Corticosteroid therapy in IgA nephropathy with minimal proteinuria and high renal pathological score: A single-center cohort study

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Abstract. Currently, there is no clear evidence that advocates the widespread use of corticosteroids for the treatment of immunoglobulin A nephropathy (IgAN) with minimal proteinuria (<1 g/day). The recent Kidney Disease: Improving Global Outcomes Clinical Practice Guideline recommends supportive corticosteroid treatment. In the present study, 45 IgAN patients with high renal pathological scores and minimal proteinuria were enrolled. The patients were randomly divided into two groups. The treatment group received methylprednisolone tablets in addition to angiotensin-converting-enzyme inhibitor (ACE-I) and/or angiotensin-receptor blocker (ARB) treatment. The control group only received ACE-I and/or ARB treatment. In the treatment group, a single dose of 1 mg/kg (maximum 60 mg/day) methylprednisolone tablets was given daily followed by gradually decreasing dosage. The follow-up time of the patients was 3 years. In addition, the underlying mechanisms were investigated. The results indicated that there was a significant reduction in the amount of urinary proteins in the treatment group compared with the control group. At the end of the follow-up, the endpoint event rate of moderate or severe proteinuria and decrease in estimated glomerular filtration rate (eGFR) in the treatment group was significantly lower than the control group. Furthermore, high levels of serum cytokines, interleukin (IL)-4, IL-17, transforming growth factor β1 and IL-21, were detected in patients with IgAN compared with a group of healthy controls. However, the expression levels of STAT5 and chaperone protein, C1GALT1 specific chaperone 1, in IgAN patients were significantly reduced compared with healthy controls. In addition, there was no significant difference in the expression of Jak2, tyrosine kinase 2, STAT1 and STAT4 between the two groups. In conclusion, for IgAN patients with minimal proteinuria and high renal pathological score corticosteroid therapy is likely to be effective. The dysregulation of serum cytokine levels in these patients with IgAN may have a role in the pathogenesis and progression of disease, which is associated with the activation of the JAK/STAT signaling pathway.

Introduction

Immunoglobulin A (IgA) nephropathy (IgAN) is the most common type of primary glomerulonephritis and a principal cause of end-stage renal disease (ESRD) worldwide. IgAN is a heterogeneous disease with different clinical and pathological phenotypes (1,2); therefore, appropriate therapy for IgAN is debated among nephrologists. The recent Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guidelines for IgA nephropathy recommend long-term angiotensin-converting enzyme (ACE) inhibitors or angiotensin-receptor blockers (ARBs) as treatment when proteinuria is 0.5-1 g/day (3). However, after some time patients may experience relapse of proteinuria or more (>1 g/day). These patients developed chronic renal insufficiency in the clinic, which indicated that treatment strategies that only depend on the severity of proteinuria were not comprehensive. Unfortunately, active treatment was frequently delayed until the late clinical stages of the disease, often beyond the time-point at which therapeutic intervention may be successful (4). In the current report, a single-center cohort study was designed to prospectively evaluate the efficiency and safety of corticosteroids for the treatment of IgA nephropathy patients with minimal proteinuria and high renal pathological score based on Haas classification (ztype II), Katafuchi semi-quantitative integration method (score, ≥2) and tubulointerstitial injury score (≥2 points).

Renal biopsy is widely considered to be the gold standard for the diagnosis of IgAN (5). However, renal biopsy has potential
complications that cannot be easily tolerated by the majority of patients, and repeated monitoring is technically difficult. Consequently, highly sensitive and specific non-invasive biomarkers that reflect disease severity and progression are urgently needed for the clinical management of patients with IgAN. A previous study demonstrated that galactose-deficient IgA1 (Gd-IgA1) is one of the key effector molecules in the pathogenesis of IgAN (6). Unfortunately, the underlying molecular mechanisms are still under extensive investigation.

The synthesis of O-glycans starts from the addition of N-acetylgalactosamine to a peptide catalyzed by the enzyme N-acetylgalactosaminyltransferase 2 (CSGALNACT2), and continues with the addition of galactose by core 1 β1-3-galactosyltransferase (CIGALT1). CIGALT1 specific chaperone 1 (Cosmc) is essential for the activity of the mammalian CIGALT (7). The level of Cosmc in B-lymphocytes was reported to be lower in patients with IgAN than in normal controls, and the level of Cosmc was negatively correlated with the level of aberrant glycosylation of IgA1 (8). Galactose-deficient IgA1 (Gd-IgA1) was reported to be elevated in metabolites that were excreted in the urine of patients with IgAN and the levels of urinary Gd-IgA1 were correlated with proteinuria (9). However, as sample preparation (isolation of the IgA1 hinge region) is complicated, the current techniques for detecting Gd-IgA1 are not suitable for clinical application. Furthermore, an increased frequency of B-lymphocytes was observed, particularly in patients with an elevated serum concentration of IgA (10). Notably, polymeric IgA1 is secreted by active polyclonal B-lymphocytes, and T-lymphocytes are involved in the secretion of IgA by B-lymphocytes.

Lymphocytes primed by antigens at mucosal sites produce abnormal amounts of deglycosylated IgA1 and polymeric IgA-IgG immunocomplexes (11,12). T-lymphocytes are critical in the control of the antigen-driven adaptive immune response. In particular, the polarization of T-helper cells can affect IgA nephropathy (13,14). In addition, each of these T-cell subtypes is characterized by certain specialized cytokines and has various immune functions that are dependent on the type of cytokine (15). Therefore, the dysregulation of T-cells may cause B-cells to secrete Gd-IgA1, which deposits in the mesangium and triggers several immune and pathological changes, culminating in the development of IgA nephropathy. In the current study, the levels of T-cell cytokines, Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway proteins and the chaperone protein, COSMC, were analyzed in peripheral blood mononuclear cells (PBMCs) of patients with IgAN prior to treatment and in the normal control group. A group of 49 healthy subjects, recruited at the Minhang Branch of Zhongshan Hospital (Shanghai, China), were selected as the normal control group. Changes in these indicators changed were analyzed, and whether these indicators could predict the severity of kidney disease in IgA nephropathy patients with minimal proteinuria and high renal pathological scores was determined.

Materials and methods

Consent and ethics approval. All healthy and patients donors provided written informed consent prior to sampling. The experiments and procedures were conducted in accordance with the Helsinki Declaration of 1975, and were approved by the Human Ethics Committee of School of Medicine, Fudan University (Shanghai, China).

Inclusion criteria of patients. A total of 45 patients with IgA nephropathy were included in the study. The inclusion criteria were as follows: Diagnosis of IgA nephropathy as confirmed by renal biopsy with minimal proteinuria (0.5-1.0 g/day); with (or without) microscopic hematuria and estimated glomerular filtration rate (eGFR) ≥90 ml/min/1.73 m²; and biopsy findings that include Haas grading of pathological classification ≥type II (16), glomerular injury score of Katafuchi integral ≥2 and/or tubulointerstitial injury score ≥2 points (17). The treatment group (22 cases) was administered with hormone and conventional treatment (ACE-I and/or ARB). The control group (23 cases) only received conventional therapy (ACE-I and/or ARB). The clinical characteristics of the patients are listed in Table I.

Treatment protocol. Patients underwent renal biopsy prior to treatment. The patients were randomly divided into two groups. The treatment group (22 cases) received methylprednisone tablets and ACE-I (Lotensin 10 mg/day) and/or ARB treatment (Losartan 50 mg/day) for 3 years. A single daily dose of 1 mg/kg (maximum 60 mg/day) methylprednisolone tablets was given to the treatment group, which was gradually decreased. When the dose of methylprednisolone was reduced to 10 mg/day, the dose was maintained for 6 months and then further reduced to 5 mg/day for another 6 months. The control group (23 cases) only received ACE-I and/or ARB treatment. The levels of T-cell cytokines and molecules involved in the JAK/STAT pathway and the chaperone protein, COSMC, were detected in the PBMCs of patients with IgAN prior to treatment and in the normal control group. For both groups, if the blood pressure increased, a calcium channel blocker, Norvasc (5 mg/day), was used to control blood pressure (130/80 mmHg). The duration of patient follow-up was 3 years.

Laboratory data. The blood pressure of all patients was measured daily prior to and following treatment. Other tests, including 24-h urinary protein, quantitative analysis of urine sediment, renal function, blood lipid, blood glucose, hemoglobin subunit α1 and eGFR, were also performed monthly prior to and following treatment. eGFR was evaluated using the CKD-EPI equation (18): eGFR (ml/min/1.73 m²) = 141 x min [serum creatinine (SCr/k, 1)] x max [SCr/k, 1]-1.209 x 0.993 age x 1.018 (if female) x 1.159 (if of African descent). The value of k is 0.7 for females and 0.9 for males. The value of α is -0.329 for females and -0.411 for males. Min refers to the minimum SCr/k and 1, and max refers to the maximum SCr/k and 1. All blood samples from patients with IgAN were collected prior to renal biopsy and treatment. The participants in the control group were from patients that underwent physical examination at the hospital.

Cell isolation. PBMCs were collected from fresh heparinized blood by the use of the Ficoll Isopaque gradient centrifugation prior to renal biopsy. Briefly, 5 ml peripheral venous blood samples were obtained from patients and healthy subjects in
a sterile heparinized test tube. The whole blood sample was diluted to a final volume of 10 ml by phosphate-buffered saline (PBS) in a 15-ml centrifuge tube. The diluted blood was then slowly poured into Ficoll-Biocoll separating solution (5 ml; Dakewe Biotech Co., Ltd., Beijing, China) in a centrifuge tube. Finally, PBMCs were isolated following density gradient centrifugation (1,071.1 × g, 20˚C, 20 min). The isolated PBMCs were then washed twice with sterile PBS (566.5 × g, 20˚C, 10 min) and carefully re-suspended in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) in 6-well plate. Cell viability was estimated by trypan blue staining, and cell viability should always be >95%. The cells were counted, and the final cell density was adjusted to 5 -6×10^6 cells/ml. The PBMCs from healthy donors were collected in the same manner. Meanwhile, serum samples were isolated from 5 ml blood prior to renal biopsy, which was centrifuged for 20 min at 2,389.5 × g to obtain ~2 ml plasma. The plasma samples were added to a 24-well culture plate and maintained at ‑20˚C for subsequent experiments. The serum samples from the normal control group were collected in the same manner.

### Analysis of the levels of cytokines

The concentrations of serum interferon-γ (IFN-γ; cat. no. EH0195), interleukin (IL)-4 (cat. no. EH0212), IL-17 (cat. no. EH0228), IL-21 (cat. no. EH0435) and TGF-β1 (cat. no. EH0304) were measured using ELISA kits (Shanghai Weiao Biotechnology Co., Ltd., Shanghai, China; http://www.biotechwell.com), following the manufacturer's protocols. The levels of cytokines were determined by measuring absorbance at 450 nm with a microplate reader. The concentrations of the cytokines in the samples were calculated using standard curves. The data are expressed as pg/ml.

### Analysis of COSMC and JAK/STAT pathway genes by reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from the harvested PBMCs according to the protocol of the TRizol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). cDNA was synthesized by RT using the PrimeScript 1st Strand cDNA Synthesis kit (Takara Bio, Inc., Otsu, Japan). qPCR reactions were prepared using SYBR Premix Ex Taq™ reagents (Takara Bio, Inc.) on a LightCycler (Roche Diagnostics, Basel, Switzerland). The PCR cycling conditions were as follows: 30 sec at 95˚C followed by 40 cycles of 95˚C for 5 sec and 60˚C for 30 sec, and a final step of 95˚C for 15 sec, 60˚C for 1 min and 95˚C for 15 sec. GAPDH served as the internal reference. The sequences of the PCR primers used are as follows: JAK1 forward, 5' -TGC  TCTGTA GTG TGT TGA GG-3' and reverse, 5'-AGG  TCA GCC AGC  TCC TTA CA-3'; JAK2 forward, 5'-GAG  CCT ATC GGC  ATG GAA TA-3' and reverse, 5'-ACT  GCC ATC CCA AGA CAT TC-3'; JAK3 forward, 5'-TCT  CAA GGA GCA GGG TGA GT-3' and reverse, 5'-GTA  GGC AGG CCT TGT AGC TG-3'; Tyk2 forward, 5' -TGA  CCC TGT ATG AGC TGC TG-3' and reverse, 5' -CTG  TCA TCT GAC CCT GAG CA-3'; STAT1 forward, 5'-TTC  AGG AAG ACC CAA TCC AG-3' and reverse, 5'-TGA  ATA TTC CCC GAC TGA GC-3'; STAT4 forward, 5'-AGC  CTT GCG AAG TTT  CAA GA-3' and reverse, 5'-ACA  CCG CAT ACA CAC TTG  GA-3'; STAT5 forward, 5'-ACA  TTT GAG GAG CTG CGA CT-3' and reverse, 5'-CCT  CCA GAG ACA CCT GCT-3'; STAT6 forward, 5'-AGC  CTG TCT CTC TCT TCT TGT-3' and reverse, 5'-AGG  TGC AGT GAG CTT GAA-3'; STAT7 forward, 5'-ACT  CAG TCC TCC TCT CAC AT-3' and reverse, 5'-CTG  TCC CAT TGG ATT TCA-3'; JAK2/STAT1 forward, 5'-TCC  CAC CAC CGG CAT TCT TGA-3' and reverse, 5'-CTG  TCA TCT GAC CCT GAG CA-3'.

### Table I. Clinical characteristics of patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Treatment group (methylprednisolone and ACE-I and/or ARB treatment)</th>
<th>Control group (ACE-I and/or ARB treatment)</th>
<th>P-value*</th>
<th>Healthy subjects</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>22</td>
<td>23</td>
<td>49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>12/10</td>
<td>11/12</td>
<td>0.736</td>
<td>39.32±7.20</td>
<td>0.816</td>
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<tr>
<td>Age (years)</td>
<td>35.12±6.10</td>
<td>34.50±7.10</td>
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</tr>
<tr>
<td>Smoking (n)</td>
<td>4</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drinking (n)</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Course of disease (months)</td>
<td>4.51±1.03</td>
<td>4.09±1.28</td>
<td>0.096</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic pressure (mmHg)</td>
<td>126.14±21.27</td>
<td>130.08±24.70</td>
<td>0.471</td>
<td>125±5.27</td>
<td>0.541</td>
</tr>
<tr>
<td>Diastolic pressure (mmHg)</td>
<td>74.82±14.25</td>
<td>77.24±13.91</td>
<td>0.474</td>
<td>70±10.25</td>
<td>0.128</td>
</tr>
<tr>
<td>HBA1c (%)</td>
<td>5.33±0.60</td>
<td>5.84±0.52</td>
<td>0.999</td>
<td>5.23±3.15</td>
<td>0.130</td>
</tr>
<tr>
<td>FBG (mmol/l)</td>
<td>5.12±1.44</td>
<td>4.86±1.32</td>
<td>0.410</td>
<td>4.58±2.34</td>
<td>0.147</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>4.61±0.31</td>
<td>5.07±0.40</td>
<td>0.909</td>
<td>4.68±0.65</td>
<td>0.087</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>2.02±0.24</td>
<td>2.06±0.17</td>
<td>0.525</td>
<td>2.14±0.68</td>
<td>0.098</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m²)</td>
<td>94.15±11.57</td>
<td>93.13±10.51</td>
<td>0.824</td>
<td>112±10.56</td>
<td>0.076</td>
</tr>
<tr>
<td>24 h Upro (g/day)</td>
<td>0.88±0.15</td>
<td>0.82±0.11</td>
<td>0.094</td>
<td>0.2±0.58</td>
<td>0.032</td>
</tr>
<tr>
<td>Microscopic hematuria (/HP)</td>
<td>22.31±10.20</td>
<td>25.13±13.11</td>
<td>0.626</td>
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<td></td>
</tr>
</tbody>
</table>

*vs. treatment group. ACE-I, angiotensin-converting-enzyme inhibitor; ARB, angiotensin-receptor blocker; FBG, fasting blood glucose; HBA1c, glycated hemoglobin; HP, high-power field; TG, triglyceride; TC, total cholesterol; eGFR, estimated glomerular filtration rate; Upro, urinary protein.
Table II. Pathological characteristics of patients.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Treatment group</th>
<th>Control group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haas II type (n)</td>
<td>11</td>
<td>12</td>
<td>0.814</td>
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<tr>
<td>Haas III type (n)</td>
<td>8</td>
<td>9</td>
<td>0.956</td>
</tr>
<tr>
<td>Haas IV type (n)</td>
<td>3</td>
<td>2</td>
<td>0.992</td>
</tr>
<tr>
<td>Glomerular score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glomerular cell proliferation score</td>
<td>1.22±0.61</td>
<td>1.14±0.52</td>
<td>0.501</td>
</tr>
<tr>
<td>Segmental lesion score</td>
<td>0.83±0.30</td>
<td>0.91±0.44</td>
<td>0.705</td>
</tr>
<tr>
<td>Glomerulosclerosis score</td>
<td>0.72±0.41</td>
<td>0.64±0.32</td>
<td>0.295</td>
</tr>
<tr>
<td>Renal tubule interstitial score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of lesions</td>
<td>1.03±0.51</td>
<td>1.12±0.61</td>
<td>0.499</td>
</tr>
<tr>
<td>Inflammatory cell infiltration</td>
<td>0.74±0.42</td>
<td>0.63±0.32</td>
<td>0.295</td>
</tr>
<tr>
<td>Interstitial fibrosis</td>
<td>0.61±0.20</td>
<td>0.60±0.31</td>
<td>0.090</td>
</tr>
<tr>
<td>Renal tubular atrophy</td>
<td>0.62±0.30</td>
<td>0.71±0.40</td>
<td>0.705</td>
</tr>
</tbody>
</table>

Table III. Clinical characteristics of patients following treatment.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Treatment group</th>
<th>Control group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic pressure (mmHg)</td>
<td>128.14±15</td>
<td>130.44±17.34</td>
<td>0.336</td>
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<tr>
<td>Diastolic pressure (mmHg)</td>
<td>79.90±15.32</td>
<td>80.43±16.74</td>
<td>0.078</td>
</tr>
<tr>
<td>HBA1c (%)</td>
<td>5.90±1.13</td>
<td>5.34±0.92</td>
<td>0.067</td>
</tr>
<tr>
<td>FPG (mmol/l)</td>
<td>5.84±1.62</td>
<td>5.33±1.1</td>
<td>0.257</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>5.22±0.43</td>
<td>5.10±0.56</td>
<td>0.452</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>2.45±0.31</td>
<td>2.28±0.22</td>
<td>0.052</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m²)</td>
<td>85.62±12.22</td>
<td>80.56±13.39</td>
<td>0.410</td>
</tr>
<tr>
<td>24 h Upro (g/day)</td>
<td>0.48±0.17</td>
<td>0.93±0.36</td>
<td>0.001</td>
</tr>
<tr>
<td>Microscopic hematuria (/HP)</td>
<td>19.42±15.30</td>
<td>33.41±16.70</td>
<td>0.991</td>
</tr>
</tbody>
</table>

TC-3'; STAT6 forward, 5'-CAACCACCTTCTACCCCA GA-3' and reverse, 5'-ATGCTCATGGAATCAGG-3'; COSMC forward, 5'-TTTGAGGTTGATGCTTG-3' and reverse, 5'-ATGCCTCATCCTCTGAAAT-3'; GAPDH forward, 5'-GCGAGATCCCTCCAAATCAA-3' and reverse, 5'-GTTCACACCCCATGACGAACAT-3'.

Safety endpoints. Patients were withdrawn from the study if they experienced moderate proteinuria or more (proteinuria >1 g/day) and decline in eGFR (eGFR <90 ml/min/1.73 m²). The duration of follow-up for patients was 3 years. During the entire study, no patient reached the safety endpoints.

Statistical analyses. The data are presented as the mean ± standard error and were analyzed using the SPSS 19.0 software (IBM Corp., Armonk, NY, USA). One-way analysis of variance and Student-Newman-Keuls test were used to analyze the data. The t-test was used to determine the statistical significance in comparison to two groups. Kaplan-Meier was used for prognostic survival analysis and the log-rank test was used to compare survival in the two groups. The Haas classification data were analyzed using χ² test. P<0.05 was considered to indicate a statistically significant difference.

Results

Clinical characteristics of the subjects. There were no statistically significant differences between the two groups (treatment and control group) in sex, age, duration of disease, long-term smoking and drinking, pathological classification and integrity of kidney, blood glucose, blood pressure, blood lipid, 24 h urine protein quantification, microscopic hematuria and eGFR. The clinical characteristics of the patients are listed in Table I, and the pathological characteristics are listed in Table II.

Efficiency. There was a significant reduction in the level of urinary protein in the methylprednisolone treatment group compared with the control treatment group (P<0.05), whereas there was no significant difference in blood glucose, blood lipid and blood pressure (Table III).

Survival analysis. The renal outcome endpoint was selected as reaching a moderate amount of proteinuria or more...
IgAN patients (6.96±1.44 pg/ml), and IL-21 (89.03±34.83) were detected in the levels of serum (31.51±15.56), IL-17 (30.69±12.85), TGF-β1 (203.06±66.63) and IL-21 (89.03±34.83) in the treatment group were decreased compared with the levels prior to treatment (P<0.01; Fig. 3).

Levels of the chaperone protein Cosmc. Research has demonstrated that the expression level of Cosmc in B-lymphocytes was lower in patients with IgAN compared with normal controls, and the level of Cosmc was negatively correlated with the expression of galactose-deficient IgA1 (8). The mRNA expression of COSMC, a chaperone protein, was analyzed from the PBMCs of IgAN patients. The mRNA expression of Cosmc was decreased in IgAN patients compared with healthy controls (P<0.01). The mRNA levels of Cosmc in patients that were treated for 1 month were increased compared with the levels prior to treatment (P=0.0233; Fig. 4).

Expression of proteins that are associated with the JAK/STAT pathway. The JAK/STAT pathway is the principal signaling mechanism for a wide array of cytokines and growth factors. To analyze the mechanism of galactose-deficient IgA1 in IgAN, the expression of Jak1, Jak2, Jak3, Tyk2, STAT1, STAT3, STAT4, STAT5 and STAT6 mRNA was analyzed in the PBMCs of patients with IgAN before treatment and healthy controls. The results revealed that the expression of Jak1, Jak3, STAT3 and STAT6 mRNA was significantly upregulated in the PBMCs of IgAN patients. However, the level of STAT5 mRNA was decreased in the PBMCs of patients IgAN compared with healthy controls. However, there was no significant difference in the levels of Jak2, Tyk2, STAT1 and STAT4 mRNA expression between the IgAN patients and the healthy control group (P>0.05; Fig. 5).

Discussion

IgAN is an immune complex-mediated disease, as circulating immune complexes are deposited exclusively in the glomerular mesangium, which leads to the occurrence and development of IgAN. The circulating immune complexes are mainly composed of Gd-IgA1 and IgG anti-Gd-IgA1 antibodies (19). Microscopic hematuria and proteinuria are the most common presentations of IgAN (20).

Renal biopsy is considered to be the gold standard for the diagnosis of IgAN. Emerging evidence suggests that the presence of histopathologic lesions to be risk factors for the development and progression of IgA nephropathy. In the past few decades, various histological parameters have been used to predict the prognosis of patients with IgAN (19). To date, pathological classifications of IgAN included glomerular score, Lee's classification and Haas classification (21). Each of the classifications has limitations. The semi-quantitative glomerular score system encompasses three pathologic presentations of IgAN (20).
closely associated with the renal outcome (17). The Lee’s and Haas classifications are also known as single-grade scoring systems, which have been widely used in the clinical practice (22,23). In this study, all three histologic classifications, Haas classification, Katafuchi semi-quantitative integral glomerular injury score and tubulointerstitial injury score, were used. In this study, a high renal pathological score considered as Haas classification ≥type II, Katafuchi semi-quantitative integral ≥2 and/or tubulointerstitial injury score ≥2 points, and this was used as a criterion for recruitment of patients.

KDIGO used of <1 g/day urinary protein as a standard for determining the prognosis of patients with primary glomerulonephritis (24). However, KDIGO did not provide a recommended treatment for IgAN patients with asymptomatic hematuria and urine protein in the range of 0.15-1 g/day. KDIGO suggests non-specific supportive treatment (particularly renin-angiotensin system blocking agents) for these patients (grade A, level 1b). However, there is currently no clear evidence that advocates the widespread use of corticosteroids for the treatment of IgAN with minimal proteinuria (<1 g/day) (3). Studies have suggested that the incidence of renal interstitial vascular disease in IgAN was high, and mild clinical manifestations of IgAN in parents may predispose offspring to severe renal pathological lesions, which may lead to severe renal dysfunction (25-27). Therefore, guiding the treatment of IgAN according to clinical proteinuria has deficiencies.
In the absence of optimal and comprehensive data from randomized trials of IgAN, the Supportive vs. Immunosuppressive Therapy of Progressive IgA Nephropathy (STOP-IgAN) trial has been conducted. The STOP-IgAN trial aimed to investigate whether immunosuppressive agents are effective for patients with high-risk IgAN. In the STOP-IgAN study, systemic steroid/immunosuppressive treatment significantly decreased proteinuria, but did not stop disease progression (28,29). In the present study, patients with minimal proteinuria (<1 g/day) and a high renal pathological score, that underwent conventional therapy (ACE-I and/or ARB treatment) and methylprednisolone corticosteroid therapy, were selected for recruitment. The endpoint for renal outcome was a moderate level of proteinuria and a high renal pathological score, who were given conventional therapy (ACE-I and/or ARB treatment) and methylprednisolone corticosteroid therapy. The moderate amount of proteinuria or more was >1 g/day and eGFR decline <90 ml/min/1.73 m². The follow-up time of patients was 3 years. At the end of the follow-up, the endpoint event rate of moderate proteinuria or more, and eGFR decline in the methylprednisolone treatment group was significantly lower than the control group that did not receive methylprednisolone (P<0.05). In addition, the results demonstrated the importance of obtaining biopsies in the clinical management of IgAN, as the pathological changes in the kidneys were serious in some patients with minimal proteinuria (<1 g/day). In IgAN patients with small amount of proteinuria (<1 g/day), for decisions on treatment options-whether to select conservative treatment involving ACE inhibitors or ARBs and whether to use combined therapy-clinicians should base the decision for treatment on the pathological grading, rather than proteinuria alone.

Renal biopsy has potential complications and repeated monitoring is technically difficult. Therefore, a clearer understanding of the molecular mechanisms should facilitate the identification of specific non-invasive biomarkers that reflect disease severity and progression. IgAN, one of the most common types of primary glomerulopathy globally, is characterized by the glomerular mesangial deposition of Gd-IgA1 in the kidney (6). The IgA1 hinge region is composed of the hypogalactosylated O-glycosides that result in an increased tendency for non-covalent self-aggregation and polymerization of circulating IgA1. Several studies have supported that Gd-IgA1 has a pivotal role in renal tissue injury (30). The origin of this galactosylation defect remains unclear. Increased synthesis of Gd-IgA1 may be the result of an imbalance between the activities of enzymes involved in post-translational galactosylation in the Golgi apparatus (31,32).

The synthesis of O-glycans begins with the addition of N-acetylgalactosamine to a peptide catalyzed by the enzyme CGALNACT2, which then continues with the addition of galactose by the enzyme CIGALT1 (33). Furthermore, CIGALT1 has been shown to be assisted by the chaperone protein, Cosmc, which is crucial for ensuring the stability and enzymatic activity of galactosyltransferase (34,35). Notably, Cosmc expression is decreased in the B-cells of patients with IgAN and is negatively correlated with Gd-IgA1 (8). In the current study, the level of Cosmc mRNA was analyzed in the PBMCs from patients with IgAN by RT-qPCR. The mRNA expression of Cosmc was lower in IgAN patients compared with healthy control subjects.

The synthesis of Gd-IgA1 is associated with imbalanced activity between enzymes, including CIGALT1 and the dysregulation of CD4+ T-cell subset. Accumulating evidence suggests that Gd-IgA1 deposits may be produced from mucosal plasma cells, and associated with T-cell dysregulation (36). There is a growing body of evidence indicating that an impaired mucosal IgA response may lead to the impaired depletion of mucosal antigens (36). Mucosal immunity depends on the equilibrium between the responsiveness and tolerance of antigens, and the CD4+ T-cell subset has a key role in maintaining or disrupting this delicate balance (37). Previous studies have demonstrated that the predominant cytokines secreted by T-lymphocytes in IgAN were the Th2 type, and that the Th2 cytokine, IL-4, may have a critical role in leading the glycosylation of the IgA1 hinge region (38) and renal fibrosis (39); this cytokine production may lead to the overproduction of Gd-IgA1, which is prone to deposition in the mesangium (40). Strong polarization toward the production of Th1 type cytokines in IgAN was also indicated by another study (41), and Th1 predominance was reported to be associated with the progression of renal injury in IgAN (41). In addition, a previous report showed that patients with IgAN exhibited increased serum levels of Th17 and Th17 family cytokines. In addition, serum levels of IL-17A and IL-21 were elevated in IgAN, and serum IL-17A was correlated with 24-h proteinuria (42). Furthermore, a recent study reported that the number of tonsillar regulatory T-cells was decreased in patients with IgAN, which was negatively correlated with the number of dimeric IgA-producing cells (43).

The JAK/STAT signaling pathway mediates the biological responses induced by numerous cytokines, and it is particularly important for differentiation of helper T-cells. Cytokines bind to the cell surface receptors of immune and non-immune cells to activate the JAK-STAT signaling pathway, which affects the function of CD4+ T-cells by upregulating the expression of specific target genes (44-46). In the present study, assays were performed to detect the levels of T-cell cytokines in serum samples and JAK/STAT pathway proteins in the PBMCs of all patients with IgAN prior to treatment and in the healthy control group (49 healthy subjects were selected as the normal control group) There was no significant difference in sex and
The results revealed that higher levels of serum IL-4, IL-17, TGF-β1 and IL-21 were detected in patients with IgAN compared with the normal controls. There was no significant difference in IFN-γ expression between patients IgAN and the normal control group. Furthermore, the patients with IgAN were treated for 1 month (AF-IgAN), and the serum levels of IL-4, IL-17, TGF-β1 and IL-21 were decreased compared with the levels before treatment. Additionally, the results indicated that the expression of Jak1, Jak3, STAT3 and STAT6 mRNA was significantly upregulated in the PBMCs of patients with IgAN. However, STAT5 mRNA expression was decreased in the PBMCs of patients with IgAN. There was no significant difference in the expression of Jak2, Tyk2, STAT1 and STAT4 mRNA between patients with IgAN and the normal control group. These results indicated that the imbalance of dysregulation of CD4+ T cells subsets in IgAN may have a role in disease pathogenesis and progression, which is associated with the activation of the JAK/STAT signaling pathway. The levels of T-cell cytokines (IL-4, IL-17, TGF-β1 and IL-21) may therefore represent novel therapeutic targets and biomarkers for the treatment and monitoring of IgAN.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article.
Authors' contributions

YT, HH and XX conceived the study, designed the study protocol and wrote the paper. YT, HH and PH performed the experiments. WS and XC analyzed the data. YT and HH wrote the final version of the paper. All authors reviewed and approved the final version of the manuscript.

Ethics approval and consent to participate

All healthy and patient donors provided written informed consent prior to sampling. The experiments and procedures were conducted in accordance with the Helsinki Declaration of 1975, and were approved by the Human Ethics Committee of School of Medicine, Fudan University.

Patient consent for publication

The patients in the present study agreed to publication of the anonymous data.

Competing interests

The authors declare that they have no competing interests.

References


