the current study is to investigate the glycosylation of IgA1 molecules in serum IgA1-containing macromolecules and their association with pathological phenotypes of IgAN. Sera was collected from 40 patients with IgAN and 20 donors. Twenty patients had mild mesangial proliferative IgAN, the remaining 20 had focal proliferative sclerosing IgAN. Polyethylene glycol 6000 was used to precipitate the macromolecules from sera of patients and controls. Biotinylated lectins were used in an enzyme-linked immunosorbent assay (ELISA) to examine different glycans on IgA1 molecules. The α2,6 sialic acid was detected by elderberry bark lectin (SNA) and the exposure of terminal galactose (Gal) and N-acetylgalactosamine (GalNAc) were detected by *Arachis hypogaea* (PNA) and *Vilsa villosa* lectin (VVL), respectively. The IgA1 glycans levels corrected by IgA1 concentrations were compared between patients and controls. Reduced terminal α2,6 sialic acid of IgA1 (79·89 ± 25·17 *versus* 62·12 ± 24·50, $P = 0·034$) was demonstrated only in precipitates from sera of patients with focal proliferative sclerosing IgAN, compared with those from controls. Reduced galactosylation of IgA1 molecules in precipitates was demonstrated in patients with both mild mesangial proliferative IgAN and focal proliferative sclerosing IgAN compared with normal controls (24·52 ± 18·71 *versus* 76·84 ± 32·59 $P = 0·000$ and 33·48 ± 25·36 *versus* 76·84 ± 32·59 $P = 0·000$). However, no significant difference was found in IgA1 glycosylation in the supernatant between patients and normal controls ($P > 0·05$). The glycosylation deficiency of IgA1 existed only in serum IgA1-containing macromolecules of patients with IgAN, and was associated with the renal pathological phenotypes. This suggests that aberrant glycosylation of IgA1 in serum macromolecules might be a contributory factor in the pathogenesis of IgAN.

Keywords: IgA1-containing macromolecules, IgA nephropathy, glycosylation, pathology, polyethylene glycol

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Introduction

IgA nephropathy (IgAN), which leads to progressive renal failure in almost one-third of patients, is the most common glomerular disease, characterized by immune complex deposits containing IgA1 as the major immunoglobulin. In most cases these deposits are located primarily in the glomerular mesangium [1–3]. The deposits of the protein in the glomerular mesangium may be implicated in pathogenesis. Previous studies have suggested that increased serum polymeric IgA1-containing immune complexes are found commonly in patients with IgAN, and the glomerular IgA1

deposits are mainly polymeric [4,5]. The polymeric IgA1-containing immune complexes could not be removed effectively from circulation by phagocytic cells or hepatocytes but could bind to human mesangial cells efficiently [6,7]. After binding of IgA1-containing immune complexes to mesangial cells a number of events could be triggered. Our previous *in vitro* studies and others have demonstrated enhanced proliferation, increased cytokine release and enhanced production of extracellular matrix of human mesangial cells [8–11], but the elevated plasma levels of IgA1 and/or IgA1-containing immune complexes alone are not sufficient to cause mesangial deposition [12]. Therefore, abnormal physicochemical properties of circulating IgA1, such as size, charge and particularly the glycosylation of IgA1, have been supposed to play a role [6,13–18,41]. This is supported by the presence of altered glycosylation of serum and mesangial IgA1 in patients with IgAN [19,20].

It is known that the histological severity of IgAN is variable and the prognosis of the disease is connected directly with the pathological phenotypes. Typical cases show slow progression of the mesangial proliferative glomerulonephritis towards glomerulosclerosis, with secondary tubulointerstitial atrophy and fibrosis. However, many patients will have mesangial IgA1 deposits for years with little or no mesangial inflammation. The reason for this different course and prognosis is unknown. In our previous studies we have demonstrated that aberrantly glycosylated serum IgA1 of patients with IgAN is associated with renal pathological phenotypes [21], but mesangial IgA deposits are mainly polymeric in nature, so whether the glycans-deficient IgA1 is in monomer, polymer or IgA1-containing immune complexes still needs to be elucidated further.

In order to investigate whether the aberrantly glycosylated IgA1 are free or linked with other proteins, and whether aberrant glycosylation of circulating macromolecular IgA1 is associated with renal histological severity, polyethylene glycol (PEG) 6000 was used to precipitate the macromolecules from sera of patients with IgAN with different histological severity, and the glycosylation of precipitated macromolecular IgA1 and IgA1 in supernatant was detected by different lectins.

Materials and methods

Patients and sera

Forty patients with IgAN were enrolled in the current study: 21 females and 19 males. Serum samples from these patients were obtained at the time of renal biopsy. Twenty of them had mild mesangial proliferative glomerulonephritis as shown from renal pathology, in which the glomeruli showed no more than a minimal increase in mesangial cellularity, without segmental sclerosis or crescents, and were defined as Haas-I, a pathological scheme of IgAN proposed by Haas [22]. Their mean age was 29·7 ± 9·9 years, and the mean duration of disease at the time of renal biopsy was 25·4 ± 36·4 months. The remaining patients had focal proliferative sclerosing IgAN, which indicated that 40% or more of the glomeruli were globally sclerosing, with a mean age of 34·7 ± 10·8 years; the mean duration of disease was 22·0 ± 32·3 months. Sera from 20 healthy volunteers with comparable age and gender distribution and a normal urinalysis were recruited as controls. The protocol of the study was approved by the ethics committee in our hospital and informed consent was obtained for sampling renal biopsy tissues and sera.

Precipitation of the macromolecules

Macromolecules were precipitated by PEG 6000, as described previously [23]. Briefly, 100 μl serum from patients with IgA nephropathy was mixed by inversion with 50 μl 0·1 mol/l sodium borate buffer (BB, PH 8·4) and 150 μl 7% PEG-borate, and the mixture was incubated overnight at 4°C followed by centrifugation at 2810 ***g*** for 30 min. Both supernatant and pellets were collected. The pellets were then washed twice with 3·5% PEG-borate. Finally, the precipitates were dissolved to the original volume of 100 μl with phosphate-buffered saline (PBS), pH 7·4.

Concentration of proteins in the precipitates

The Bio-Rad DC protein assay was used to detect the protein concentration precipitated by PEG 6000. In brief, a standard curve was established by preparing six dilutions of bovine serum albumin (BSA) dissolved in PBS containing 0·3125–10 mg/ml protein. The samples were diluted 1 : 4 in PBS. Twenty μl of standards and samples were pipetted into a clean, dry microtitre plate (Costar, Cambridge, MA, USA), then 10 μl of reagent A and 80 μl of reagent B were added into each well, respectively. After 15 min, the absorbances were read at 655 nm. According to the standard curves, the protein concentration of each sample was calculated.

Relative content of IgG bound to IgA1 in the precipitates

Rabbit anti-human IgA (Dako, Glostrup, Denmark) diluted to 5·5 μg/ml in 0·05 M bicarbonate buffer PH 9·6 were coated to the wells of one-half of a polystyrene microtitre plate (Costar). The wells in the other half were coated with bicarbonate buffer alone to act as antigen-free wells. The volumes of each well for this step and for subsequent steps were 100 μl; all incubations were carried out at 37°C for 1 h and the plates were washed three times with 0·01 M phosphate-buffered saline containing 0·1% Tween 20 (PBST). The plate was then blocked with PBST containing 1% BSA (PBST/BSA). The precipitates diluted 1 : 100 in PBST/BSA were added in duplicate to both antigen-coated and antigen-free wells. Each plate contained a blank control (PBST/BSA) and a known normal serum was used as a positive control.

After incubation and washing, horseradish peroxidase (HRP)-conjugated sheep anti-human IgG (Zhongshan Biotech, Beijing, China) with a dilution of 1 : 2000 was added to detect the IgG bound to IgA1 in the precipitates. The reaction was revealed with 0·1 M citrate phosphate buffer pH 5·0 containing 0·04% o-phenylenediamine (OPD) and 0·1% H_2O_2 (V/V), then the reaction was stopped with 1 M H_2SO_4. The absorbance at 490 nm (A) was recorded in an enzyme-linked immunosorbent assay (ELISA) reader (Bio-Rad 550, Tokyo, Japan). The relative concentration of IgG was calculated as follows: the A value of the blank control was defined as 0 and the A value of the known control was defined as 100%; the A value of each sample was calculated by log-transformed data.

Glycosylation of IgA1 in the supernatant and precipitates

The O-glycans in the hinge region of IgA1 were detected by specific lectin binding ELISA, as reported previously [21]. The IgA1-catching procedures were as described above. The supernatant was diluted 1 : 66·7, which was equivalent to a dilution of 1 : 200 in the original serum, and the precipitates diluted 1 : 100 in PBST/BSA were added in duplicate to both antigen-coated and antigen-free wells. Each plate contained a blank control (PBST/BSA) and IgA1 affinity by jacalin was used as a positive control in IgA1 concentration and α2,6 sialic acid detection; IgA1 digested by neuraminidase and galactosidase was used as a positive control in detecting the GalNAc. After incubation and washing, the biotinylated second antibodies or lectins in PBST/BSA were added, including 1 : 20000 diluted monoclonal anti-human IgA1 (clone no. A1-18; Sigma, St Louis, MO, USA) to detect the IgA1 concentrations; 1 : 500 diluted elderberry bark (*Sambucus nigra* agglutinin, SNA; Vector Laboratories, Burlingame, CA, USA) to detect α2,6 sialic acid of IgA1 molecules; 1 : 200 diluted *Arachis hypogaea* (PNA; Sigma, USA) or *Vilsa villosa* lectin (VVL; Vector Laboratories) to detect galactose (Gal) and N-acetylgalactosamine (GalNAc) of IgA1 molecules, respectively. The wells were then incubated with 1 : 20000 diluted avidin–HRP (Sigma). The subsequent steps were the same as described above.

The relative concentration of IgA1 and their levels of glycans were calculated as follows: the A value of the blank control was defined as 0 and the A value of the known control was defined as 100%; the A value of each sample was calculated by log-transformed data. The relative lectin binding per unit IgA1 was calculated as the A value of lectin binding over the A value of IgA1 concentration.

Statistics

For statistical analysis, statistical software SPSS 11·0 (SPSS, Chicago, IL, USA) was employed. Quantitative data were expressed as mean ±s.d. and median with range (minimum, maximum). For comparison between patients and controls, the Student's *t*-test, two-way analysis of variance and Mann–Whitney *U*-test were used. Statistical significance was considered as $P < 0·05$.

Results

Protein concentrations and relative concentrations of IgG binding to IgA1 in the precipitates

The protein concentrations of normal controls, patients with mild mesangial proliferative IgAN and patients with focal proliferative sclerosing IgAN were 2·27 ± 0·73 mg/ml, 2·51 ± 1·03 mg/ml and 2·47 ± 1·64 mg/ml, respectively. There was no significant difference between groups ($P > 0·05$) (see Table 1).

The relative concentrations of IgG bound to IgA1 molecules in the precipitates of normal controls, patients with mild mesangial proliferative IgAN and patients with focal proliferative sclerosing IgAN were 104·51 ± 36·33, 90·65 ± 32·98 and 81·47 ± 40·91, respectively. There was no significant difference between groups ($P > 0·05$) (see Table 1).

Relative concentrations of IgA1 in the supernatant and precipitates

The relative concentration of IgA1 in the supernatant of normal controls, patients with mild mesangial proliferative IgAN and patients with focal proliferative sclerosing IgAN were 102·04 ± 14·58, 103·90 ± 7·36 and 100·75 ± 8·65, respectively. There was no significant difference between groups ($P > 0·05$) (see Table 2).

The relative concentrations of IgA1 in the precipitates of normal controls and patients with mild mesangial

Table 1. The protein concentration and relative concentration of IgG bound to IgA1 in the precipitates.

Groups	*n*	Protein concentration (mg/ml)	Relative concentration of IgG
Controls	20	2·27 ± 0·73	104·51 ± 36·33
Mild mesangial proliferative IgAN patients	20	2·51 ± 1·03	90·65 ± 32·98
Focal proliferative sclerosing IgAN patients	20	2·47 ± 1·64	81·47 ± 40·91
		Protein concentration	Relative concentration of IgG
Controls *versus* mild IgAN		$P = 0·515$	$P = 0·240$
Controls *versus* focal IgAN		$P = 0·600$	$P = 0·053$
Mild IgAN *versus* focal IgAN		$P = 0·899$	$P = 0·434$

Table 2. The relative concentration of IgA1 in the supernatant and precipitates.

Groups	*n*	Relative concentration of IgA1 in supernatant	Relative concentration of IgA1 in precipitates
Controls	20	102·04 ± 14·58	87·72 ± 28·87
Mild mesangial proliferative IgAN patients	20	103·90 ± 7·36	95·90 ± 24·71
Focal proliferative sclerosing IgAN patients	20	100·75 ± 8·65	76·45 ± 33·72
		Supernatant	Precipitates
Controls *versus* mild IgAN		$P = 0·533$	$P = 0·381$
Controls *versus* focal IgAN		$P = 0·666$	$P = 0·229$
Mild IgAN *versus* focal IgAN		$P = 0·292$	$P = 0·040$

proliferative IgAN were 87·72 ± 28·87 and 95·90 ± 24·71, respectively. However, the relative concentration of IgA1 in the precipitates of patients with focal proliferative sclerosing IgAN were 76·45 ± 33·72: less than the IgA1 in the precipitates of patients with mild mesangial proliferative IgAN ($P = 0·04$) (see Table 2).

α2,6 Sialic acid levels of IgA1 molecules in the supernatant and precipitates

The α2,6 sialic acid levels of IgA1 molecules in the supernatant of normal controls, patients with mild mesangial proliferative IgAN and patients with focal proliferative sclerosing IgAN were 80·17 ± 15·63, 90·44 ± 28·55 and 90·91 ± 21·32, respectively. There was no significant difference between groups ($P > 0·05$) (see Table 3).

The sialic acid level of IgA1 molecules in the precipitates of patients with mild mesangial proliferative IgAN was comparable with that of controls (82·00 ± 23·41 *versus* 79·89 ± 25·17, $P = 0·799$); however, the sialic acid level of IgA1 in the precipitates of patients with focal proliferative sclerosing IgAN was decreased significantly (62·12 ± 24·50 *versus* 79·89 ± 25·17, $P = 0·034$) (see Table 3).

More importantly, the sialic acid level of IgA1 molecules was significantly lower in patients with focal proliferative sclerosing IgAN than that of patients with mild mesangial proliferative IgAN (62·12 ± 24·50 *versus* 82·00 ± 23·41, $P = 0·018$) (see Table 3).

Galactose levels of IgA1 molecules in the supernatant and precipitates

The galactose levels of IgA1 molecules in the supernatant of normal controls, patients with mild mesangial proliferative IgAN and patients with focal proliferative sclerosing IgAN were 20·11 ± 11·74, 24·56 ± 10·80 and 26·21 ± 9·96, respectively. There was no significant difference between groups ($P > 0·05$) (see Table 4).

The galactose levels of IgA1 molecules in the precipitates of patients with mild mesangial proliferative IgAN and

Table 3. The A2,6 sialic acid levels of IgA1 in the supernatant and precipitates.

Groups	*n*	Sialic acid level of IgA1 in supernatant	Sialic acid level of IgA1 in precipitates
Controls	20	80·17 ± 15·63	79·89 ± 25·17
Mild mesangial proliferative IgAN patients	20	90·44 ± 28·55	82·00 ± 23·41
Focal proliferative sclerosing IgAN patients	20	90·91 ± 21·32	62·12 ± 24·50
		Supernatant	Precipitates
Controls *versus* mild IgAN		$P = 0·095$	$P = 0·779$
Controls *versus* focal IgAN		$P = 0·435$	$P = 0·034$
Mild IgAN *versus* focal IgAN		$P = 0·367$	$P = 0·018$

Table 4. The galactose levels of IgA1 in the supernatant and precipitates.

Groups	*n*	Gal level of IgA1 in supernatant	Gal level of IgA1 in precipitates
Controls	20	20·11 ± 11·74	76·84 ± 32·59
Mild mesangial proliferative IgAN patients	20	24·56 ± 10·80	24·52 ± 18·71
Focal proliferative sclerosing IgAN patients	20	26·21 ± 9·96	33·48 ± 23·36
		Supernatant	Precipitates
Controls *versus* mild IgAN		$P = 0·365$	$P = 0·000$
Controls *versus* focal IgAN		$P = 0·216$	$P = 0·000$
Mild IgAN *versus* focal IgAN		$P = 0·737$	$P = 0·292$

Table 5. The GalNAc levels of IgA1 in the supernatant and precipitates.

Groups	*n*	GalNAc level of IgA1 in supernatant	GalNAc level of IgA1 in precipitates
Controls	20	1·48 ± 2·24	5·89 ± 4·68
Mild mesangial proliferative IgAN patients	20	0·02 ± 0·04	2·66 ± 3·38
Focal proliferative sclerosing IgAN patients	20	0·08 ± 1·18	6·21 ± 8·36
		Supernatant	Precipitates
Controls *versus* mild IgAN		$P = 0·778$	$P = 0·122$
Controls *versus* focal IgAN		$P = 0·896$	$P = 0·877$
Mild IgAN *versus* focal IgAN		$P = 0·880$	$P = 0·089$

patients with focal proliferative sclerosing IgAN were both significantly lower compared with that of controls (24·52 ± 18·71 *versus* 76·84 ± 32·59, $P < 0·001$; 33·48 ± 23·36 *versus* 76·84 ± 32·59, $P < 0·001$). However, the galactose levels between the two groups of IgAN were comparable (24·52 ± 18·71 *versus* 33·48 ± 23·36, $P = 0·292$) (see Table 4).

GalNAc levels of IgA1 molecules in the supernatant and precipitates

The GalNAc levels of IgA1 molecules in the supernatant of normal controls, patients with mild mesangial proliferative IgAN and patients with focal proliferative sclerosing IgAN were 5·89 ± 4·68, 2·66 ± 3·38 and 6·21 ± 8·36, respectively. There was no significant difference between groups ($P > 0·05$) (see Table 5).

The GalNAc levels of IgA1 molecules in precipitates of normal controls, patients with mild mesangial proliferative IgAN and patients with focal proliferative sclerosing IgAN were 1·48 ± 2·24, 0·02 ± 0·04 and 0·08 ± 1·18, respectively. There was no significant difference between groups ($P > 0·05$) (see Table 5).

Discussion

In our previous studies we have demonstrated that the heat-aggregated IgA1 separated from sera of patients with IgAN had a higher binding capacity to human mesangial cells and stronger biological effects than IgA1 from healthy controls. It was suggested that the characteristics of IgA1 produced by patients might be different to healthy controls [8,9]. Human IgA1 is highly glycosylated and bears five O-linked glycans that are linked to serine or threonine residues between the CH1 and CH2 domains in the hinge region of each heavy chain. GalNAc is linked with the amino acid residues, and Gal is linked in β1,3 to GalNAc. Sialic acid has an α2,6 linkage with GalNAc and an α2,3 linkage with Gal residues [24]. Interest has been focused recently on the potential pathogenetic role of aberrantly glycosylated IgA1 molecules in the development of IgAN. We have confirmed that the glycans deficiency of serum IgA1 molecules is associated with renal histological phenotypes of IgAN; the sialic acid and Gal deficiencies of IgA1 molecules in sera from patients with severe renal pathological damage were more profound than that found in the mild type [21]. The finding of a relationship between the extent of altered IgA1 glycosylation and injury might shed new light on the possibility that glycosylation might also influence inflammation and injury.

This study has added further evidence that patients with IgAN have an altered glycosylation of circulating IgA1. Moreover, it is reported for the first time that the altered glycosylation of IgA1 exists only in the IgA1-containing macromolecules precipitated by PEG 6000, while the glycosylation of IgA1 in the supernatant is similar to that of healthy controls. As we know, 3·5% PEG 6000 could precipitate circulating immune complexes of serum; however, the relative concentration of IgA1 in the precipitates of patients with focal proliferative and sclerosing IgAN is less than patients with mild mesangial proliferative IgAN. It is suggested that qualitative, more than quantitative, changes of serum IgA1 molecules might be responsible for the mesangial trapping of IgA1 in IgAN. Therefore it could explain the rare occurrence of IgA1-associated glomerular damage in patients with acquired immune deficiency syndrome, despite very high levels of circulating polyclonal IgA1 and in patients with multiple myeloma, despite very high levels of circulating monoclonal IgA1 [25,26]. More importantly, the current study again found further evidence that the altered glycosylation is associated closely with renal pathological phenotypes of IgAN. It is well known that the advanced renal pathology usually indicates a worse prognosis in patients with IgAN. Our current study provides strong support to Coppo's speculation that defects in IgA1 glycosylation might influence the presentation and natural history of patients with IgAN [28]. Furthermore, it suggests that the deglycosylation of macromolecular IgA1 might be a contributory factor for the pathogenesis of IgAN, rather than monomolecular IgA1. This coincides well with the finding that deficiency of sialylation and galactosylation of IgA1 in the macromolecules might be involved in the enhanced mesangial proliferation and inflammation observed by Novak *et al.* [27].

We speculate that sialic acid could have an important role in preventing IgA1 deposition in kidney. This study and our previous work both demonstrate that sialic acid deficiency exists mainly in patients with focal proliferative sclerosing

IgAN. Many studies have confirmed that removal of sialic acid from the IgA1 molecules could result in self-aggregation of IgA1 molecules; it has been suggested that the binding sites of the IgA1–IgA1 interaction were exposed by removing sialic acid and that this molecule, attached to the IgA1, played a protective role against IgA1 self-aggregation [29–31]. Because sialic acid has a negative charge and a relatively bulky volume, it was thought that electrostatic repulsion and steric hindrance due to the sialic acid residues contribute to this protective role. However, the fact that IgA1 self-aggregation could not be changed by the additional removal of Gal and GalNAc suggests that the binding sites do not include Gal and GalNAc residues on the IgA1 molecule. Many studies have also demonstrated that the IgA1–extracellular matrix binding is related to the aberrant O-glycosylation of IgA1 molecules observed in IgAN. Removal of the sialic acid residues from the IgA1 molecules could result in a significant increase in the adhesion activities to type IV collagen, fibronectin and laminin compared with naive IgA1 [32]. These suggest that the sialic acid residues might play an important role in preventing the IgA1–extracellular matrix interaction as well as IgA1 self-aggregation. Two other studies have shown the presence of highly under-galactosylated IgA1 in the elutes of renal tissue containing mesangial IgA1 [18,19]. Altered O-glycosylation of serum IgA1 might favour complex formation and it had been found that under-galactosylated IgA1-containing immune complexes could bind more efficiently to mesangial cells than circulating immune complexes from healthy controls [33–36].

The presence of IgA–IgG circulating immune complexes in most IgAN patients has been reported in many studies [33–36]. However, mesangial IgG deposits were not found in all cases of IgAN. The binding of serum IgA1 to Fab fragments of IgA1 with hinge region glycans containing free terminal GalNAc residues indicated that, in addition to IgG, naturally occurring IgA1 antibodies with specificity to hinge region glycans could also be found in sera from patients with IgAN and healthy individuals. It has been suggested that a non-immunological mechanism might be responsible for the formation of macromolecular IgA1 by self-aggregation of aberrantly glycosylated IgA1 molecules in IgAN patients [37–39]. In this study, the relative concentration of IgG bound to IgA1 in the precipitates was comparable between patients and controls. However, the glycosylation of IgA1 molecules in the precipitates decreased significantly in patients with severe pathological phenotype; this might provide evidence that the key contributory factor of IgAN is not the formation of IgA1–IgG complexes but the aberrant glycosylation of IgA1 molecules. The results agree with the hypothesis of Paul *et al.*, that increased macromolecular IgA production alone is not sufficient to develop IgAN – the physicochemical properties of circulating IgA are likely to play a role [40].

The detailed components of macromolecules precipitated by PEG 6000 have not been defined clearly; there might be polymeric IgA1, IgA1–IgG complex, IgA1–CD89 complex and others, such as IgA1 combined with extracellular matrix, etc. The detailed components of the precipitates still need to be analysed further and important information could be found. The increase of macromolecular IgA1 in patients with IgAN has been clarified by many studies. In this work, although a tendency was observed towards an increasing precipitated protein concentration in patients with IgAN, a significant difference was not reached and this might be due to the limited number of patients.

In conclusion, the glycosylation of IgA1 in macromolecules precipitated by PEG 6000 and the glycosylation of IgA1 molecules in the supernatant were detected; aberrant glycosylation of IgA1 could be found only in macromolecules in sera from patients with IgAN. Moreover, the deficiency of sialic acid of IgA1 is associated with the pathological phenotype of IgAN. It is suggested that the aberrant glycosylation of IgA1 molecules might be a sticking factor in pathogenesis of IgAN.

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