

Association of TLR8 Expression in Peripheral Blood Mononuclear Cells with Clinical and Histopathological Severity in IgA Nephropathy

Na Jiang

Nantong University

Qian Meng

Nantong University

Yingjie Cao

nt.fycao@126.com

Affiliated Hospital of Nantong University

Research Article

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Abstract

Background

IgA nephropathy (IgAN) is characterized by mesangial IgA deposition and variable clinical and histopathological severity. Toll-like receptor 8 (TLR8) is an innate immune receptor that may participate in inflammatory signaling, but its association with IgAN severity remains insufficiently defined. This study evaluated TLR8 expression in peripheral blood mononuclear cells (PBMCs) and renal tissue and examined its relationships with galactose-deficient IgA1 (Gd-IgA1), clinical parameters, and renal pathological severity.

Methods

Sixty treatment-naïve patients with biopsy-proven IgAN enrolled between December 2022 and February 2025 were stratified into mild (Katafuchi score 1–4, $n = 25$), moderate (5–8, $n = 21$), and severe (9–12, $n = 14$) groups. Twenty patients undergoing renal tumor resection who provided peritumoral renal tissue served as controls. Serum Gd-IgA1 and PBMC TLR8 were measured by ELISA. TLR8 and Gd-IgA1-specific staining in renal tissue was assessed by immunohistochemistry. Associations with 24-h urinary protein excretion, estimated glomerular filtration rate (eGFR), serum albumin, and pathological score were evaluated using Spearman rank correlation.

Results

Serum Gd-IgA1 and PBMC TLR8 were higher in IgAN than in controls and increased across pathological severity groups. PBMC TLR8 correlated positively with 24-h urinary protein excretion ($r = 0.55$, $P < 0.001$) and negatively with eGFR ($r = -0.48$, $P < 0.001$) and serum albumin ($r = -0.36$, $P < 0.05$). Renal tissue TLR8 showed similar associations with 24-h urinary protein excretion ($r = 0.60$, $P < 0.001$), eGFR ($r = -0.52$, $P < 0.001$), and albumin ($r = -0.49$, $P < 0.01$). PBMC TLR8 was positively correlated with circulating Gd-IgA1 ($r = 0.50$, $P < 0.001$), while renal tissue TLR8 was positively correlated with renal tissue Gd-IgA1 staining ($r = 0.67$, $P < 0.001$). PBMC TLR8 was strongly correlated with renal pathological score ($r = 0.74$, $P < 0.001$).

Conclusions

In this cross-sectional cohort, higher TLR8 expression in PBMCs and renal tissue was associated with greater clinical and histopathological severity of IgAN and with higher Gd-IgA1 levels. These findings support further investigation of TLR8 as a candidate biomarker of disease severity; longitudinal and mechanistic studies are needed before its clinical utility or therapeutic relevance can be established.

Introduction

IgA nephropathy (IgAN) is a common primary glomerular disease characterized by predominant mesangial deposition of IgA-containing immune complexes. Its clinical course is heterogeneous, and a substantial proportion of patients develop progressive kidney dysfunction over time [1, 2]. Galactose-deficient IgA1 (Gd-IgA1) is a central component of the current pathogenic model of IgAN. Aberrant O-glycosylation of IgA1 contributes to the formation of circulating immune complexes and subsequent glomerular injury [2–4]. Circulating and tissue-associated Gd-IgA1 have also been associated with disease activity and histopathological severity [4–7].

Renal biopsy remains essential for establishing the diagnosis and assessing pathological severity, whereas convenient biomarkers that reflect disease burden and pathological activity remain an important research need. Innate immune signaling has attracted increasing attention in IgAN. Toll-like receptors (TLRs) recognize pathogen- or damage-associated molecular patterns and can connect innate immune activation with adaptive immune responses. Previous work has demonstrated altered expression of several TLRs in peripheral blood mononuclear cells (PBMCs) from patients with IgAN, with some TLR expression patterns associated with proteinuria or other clinical features [8].

TLR8 is an endosomal nucleic-acid-sensing receptor expressed predominantly in myeloid-lineage cells. TLR8 signaling can activate MyD88-dependent inflammatory pathways and regulate cytokine production [9–11]. Experimental and clinical studies have implicated TLR signaling in renal inflammation, while TLR7/TLR9 signaling has also been investigated in IgAN [12, 13]. However, the relationship between TLR8 expression, Gd-IgA1, and renal pathological severity in IgAN has not been adequately characterized.

Accordingly, this study investigated TLR8 expression in PBMCs and renal tissue in patients with IgAN and examined its associations with circulating and renal Gd-IgA1-related staining, clinical indicators, and pathological severity. Because the study is cross-sectional, the analyses were designed to evaluate associations rather than causality.

Materials and Methods

Study population

A total of 60 treatment-naïve patients with biopsy-proven IgAN (32 males and 28 females; mean age 44.52 ± 14.73 years) were enrolled at the Affiliated Hospital of Nantong University between December 2022 and February 2025. Patients with secondary IgAN, diabetes, malignant tumors, complicated cardiovascular or cerebrovascular disease, or severe infection were excluded. The IgAN cohort was stratified according to the Katafuchi semi-quantitative pathological score into mild (1–4 points, $n = 25$), moderate (5–8 points, $n = 21$), and severe (9–12 points, $n = 14$) groups.

The control group comprised 20 patients undergoing renal tumor resection (8 males and 12 females; mean age 43.95 ± 5.04 years) from whom peritumoral renal tissue was obtained. The control group was used as a tissue comparator; because these specimens were obtained from patients undergoing tumor surgery rather than healthy volunteers, residual differences between peritumoral tissue and normal kidney tissue cannot be completely excluded.

The study was approved by the hospital ethics committee, and written informed consent was obtained from all participants.

Clinical and pathological assessment

Clinical variables included 24-h urinary protein excretion, serum albumin, estimated glomerular filtration rate (eGFR), hemoglobin, platelet count, C-reactive protein, alanine aminotransferase, erythrocyte sedimentation rate, white blood cell count, neutrophil count, and β 2-microglobulin. Renal pathological severity was represented by the Katafuchi semi-quantitative score, with total scores ranging from 1 to 12 points in the present classification.

Isolation of PBMCs and measurement of TLR8

Fasting venous blood (2 mL) was collected into EDTA-anticoagulated tubes. Samples were diluted and layered over Ficoll separation medium, followed by centrifugation at $400\times g$ for 25 min at 25°C . The PBMC layer at the plasma–Ficoll interface was collected, washed twice with phosphate-buffered saline by centrifugation at $250\times g$ for 10 min, and stored at -80°C .

PBMC lysates were analyzed using a TLR8 ELISA kit. Samples were added to assay wells and incubated at 37°C for 1 h, followed by incubation with a biotinylated detection antibody and enzyme conjugate. TMB substrate was used for color development, the reaction was terminated with 2 M sulfuric acid, and absorbance was measured at 450 nm. TLR8 concentrations were calculated from a standard curve.

Measurement of serum Gd-IgA1

Microtiter plates were coated with an anti-human Gd-IgA1 monoclonal antibody. Standards and serum samples were added and incubated at 37°C for 90 min. After incubation with an HRP-labeled secondary antibody for 60 min, TMB substrate was added. The reaction was terminated with 2 M sulfuric acid, and absorbance was measured at 450 and 650 nm. Gd-IgA1 concentrations were calculated using a four-parameter logistic standard curve.

Immunohistochemistry of renal tissue

Renal tissue specimens were fixed in 4% paraformaldehyde, paraffin-embedded, and sectioned. Sections were deparaffinized and rehydrated, followed by citrate-buffer antigen retrieval. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide, and nonspecific binding was blocked for 30 min. Sections were incubated overnight at 4°C with primary antibodies specific for Gd-IgA1 and TLR8 (1:100), followed by secondary antibody incubation at room temperature for 30 min. Diaminobenzidine was used for chromogenic detection and hematoxylin for counterstaining. Positive staining was evaluated microscopically and quantified.

Statistical analysis Given the cross-sectional design and sample size, these correlation analyses were considered exploratory and were not interpreted as evidence of causality or independent predictive performance.

Statistical analyses were performed using GraphPad Prism 10.3.0 and IBM SPSS Statistics 27. Normally distributed continuous variables were summarized as mean±standard deviation (SD) and compared using Student's t-test or one-way analysis of variance (ANOVA). Non-normally distributed variables were summarized as median (interquartile range) and analyzed using the Mann–Whitney U test or Kruskal–Wallis H test. Associations between continuous or ordinal variables were assessed using Spearman rank correlation coefficients. All tests were two-sided, and $P < 0.05$ was considered statistically significant.

Results

Clinical characteristics

The IgAN cohort included 60 patients and the control group included 20 participants. There were no statistically significant differences in age or sex between the groups. The baseline clinical characteristics of the study participants are summarized in Table 1.

Table 1
Baseline clinical characteristics of patients with IgAN and controls

Variable	IgAN (n = 60)	Control (n = 20)	P value
Male, n (%)	32 (53.3)	8 (40.0)	0.302
Age, years	44.52 ± 14.73	43.95 ± 5.04	0.522
Hemoglobin, g/L	132.38 ± 22.73	130.00 ± 9.06	0.394
Platelets, ×10 ⁹ /L	180.87 ± 60.33	207.45 ± 58.99	0.0717
C-reactive protein, mg/L	2.64 ± 6.95	2.35 ± 5.52	0.493
Alanine aminotransferase, U/L	31.40 ± 8.57	37.35 ± 6.47	0.119
ESR, mm/h	8 (4, 10.75)	7.5 (4.25, 10)	0.982
WBC, ×10 ⁹ /L	4.93 (4.27, 5.36)	4.90 (4.20, 6.28)	0.661
Neutrophils, ×10 ⁹ /L	3.34 (2.91, 4.21)	4.11 (3.23, 4.91)	0.098
β2-microglobulin, µg/mL	1.805 (1.59, 2.19)	1.65 (1.34, 2.26)	0.250

Serum Gd-IgA1 and PBMC TLR8 are associated with clinical severity

Serum Gd-IgA1 was significantly higher in patients with IgAN than in controls (median 5.99 [4.40–8.95] vs. 2.45 [1.52–3.19] pg/mL, $P < 0.001$). Serum Gd-IgA1 increased progressively across the mild, moderate, and severe IgAN groups (4.39 [3.31–4.79], 6.40 [5.29–7.85], and 10.35 [9.41–11.53] pg/mL, respectively; Kruskal–Wallis $H = 61.18$).

PBMC TLR8 expression was significantly increased in IgAN compared with controls and increased with pathological severity. Spearman analysis showed that PBMC TLR8 was positively correlated with 24-h urinary protein excretion ($r = 0.55$, $P < 0.001$) and negatively correlated with eGFR ($r = -0.48$, $P < 0.001$) and serum albumin ($r = -0.36$, $P < 0.05$). Serum Gd-IgA1 showed corresponding correlations with 24-h urinary protein excretion ($r = 0.60$, $P < 0.001$), eGFR ($r = -0.57$, $P < 0.001$), and albumin ($r = -0.43$, $P < 0.001$).

Renal tissue TLR8 and Gd-IgA1-specific staining

Renal tissue TLR8 staining was higher in IgAN than in controls and increased across pathological severity groups. The reported median staining values were 0.06 (0.08–0.09) in controls, 0.54 (0.45–0.63) in mild IgAN, 0.84 (0.78–0.92) in moderate IgAN, and 1.01 (0.94–1.10) in severe IgAN ($H = 62.49$).

Renal tissue staining described in the source as Gd-IgA1 was also higher in IgAN and increased with pathological severity, with reported values of 0.03 (0.06–0.07), 0.20 (0.19–0.23), 0.38 (0.28–0.48), and 0.70 (0.46–0.82) in the control, mild, moderate, and severe groups, respectively ($H = 58.28$). Renal tissue

Gd-IgA1 staining was assessed using the Gd-IgA1-specific immunohistochemical protocol used in this study.

Associations of renal tissue markers with clinical parameters

Renal tissue Gd-IgA1-related staining was positively correlated with 24-h urinary protein excretion ($r = 0.53$, $P < 0.001$) and negatively correlated with eGFR ($r = -0.51$, $P < 0.001$) and serum albumin ($r = -0.39$, $P < 0.05$). Renal tissue TLR8 showed positive correlation with 24-h urinary protein excretion ($r = 0.60$, $P < 0.001$) and negative correlations with eGFR ($r = -0.52$, $P < 0.001$) and albumin ($r = -0.49$, $P < 0.01$).

Association between TLR8 and Gd-IgA1

TLR8 expression in renal tissue was positively correlated with the corresponding renal tissue Gd-IgA1-related staining ($r = 0.67$, $P < 0.001$). PBMC TLR8 was positively correlated with circulating serum Gd-IgA1 ($r = 0.50$, $P < 0.001$). These findings indicate an association between TLR8 expression and Gd-IgA1-related measures, although they do not establish a causal relationship.

Association with renal pathological severity

TLR8 expression in renal tissue was positively correlated with renal pathological score ($r = 0.70$, $P < 0.001$). Notably, PBMC TLR8 showed an even stronger positive correlation with pathological score ($r = 0.74$, $P < 0.001$). Renal tissue Gd-IgA1-related staining and circulating serum Gd-IgA1 were also positively correlated with pathological score ($r = 0.63$ and $r = 0.55$, respectively; both $P < 0.001$).

Discussion

The principal finding of this study is that TLR8 expression in both PBMCs and renal tissue was associated with clinical and histopathological severity in patients with IgAN. In particular, PBMC TLR8 showed a strong correlation with renal pathological score ($r = 0.74$, $P < 0.001$), together with positive association with proteinuria and inverse associations with eGFR and serum albumin. These findings suggest that circulating immune-cell TLR8 expression may reflect disease burden in IgAN and provide a rationale for further biomarker studies.

The present findings are consistent with the broader concept that aberrant immune activation and Gd-IgA1-related responses contribute to IgAN. Gd-IgA1 is a central component of the current multi-hit model of IgAN, and previous studies have linked higher Gd-IgA1 levels with disease activity and pathological injury [4–7]. In the present cohort, circulating Gd-IgA1 was associated with proteinuria, lower eGFR, lower albumin, and higher pathological scores, supporting the clinical relevance of the measured biomarker.

The observation that TLR8 was elevated in PBMCs and renal tissue and increased across pathological severity groups extends previous work on TLR signaling in IgAN. Earlier studies have reported altered expression of TLRs in PBMCs from patients with IgAN, including associations between selected TLR transcripts and proteinuria [8]. Studies of TLR7/TLR9 signaling have further suggested that nucleic-acid-

sensing innate immune pathways may participate in IgAN pathogenesis [12, 13]. However, these observations should not be interpreted as evidence that TLR8 itself causes disease progression.

The positive association between TLR8 and Gd-IgA1 is biologically interesting but remains hypothesis-generating. TLR8 signaling can activate MyD88-dependent inflammatory pathways and regulate cytokines [9–11]. Inflammatory signaling can influence B-cell activity and IgA production, and cytokine-related abnormalities have been implicated in Gd-IgA1 production [14, 15]. Nevertheless, the present study did not include TLR8 inhibition, genetic manipulation, longitudinal sampling, or direct measurement of downstream signaling. Therefore, whether TLR8 lies upstream of Gd-IgA1 production, acts downstream of immune-complex-mediated inflammation, or reflects a shared inflammatory state cannot be determined from the present data.

The correlation between PBMC TLR8 and pathological score is potentially relevant to biomarker development because PBMC sampling is less invasive than renal biopsy. However, a correlation coefficient alone does not establish diagnostic accuracy or clinical utility. Prospective studies should evaluate whether PBMC TLR8 adds predictive value beyond established clinical variables, determine its reproducibility across centers, and assess whether changes in TLR8 over time track changes in disease activity or kidney function.

Several limitations should be considered. First, this was a relatively small, cross-sectional study from a single institution, limiting causal inference and generalizability. Second, the control tissue was obtained from patients undergoing renal tumor resection, and residual differences between peritumoral tissue and truly healthy kidney tissue may influence comparisons. Third, the current dataset does not provide longitudinal renal outcomes or an external validation cohort. Fourth, the study used univariate Spearman correlations; therefore, whether the observed associations remain independent after adjustment for established clinical and pathological covariates cannot be determined. Finally, the present study does not establish diagnostic accuracy or longitudinal prognostic value. The use of the Katafuchi score also limits direct comparison with studies using the Oxford MEST-C system. In addition, because multivariable analyses were not performed, the independent association of TLR8 with pathological severity after adjustment for clinical covariates cannot be determined.

Overall, the data support an association between higher TLR8 expression and greater clinical and pathological severity in IgAN. The findings justify further validation of PBMC TLR8 as a candidate biomarker, but they do not by themselves establish TLR8 as a therapeutic target.

Conclusions

TLR8 expression in PBMCs and renal tissue was increased in patients with IgAN and was associated with proteinuria, eGFR, serum albumin, Gd-IgA1-related measures, and renal pathological severity. PBMC TLR8 showed a strong correlation with pathological score, suggesting potential value as a candidate circulating biomarker of disease severity. Prospective, multicenter, and mechanistic studies are required to determine its reproducibility, incremental predictive value, and biological role.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the Affiliated Hospital of Nantong University. Written informed consent was obtained from all participants.

Consent for publication

Not applicable. This manuscript does not contain any individual person's data in any form that could identify a participant.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

NJ and QM contributed equally to this work. NJ and QM participated in the conception and design of the study, patient recruitment, clinical data collection, laboratory experiments, and data analysis. NJ and QM contributed to the measurement of PBMC TLR8 and serum Gd-IgA1, as well as the evaluation of renal tissue TLR8 and Gd-IgA1-specific staining. NJ and QM contributed to the interpretation of the results and manuscript preparation. YC supervised the study, provided critical scientific guidance, and critically revised the manuscript. All authors reviewed and approved the final manuscript.

Data availability

The datasets generated and/or analyzed during the current study are not publicly available because they contain potentially identifiable clinical information and are subject to institutional and ethical restrictions, but are available from the corresponding author on reasonable request, subject to approval by the relevant institutional authorities.

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