DIAGNOSTIC EFFICACY OF SERUM LOW GLYCOSYLATION IGA1 AND URINARY ANGIOTENSINOGEN DETECTION IN EARLY RENAL INJURY OF CHILDREN WITH HENOCH-SCHONLEIN PURPURA

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ABSTRACT

Objective*: The aim of this study was to analyze the diagnostic efficacy of serum low glycosylation IgA1 and urinary angiotensinogen detection for early renal injury in children with Henoch-Schonlein purpura (HSP).*

Methods: *Sixty-one children with HSP treated in the pediatric ward of our hospital between January 2016 and December 2017 were selected, including 31 children with HSP comprising the HSP group, 30 children with HSP nephritis (HSPN) comprising the HSPN group, and 25 healthy children comprising the control group. The levels of blood urea nitrogen (BUN), creatinine (Cr), low glycosylation (Gd-IgA1), angiotensin (AGT), complement C3, and urine angiotensin (AGT) were measured. The correlations between Gd-IgA1, AGT, and renal function were analyzed using a Pearson correlation analysis. The relationship between Gd-IgA1/C3, uAGT/uCr, and early renal injury in children with HSP were analyzed using a multiple logistic regression model. An ROC curve was used to evaluate the sensitivity and specificity of Gd-IgA1/C3 and uAGT/uCr in the diagnosis of early renal injury in children with HSP.*

Results: *The ratio of serum Gd-IgA1 to Gd-IgA1/C3 in the HSPN group was significantly higher than that in the HSP group and normal control group ($p<0.05$). The serum Gd-IgA1 level in the HSP group was significantly higher than that of the normal control group ($p<0.05$). There was no significant difference in the ratio of Gd-IgA1/C3 between the HSP group and the normal control group ($p>0.05$). The uAGT/uCr level of HSPN group was markedly higher than that of HSP group and control group, and the difference was statistically significant ($p<0.05$). Moreover, the uAGT/uCr level in the HSP group was significantly higher than that of the control group ($p<0.05$). There was no significant difference in AGT level among the three groups ($p>0.05$). The Gd-IgA1/C3 ratio and uAGT/uCr level in the HSPN group was positively correlated with the serum Cr. The elevated levels of Gd-IgA1/C3 and uAGT/uCr were independent risk factors for HSPN occurrence (OR (95% CI) = 1.637 (1.068-2.435), OR (95% CI) = 1.952 (1.157-3.062)). The area under the ROC curve (AUC) of the serum Gd-IgA1/C3 and uAGT/uCr in the diagnosis of HSPN was 0.696 (95% CI: 0.531-0.816, $p = 0.018$) and 0.715 (95% CI: 0.543-0.832, $p = 0.007$), respectively. The selected cut-off points were 3.65 and 8.67, the corresponding sensitivities were 66.8% and 63.5%, and the specificities were 75.3% and 82.6%, respectively.*

Conclusion: *The serum Gd-IgA1 and uAGT/uCr levels of children with HSPN were significantly increased, indicating that Gd-IgA1 and uAGT may be closely related to the progression of HSP and renal injury in children, and can be used as an early diagnosis of HSPN to predict the progression and prognosis of HSP renal injury.*

Keywords: *Low serum glycosylation IgA1, urinary angiotensinogen, HSPN, diagnosis.*

DOI: 10.19193/0393-6384_2021_1_28

Received *November 30, 2019;* ***Accepted*** *January 20, 2020*

Introduction

Henoch-Schonlein purpura (HSP) is a common leukocyte fragmentation vasculitis that occurs in childhood. The main clinical symptoms are non-thrombocytopenic purpura, joint swelling and pain, abdominal pain, hematuria and proteinuria[(1)]. Kidney damage is the main cause of the HSP prognosis. HSP nephritis (HSPN) can be diagnosed in children with hematuria or proteinuria during the course of HSP (usually within half a year), and severe cases can progress to renal failure. Thus far, the pathogenesis of this disease has been unclear. Therefore, it is of great clinical value to explore its pathogenesis and treatment measures[(2)]. The serum and tissues of HSP children are often accompanied by the deposition of immunoglobulin A (Ig A), which can easily be gathered and combined with IgG to form a macromolecular immune complex deposited on the vascular wall, playing a key role in the tissue and organ dam-

age of patients with HSP[3]. Studies have shown that activation of the renin-angiotensin system (RAS) has an important role in kidney disease, in which angiotensinogen (AGT) is a vital initial substrate for a rate-limiting reaction that directly affects RAS activity and renal development and prognosis[4]. This study compared the changes of Gd-IgA1 and AGT levels in children with HSP, children with HSPN and normal children, and further analyzed the relationship between Gd-IgA1, AGT and the incidence of child HSP and renal involvement.

Data and methods

Sample

The data for the present study was collected from 61 children with HSP treated in the pediatric ward of our hospital between January 2016 and December 2017 as well as 25 healthy children. Among the HSP patients, there were 30 cases of children with HSPN, including 16 males and 14 females, aged 5 to 13 years old, with an average age of 9.12±2.55 years. the remaining 30 cases of children with HSP, included 19 males and 12 females, aged 3 to 14 years old, with a mean age of 7.97±2.56 years.

There were five inclusion criteria for the data. First, all HSP diagnoses must have been in accordance with the European committee for the prevention and control of rheumatism and the European commission for the prevention and control of childhood kidney disease[5]. Second, the diagnosis of HSPN was based on the criteria outlined in the Chinese Journal of Integrated Traditional and Western Nephrology[6]. Third, the age of first onset must have been between the ages of 3 and 14 years Fourth, patients could not have received glucocorticoid or other immunosuppressive therapies. Finally, the patients and their families were informed and signed the informed consent; those who did not consent to participation were not included in the study.

The exclusion criteria for the study were threefold:

• Patients with severe liver and kidney function or cardiovascular diseases;

• Hemorrhagic diseases, such as septicemia or thrombocytopenic purpura; or mental illness were not included.

The 25 healthy children selected for the control group included 14 males and 11 females aged 6 to 12 years old, with an average age of 9.21±1.50 years old. There was no statistically significant difference in age or gender composition among the three groups ($p>0.05$). This research program was reviewed and approved by the hospital ethics committee and the subjects all gave informed consent.

Observation indicators

On the second day of enrollment, 4 ml of fasting venous blood were extracted from both the HSP group and the HSPN group and placed at room temperature for one hour. The fasting venous blood was centrifuged at 3000 r/min for 15 minutes. Meanwhile, 5 ml of fresh urine were collected, and centrifuged at 4000 r/min for 15 minutes at 4oC. The supernatant was respectively packed into EP tubes and stored in a refrigerator at -80oC for further use. The normal control group comprised the blood samples taken from healthy children during a physical examination, and the treatment was the same as above.

Blood urea nitrogen (BUN) and serum creatinine (SCr) in the three groups of children were detected using an AU-400 automatic biochemical analyzer (OLYPUS, Japan). The changes in Gd-IgA1 and uAGT levels were determined from an enzyme linked immunosorbent assay (ELISA). Serum complement C3 was detected by immune scattering turbidimetry and the Gd-IgA1/C3 ratio was also measured. The specific operations for each test were carried out according to the specifications provided by the manufacturers.

Statistical methods

All of the data in this study were analyzed using the SPSS version 19.0 software package. The measurement data consistent with the normal distribution were expressed as mean ± standard deviation ($\bar{x}\pm SD$). A t-test was used to compare the two study groups, and a one-way analysis of variance (ANOVA) was used to compare results between groups. A Pearson correlation analysis was used for the correlation analysis, and a multivariate logistic regression model was used for risk factor analysis. A p-value of less than 0.05 indicated statistical significance.

Results

Changes in serum Gd-IgA1 and Gd-IgA1/C3 ratio in children in each group

The ratio of serum Gd-IgA1 and Gd-IgA1/C3 in the HSPN group was significantly higher than that in the HSP group and the normal control group ($p<0.05$). The serum Gd-IgA1 level of the HSP group was also significantly higher than that of normal control group ($p<0.05$). There was no significant differ-

ence in the Gd-IgA1/C3 ratio between the HSP group and the normal control group (p>0.05). The results are shown in Table 1 and Figure 1.

Groups	Cases (*n*)	Gd-IgA1 (mg/L)	Gd-IgA1/C3
HSP group	31	3.73±1.35*	3.22±1.34
HSPN group	30	5.10±1.21*#	4.47±1.32*#
Normal control group	25	2.53±0.91	2.55±0.90
F		11.976	7.413
p		<0.001	<0.001

Table 1: Comparison of serum Gd-IgA1 and Gd-IgA1/C3 ratio in each group.
*Notes: *represents a comparison with the normal control group, p<.05; #indicates a comparison with the HSP group, p<.05.*

Correlation analysis between Gd-IgA1/C3 ratio and renal function index of children in the HSPN group

The Gd-IgA1/C3 ratio of children in the HSPN group was positively correlated with serum Cr, as shown in Figure 1 (r = 0.684, Pr = 0.001), but was not clearly correlated with BUN (r = 0.008, p = 0.973).

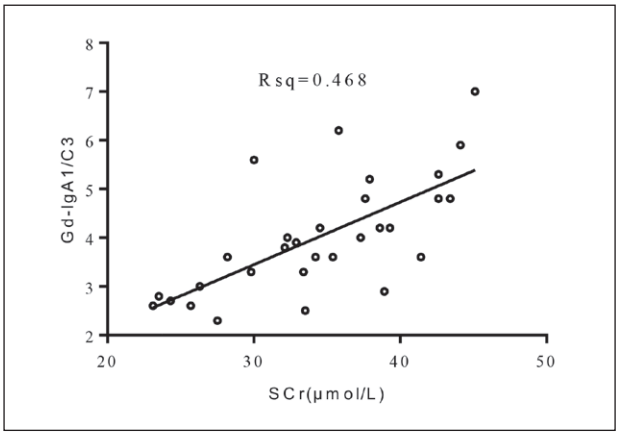

Figure 1: Correlation analysis between serum Gd-IgA1/C3 ratio and serum Cr.

Comparison of serum AGT and uAGT/uCr levels of children in each group

There were no significant differences in AGT level among the three groups (p>0.05).

The uAGT/uCr level of the HSPN group was markedly higher than that of the HSP group and control group, and the difference was statistically significant (p<0.05). Moreover, the uAGT/uCr level of the HSP group was significantly higher than that of the control group (p<0.05). The results are outlined in Table 2.

Correlation analysis between uAGT/uCr level and renal function of children in the HSPN group

The uAGT/uCr levels were positively correlated with the serum Cr in the HSPN groups, as shown in Figure 2 (r = 0.540, p = 0.002), but they were not correlated with the BUN levels (r = 0.011, p = 0.955).

Groups	Cases (*n*)	Serum AGT	uAGT/uCr
HSP group	31	27.06±17.33	6.97±3.50*
HSPN group	30	27.38±10.69	19.08±9.14*#
Normal control group	25	31.05±14.94	3.40±2.08
F		0.616	29.067
p		.543	<.001

Table 2: Comparison of uAGT/uCr and AGT levels in the three groups.
*Notes: *represents a comparison with the normal control group, p<.05; #indicates a comparison with the HSP group, p<.05.*

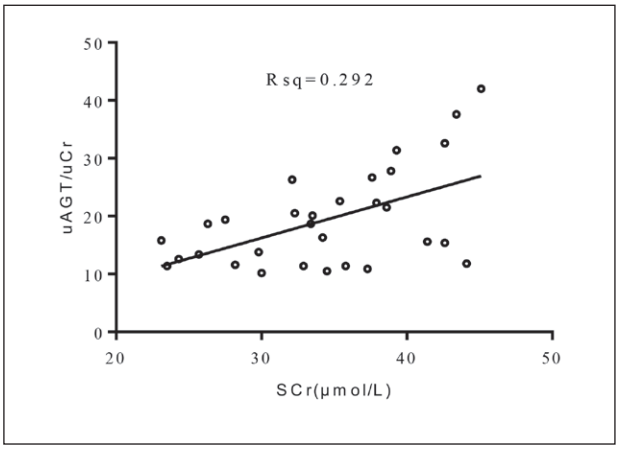

Figure 2: Correlation analysis between uAGT/uCr and serum Cr.

Analysis of the relationship between Gd-IgA1/C3, uAGT/uCr, and HSPN by multivariate logistic regression

The HSPN group and the non-HSPN group were used as a dichotomous variable, and Gd-IgA1/C3 and uAGT/uCr were used as the independent variables in the multivariate logistic regression model. The results indicated that the elevated levels of Gd-IgA1/C3 and uAGT/uCr were independent risk factors for the occurrence of HSPN (OR (95% CI) = 1.637 (1.068-2.435), OR (95% CI) = 1.952 (1.157-3.062)). The results are provided in Table 3.

Factor	β	SE(β)	Wald	OR	95% CI	*p*
Gd-IgA1/C3	0.464	0.157	5.135	1.637	1.068-2.435	0.013
uAGT/uCr	0.538	0.207	6.117	1.952	1.157-3.062	0.009

Table 3: Multivariate logistic regression model of the relationship between Gd-IgA1/C3, uAGT/uCr, and HSPN.

The efficacy of serum Gd-IgA1/C3 and uAGT/uCr in the diagnosis of HSPN

The AUC for the ROC curve of serum Gd-IgA1/C3 and uAGT/uCr in the diagnosis of HSPN

were 0.696 (95% CI: 0.531-0.816, p = 0.018) and 0.715 (95% CI: 0.543-0.832, p = 0.007), respectively. The selected cut-off points were 3.65 and 8.67, the corresponding sensitivities were 66.8% and 63.5%, and the specificities were 75.3% and 82.6%, respectively. The results were shown in Figure 3 and Table 4.

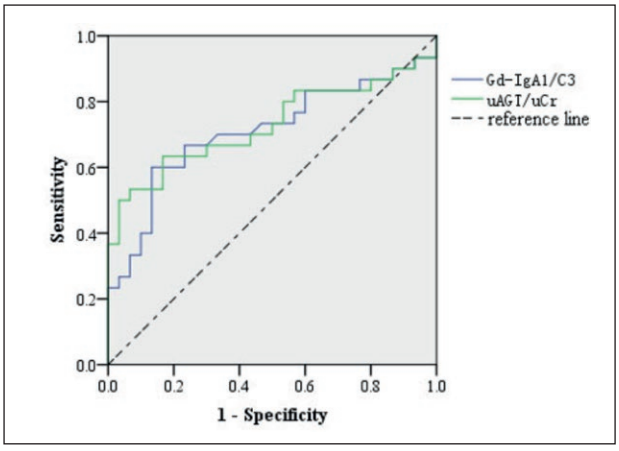

Figure 3: The efficacy of serum Gd-IgA1/C3 and uAGT/uCr in the diagnosis of HSPN.

Factor	Cut-off points	AUC	95% CI	p	Sensibility	Specificity
Gd-IgA1/C3	3.65	0.696	0.531-0.816	0.018	66.8	75.3
uAGT/uCr	8.67	0.715	0.543- 0.832	0.007	63.5	82.6

Table 4: The efficacy of serum Gd-IgA1/C3 and uAGT/uCr in the diagnosis of HSPN.

Discussion

HSP is a common glomerular disease in children, and the incidence and hospitalization rate is among the highest in urinary system diseases in children. Patients with this disease experience different renal pathological changes based on individual differences, vasculitis involving organs, and varying degrees of the disease. Studies have shown that the incidence of HSPN in China is 29 to 56%, of which 5 to 18% can progress to end-stage kidney disease. Thus, kidney injury is the main factor affecting the treatment and prognosis of HSP in children[7]. The pathogenesis of HSP has yet to be fully elucidated, and it is generally believed that an abnormal glycosylation of the IgA1 molecular, an abnormal expression of inflammatory cytokines, a disturbance of RAS system regulation, and genetic factors are all involved in the pathogenesis of HSP. It has been reported that abnormal glycosylation of IgA1 and RAS system disorders play a key role in HSP pathogenesis, especially in the progression of HSPN[8].

In this study, the levels of Gd-IgA1 and uAGT in children with HSP were determined by the ELISA method, and the relationships between Gd-IgA1, uAGT, and other renal function indexes in children with HSP were further analyzed, providing a new idea for the clinical treatment of children with HSP.

During the pathogenesis of HSPN, various stimulating factors stimulate the cloning and amplification of B lymphocytes, resulting in significantly increased IgA1 production. The O-linked glycosyl group of the normal IgA1 molecule is N-acetyl galactosamine. The abnormality of the O-linked glycosyl group clearly affects the properties of the IgA1 molecule. Such an IgA1 molecule with an abnormal O-linked glycosyl group is prone to the pathogenesis of HSP[9]. Several other reasons can also explain this[10-11]. First, Gd-IgA1 has the tendency to accumulate to form antigen complexes, but cannot be removed normally by the body due to the decomposition of the IgA1 molecule. It is therefore deposited in the glomerular mesangial area, resulting in organ damage. Second, Gd-IgA1 cannot be recognized, ingested, or removed by the asialoglycoprotein receptor on the surface of hepatocytes, so a large amount of Gd-IgA1 is deposited in the small vascular wall of the skin and the glomerular mesangial area. Third, due to the change in three-dimensional conformation of the Gd-IgA1 molecule, the new exposed epitopes are combined with the induced autoantibodies to form the IgA1 or IgA1-IgG circulating immune complex, which can damage the glomerulus through the window of the glomerular endothelial cells.

The results of this study showed that the Gd-IgA1 level of children in the HSPN group was significantly higher than that in the HSP group and the normal control group, which suggested that the IgA1 in children had low glycosylation. It was thus concluded that Gd-IgA1 level played a key role in HSPN pathogenesis. In children with HSPN, the complement deposited in the skin capillary wall and the glomerular mesangial area could lead to kidney damage after activation. Some data showed that the components of the sedimentary complement were mainly C3c and C4+C3+C3d complexes. The IgA1/C3 value in HSP children was higher than that in normal children, which was an indicator of the disease activity stage[12]. In this study, Gd-IgA1 and C3 were combined to investigate [WHAT], and it was found that the serum Gd-IgA1/C3 ratio of children in the HSPN group was remarkably higher than that of children in the HSP group and the normal control group. Moreover, there was a significant positive

correlation between the serum Gd-IgA1/C3 ratio and the serum Cr of children. Therefore, this study considered Gd-IgA1/C3 as an observation indicator of renal injury in children with HSPN.

RAS is an endocrine system, mainly involved in regulating the body's blood pressure, water, and electrolyte balance, among others. However, it is also likely to participate in a variety of clinical immune inflammatory nephropathy disease processes. AGT is the only rate-limiting reaction initial substrate of RAS that can control RAS activity through changes in level[13]. The experiments on nephrotic rat models were conducted by some researchers and demonstrated that the enhancement of AGT expression in the kidney was related to the production of renal angiotensin II (Ang II), which promotes the development of glomerular injury. It has also been reported that urinary AGT (uAGT) levels reflect intrarenal AngII activity and are associated with increased risk of kidney deterioration in patients with chronic kidney disease (CKD)[14].

The results of the present study showed that the uAGT/uCr level of the HSPN group was significantly higher than that of the HSP group and the normal control group, and was positively correlated with the serum Cr of children, but there was no statistically significant difference in the level of uAGT/uCr between the HSP group and the control group. This indicates that local renal RAS may be activated during kidney injury in children with HSP, resulting in excessive local AGT production, which plays an important role in the process of kidney injury in children with HSP. This study also found that although the uAGT/uCr levels of the three groups were different, there were no significant differences in serum AGT levels in the three groups. Studies have shown that uAGT levels are associated with renal tissue AGT gene expression, and that uAGT is a sound marker of intrarenal RAS status. Some scholars have injected human AGT into rats with hypertension and normal blood pressure, and the injected AGT was detected in the serum of rats, but not in the urine[15].

This indicates that uAGT production is mainly derived from the kidney rather than through filtration in circulation. uAGT can be used as an active marker of RAS in the kidney to reflect the status of intrarenal RAS, which can promote kidney injury jointly with various humoral pathogenic factors and also lead to kidney damage, along with a series of changes in kidney function in the process of HSP kidney injury. The elevated levels of Gd-IgA1/C3 and uAGT/uCr were independent risk factors for HSPN occurrence, OR (95% CI) = 1.637 (1.068-2.435), OR (95% CI) = 1.952 (1.157-3.062). The AUC of the ROC curve of serum Gd-IgA1/C3 and uAGT/uCr in the diagnosis of HSPN were 0.696 (95% CI: 0.531-0.816, p = 0.018) and 0.715 (95% CI: 0.543-0.832, p = 0.007), respectively. The selected cut-off points were 3.65 and 8.67, the corresponding sensitivities were 66.8% and 63.5%, and the specificities were 75.3% and 82.6%, respectively. These results suggested that serum Gd-IgA1/C3 and uAGT/uCr may serve as a new diagnostic and prognostic marker and as a new direction for HSPN therapy.

In conclusion, the serum Gd-IgA1 and uAGT/uCr of children with HSPN were significantly higher than those of normal children, suggesting that Gd-IgA1 and uAGT may be closely related to the incidence of HSPN. The detection of the Gd-IgA1/C3 ratio and uAGT level can be used as a new monitoring index for renal injury in children with HSPN. Combined detection of the two indexes could further predict the progress and prognosis of HSP. The number of cases in this study was small, and thus this study needs to be expanded and replicated through multi-center collaboration.

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Acknowledgement:
This work was supported by the Shandong Medical and Health Science and Technology Development Program Project (No. 2016WS0707).

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