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Original Article

Diagnostic performance of urinary proteins as biomarkers in evaluating Henoch Schonlein purpura nephritis

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Abstract: Objective: The aim of this retrospective study was to investigate diagnostic performance of urinary proteins as biomarkers in evaluating pathological damage of children with Henoch Schonlein purpura nephritis (HSPN). Methods: Forty-five children who received renal needle biopsy and were diagnosed with HSPN were retrospectively analyzed from Jan 2015 to Dec 2017 from North China Petroleum Administration Bureau General Hospital. Of the included 45 cases, pathology grade I was in 9 cases, grade II in 14 cases, and grade III in 22 cases. The urinary microalbumin (MAlb), Transferrin (TfR), beta 2-microglobulin (beta 2-MG), and N-acetyl-β-glucosaminidase (NAG) were recorded and compared among the four groups. Diagnostic performance of urinary proteins as biomarkers in evaluating the pathological damage of children with HSPN was assessed. Results: Urinary MAlb, TfR, and NAG level were significantly different for grade I, II and III disease. With the development of pathology grading, the urinary β2-MG, MAlb, TfR and NAG level were also elevated. The distribution of simple hematuria, mild proteinuria, moderate proteinuria, and severe proteinuria in grade I, II and III were statistical different ($p < 0.05$). For grade I HSPN, most of the cases were simple hematuria (66.7%) and mild proteinuria (33.3%) disease. There were no cases of moderate proteinuria and severe proteinuria in the grade I HSPN group. However, for grade II and III HSPN, most cases were moderate proteinuria and severe proteinuria. Furthermore, there were no cases of grade simple hematuria in grade HSPN. Pearson correlation test showed significant positive correlation between urinary protein levels except for urinary β2-MG and NAG ($P > 0.05$). The sensitivity and specificity of urinary MAlb, TfR, β2-MG, and NAG as biomarkers for HSPN grading ranged from 64.29% to 95.45% and 70.00% to 100.00%. Conclusions: Urinary MAlb, TfR, β2-MG, and NAG were increased with the increase of renal pathological grade. These can be used as promising biological markers for reflecting the degree of renal pathology damage in children with HSPN.

Keywords: Henoch schonlein purpura nephritis, biological markers, urinary microalbumin, diagnosis

Introduction

Henoch-Schonlein purpura nephritis (HSPN), also known as IgA vasculitis secondary to Henoch-Schonlein purpura (HSP), is the most common secondary glomerulonephritis in childhood [1]. At present, HSPN is considered as a sequela of HSP, in which kidney histology is altered despite normal urine quality [2, 3]. HSPN is classified according to the degree in severity, and the degree of kidney involvement determines the prognosis of the disease [4]. Renal biopsy is the gold standard for assessing the extent of kidney damage in HSPN. However, because of the invasiveness of this approach, patients and their families often refuse to undergo the operation. Hence, renal biopsy cannot be widely used in children clinically [5, 6]. Therefore, clinical indicators are needed in the early stage of the disease and during follow-ups to determine the severity of kidney damage in children with HSPN. In recent years, urinary biomarkers have been widely used to evaluate early renal injury [7, 8] in glomerular diseases. However, the relationship between biomarkers and disease progression remains unclear. In our present work, we evaluated the relationship between urine biomarkers and the degree of renal damage.

Table 1. Urinary β2-MG, MAlb, TfR, and NAG level of different grading HSPN ($\bar{x}\pm s$)

Pathological grading	N	β2-MG (mg/L)	MAlb (mg/L)	TfR (mg/L)	NAG (U/L)
I	9	0.25±0.15	101.70±61.30	7.92±6.55	8.78±4.88
II	14	0.44±0.33	367.8±157.01	42.64±31.63	23.01±13.31
III	22	0.83±0.43	654.9±275.1	78.21±43.73	45.01±24.34
F		10.64	22.82	13.26	13.58
P-value		< 0.05	< 0.05	< 0.05	< 0.05

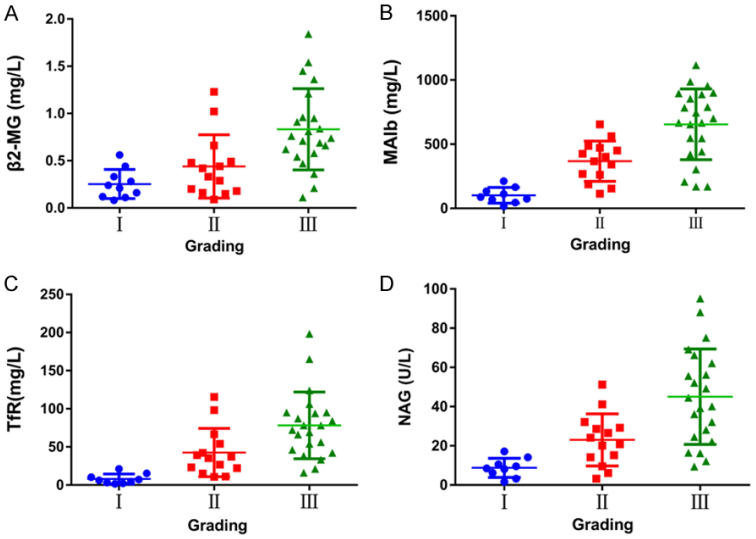

Figure 1. Scatter plot of urinary β2-MG, MAlb, TfR, and NAG level (A: β2-MG; B: MAlb; C: TfR; D: NAG).

Material and methods

Patients

Forty-five children who received renal needle biopsy and were previously diagnosed of HSPN were retrospectively analyzed from Jan 2015 to Dec 2017 from North China Petroleum Administration Bureau General Hospital. The research related to human use was complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Declaration of Helsinki, and has been approved by the North China Petroleum Administration Bureau General Hospital's Institutional Review Board or equivalent committee. Informed consent was obtained from all the included children's guardians. The patient's inclusion criteria were: (1) diagnoses of HSPN confirmed by pathology; (2) first diagnosis without previous treatment; (3) clinical data such as urinary microalbumin (MAlb), Transferrin (TfR), beta 2-microglobulin (beta 2-MG), and N-acetyl-β-glucosaminidase (NAG) extracted from the medical records; (4) diagnosis of HSPN should conform to: a clear history of skin purpura; proteinuria and/or hematuria was found within 6 months of the disease. Systemic diseases such as thrombocytopenic purpura, systemic lupus erythematosus, and hepatitis B virus infection not found.

Urinary protein examination

Urine samples were collected at 24-hours for all patients for urine protein quantification. Urine samples were collected for testing urine albumin, transferrin, β2-MG, and NAG through turbidimetric method using a Roche Cobas 8000c701 automatic biochemical analyzer.

Clinical classification

The children were divided into four groups according to the degree of proteinuria: (1) simple hematuria type, 24-hour urine protein ≤ 150 mg; (2) mild proteinuria type, 24-hour urine protein > 150 mg but < 25 mg/kg·d; (3) moderate proteinuria type, 24-hour urine protein 25-50 mg/kg·d; and (4) severe proteinuria type, 24-hour urine protein ≥ 50 mg/kg·d.

Pathological grading

Pathologic grades were divided into six levels according to the ISKDC classification guidelines [9]: Grade I, slight glomerular abnormality; Grade II, simple mesangial hyperplasia, IIa. Focal/Segmental, IIb. Diffuse; Grade III, mesangial hyperplasia, accompanied by the focal/

Table 2. Correlation between pathological grading and urinary albuminuria level [n,(%)]

Grading	N	Simple hematuria	Mild proteinuria	Moderate proteinuria	Severe proteinuria
I	9	6 (66.7)	3 (33.3)	0 (0.0)	0 (0.0)
II	14	0 (0.0)	3 (21.4)	5 (35.7)	6 (42.9)
III	22	0 (0.0)	4 (18.2)	8 (36.4)	10 (45.5)

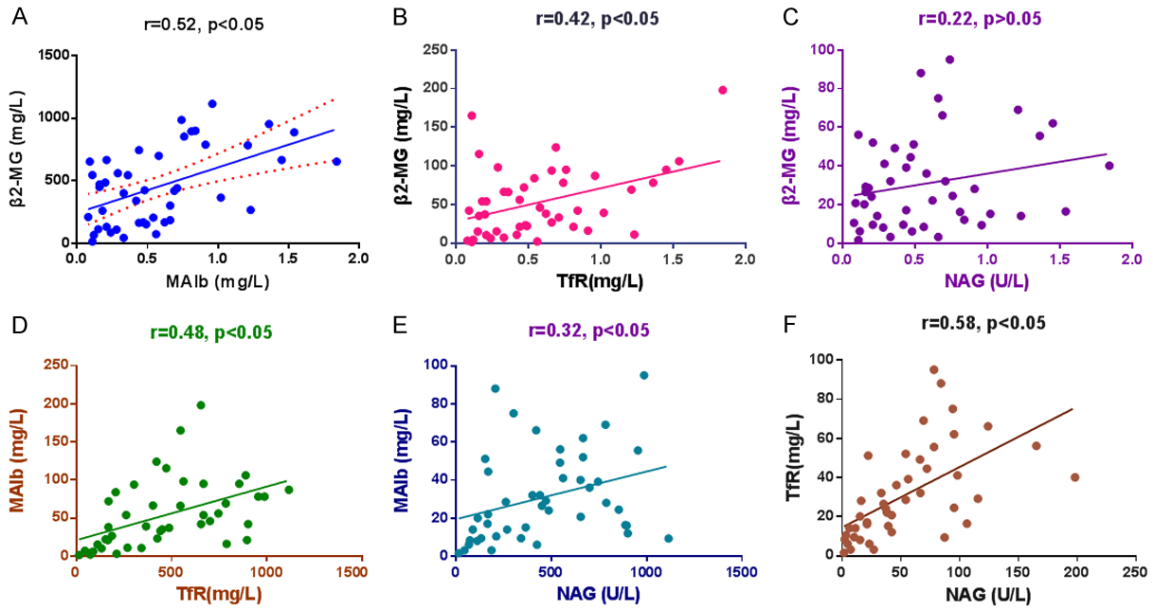

Figure 2. Pearson correlation test in evaluation correlation among urinary β2-MG, MAlb, TfR, and NAG (A: β2-MG vs MAlb; B: β2-MG vs TfR; C: β2-MG vs NAG; D: MAlb vs TfR; E: MAlb vs NAG; F: TfR vs NAG).

segmental lesions (such as sclerosis, adhesion, thrombosis, and necrosis) with > 50% glomerular crescent body, IIIa. Focal/Segmental, IIIb. Diffuse; Grade IV, lesions similar to Grade III, 50%-75% glomerular crescent body accompanied by the above-mentioned lesions, IVa. Focal/segmental, IVb. Diffuse; Grade V, lesions similar to Grade III, > 75% glomerular crescent body accompanied by the above-mentioned lesions, Va. Focal/Segmental, Vb. Diffuse; and Grade VI, membranous proliferative glomerulonephritis.

Statistical analysis

SPSS16.0 software was used for data processing. Measurements are represented as the mean and standard deviation ($\bar{x}\pm s$). One-way analysis of variance was used to make comparisons among groups, whereas *LST-t* method was adopted for multiple comparisons. Counting data are expressed as percentages. Spearman correlation test was used to evaluated the correlation between urinary proteins. Two tails $p < 0.05$ was considered as statistically significant.

Results

Urinary β2-MG, MAlb, TfR, and NAG level

Urinary MAlb, TfR, and NAG level were significantly different for grade I, II and III disease (**Table 1**). With development of pathology grading, the urinary β2-MG, MAlb, TfR, and NAG level were also elevated (**Figure 1**).

Correlation between pathological grading and urinary albuminuria level

The distribution of simple hematuria, mild proteinuria, moderate proteinuria and severe proteinuria in grade I, II and III were statistical different (**Table 2**). For grade I HSPN most of the cases were simple hematuria (66.7%) and mild proteinuria (33.3%) disease. There were no cases of moderate proteinuria and severe proteinuria in grade I HSPN group. However, for

Table 3. The differential diagnostic of different grading HSPN by urinary proteins (95% CI)

Marker	Sen	Sep	AUC	Cut-off
β2-MG				
I vs II	64.29 (35.14-87.24)	70.00 (34.75-93.33)	0.68 (0.46-0.89)	0.28
II vs III	81.82 (59.72-94.81)	78.57 (49.20-95.34)	0.79 (0.63-0.95)	0.52
I vs III	86.36 (65.09-97.09)	80.00 (44.39-97.48)	0.98 (0.82-1.00)	0.40
MAlb				
I vs II	78.57 (42.90-95.34)	88.89 (51.75-99.72)	0.95 (0.87-1.00)	199.60
II vs III	72.73 (49.78-89.72)	85.71 (57.19-98.22)	0.81 (0.66-0.95)	516.60
I vs III	86.36 (65.09-97.09)	100.00 (66.37-100.00)	0.98 (0.94-1.00)	257.50
TfR				
I vs II	92.86 (66.13-99.82)	77.78 (39.99-97.19)	0.95 (0.87-1.00)	10.88
II vs III	77.27 (54.63-92.18)	71.43 (41.90-91.61)	0.76 (0.59-0.93)	44.18
I vs III	95.45 (77.16-99.88)	100.00 (66.37-100.00)	0.99 (0.98-1.00)	21.13
NAG				
I vs II	78.57 (49.20-95.34)	88.89 (51.75-99.72)	0.84 (0.67-1.00)	14.14
I vs III	81.82 (59.72-94.81)	100.00 (66.37-100.00)	0.96 (0.89-1.00)	19.64
II vs III	77.27 (54.63-92.18)	71.43 (41.90-91.61)	0.76 (0.59-0.93)	44.18

grade II and III HSPN, most cases were moderate proteinuria and severe proteinuria. There were no cases of grade simple hematuria in grade HSPN.

Correlation between urinary β2-MG, MAlb, TfR and NAG

Correlation between urinary β2-MG, MAlb, TfR, and NAG were assessed by Pearson correlation test (**Figure 2**). Significant positive correlation was found between urinary protein levels except for urinary β2-MG and NAG ($P > 0.05$).

Diagnostic performance of urinary β2-MG, MAlb, TfR, NAG

The differential diagnosis efficacy of different grading HSPN (I, II and III) is demonstrated in **Table 3**. The area under the ROC is shown in **Figure 3**.

Discussion

HSPN is a common type of kidney disease in pediatric patients, and the prognosis of children with HSPN is closely related to the degree of renal injury [10]. In this present study, the degree of proteinuria in children with HSPN was found to correlate with pathology grading. For the grade I HSPN, most cases were simple hematuria (66.7%) and mild proteinuria (33.3%) disease. There were no cases of oderate proteinuria and severe proteinuria in grade I HSPN group. However, for grade II and III HSPN, most of the cases were moderate proteinuria and severe proteinuria. Furthermore, there were no cases of grade simple hematuria in grade HSPN. This result was consistent with previously published studies [11, 12]. However, children were also found with mild proteinuria exhibiting pathologic changes similar to Grade III. This indicated that pathological damage in HSPN was not exactly in parallel with the degree of proteinuria. Therefore, in children with HSPN who do not exhibit kidney pathologies examination, clinical laboratory indices should be utilized to assess the severity of kidney damage. Detection of biomarkers, such as urinary microalbumin and urinary enzymes, exhibits advantages of easy accessibility, non-invasiveness, high sensitivity, and specificity [13, 14].

Urine microalbumin (Malb) [15, 16] and transferrin (TfR) [17, 18] are glomerular proteins, and their appearance in urine suggests increased glomerular permeability. NAG is an important lysosomal acid hydrolase in the human body that is mainly found in renal proximal tubule epithelial cells. When the kidney and proximal convoluted tubules in particular are injured, urine NAG is the first urinary enzyme to become significantly elevated. Thus, NAG serves as a highly sensitive and reliable indicator of renal tubular injury [11, 19, 20]. In this study, urinary

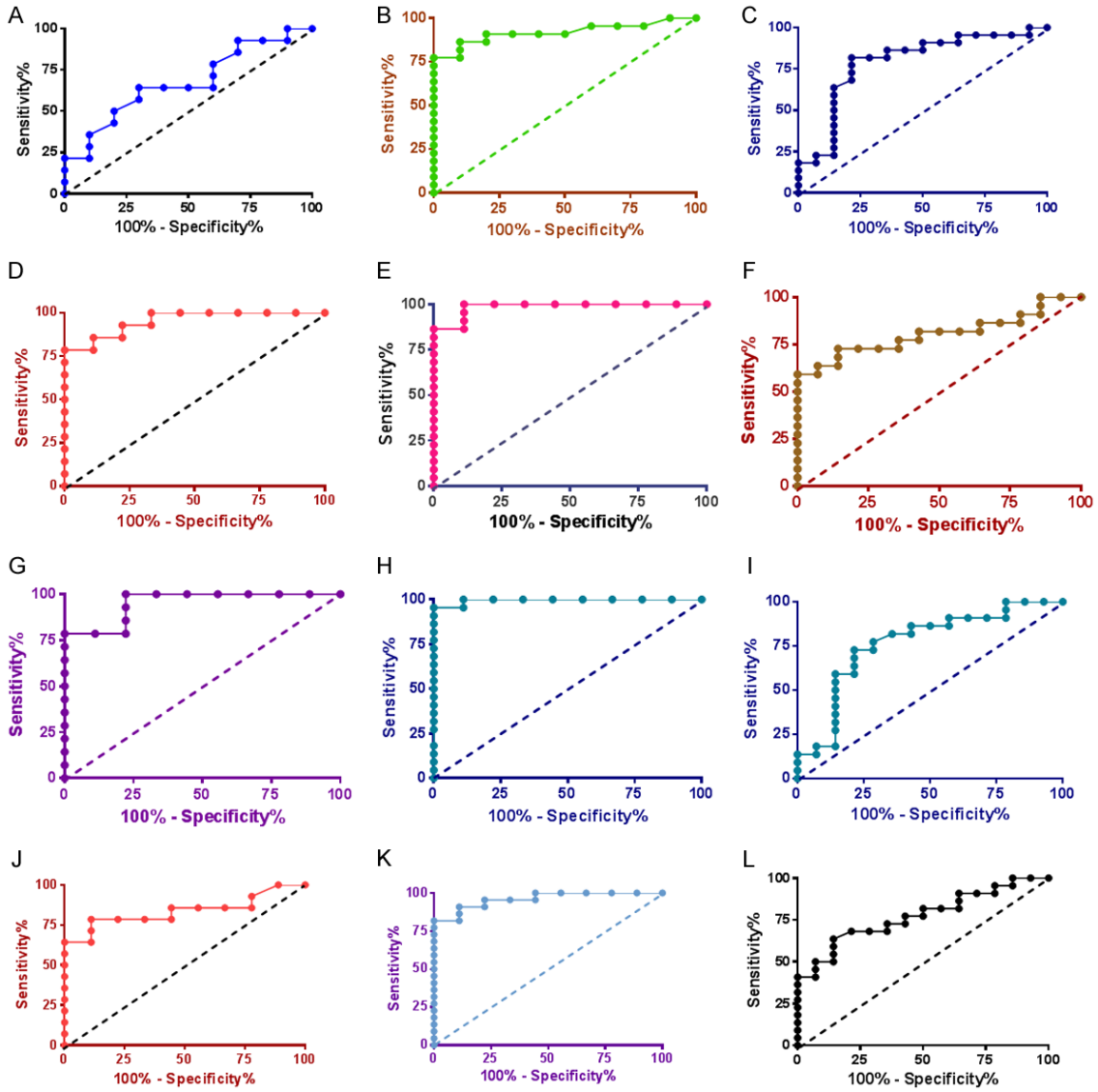

Figure 3. ROC curve of urinary protein in differential diagnosis of different grading HSPN (A: β2-MG I vs II; B: β2-MG I vs III; C: β2-MG II VS III; D: MAlb I vs III; E: MAlb I vs III; F: MAlb II VS III; G: TfR I vs II; H: TfR I vs III; I: TfR II VS III; J: NAG I vs II; K: NAG I vs III; L: NAG II VS III).

NAG was significant elevated with the development of renal pathology grading. The sensitivity and specificity of urinary NAB in differential diagnosis of renal pathology grading (I vs III) were 81.82% and 100.00% with the AUC of 0.96. This demonstrated urinary NAG is a promising biomarker for pathology injury grading differentiation. Previously studies have also found urine NAG to be a reliable index for membranous nephropathy, focal segmental glomerular sclerosis, and lesions in renal tubules [21]. Mishra OP [13, 22] et al. found that the urine NAG level in children with steroid-resistant nephrotic syndrome is significantly higher than those in hormone-sensitive patients. This results were in accordance with our conclusions.

The severity of renal damage affects the long-term prognosis of children with HSPN. Kidney biopsy can accurately reveal the degree of kidney damage, and this information is useful in guiding treatment and in assessing prognosis [23-25]. However, renal needle biopsy is invasive and not all the parents accepted this invasive examination especially in China [26, 27]. Therefore, serum or urinary biomarkers which

can provide the same information is important for clinical practices in evaluation the renal damage in children with HSPN. Here, urinary MAlb, TfR, β2-MG, and NAG were found to be increased according to the renal pathology grading scale and are therefore promising biological markers for reflecting the degree of renal pathological damage in children with HSPN. However, only 45 cases were included in our study. The statistical power was limited with small sample size. Therefore, a prospective multicenter diagnosis studies of large sample were needed to further evaluated the clinical diagnostic performance of urinary proteins as biomarkers in evaluating the pathological damage of children with HSPN.

Disclosure of conflict of interest

None.

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