Original Article

Uric acid correlates with the severity of histopathological parameters in IgA nephropathy

Juhani Myllymäki¹, Teemu Honkanen², Jaana Syrjänen¹,³, Heikki Helin⁴, Immo Rantala², Amos Pasternack¹ and Jukka Mustonen¹,³

¹Medical School, University of Tampere, ²Department of Pathology and ³Department of Internal Medicine, Tampere University Hospital and ⁴Division of Pathology, HUSLAB, Helsinki University Hospital, Finland

Abstract

Background. Immunoglobulin-A nephropathy (IgAN) is the most common chronic glomerulonephritis worldwide. Many clinical and histopathological risk factors for progression have been found previously. Recently, metabolic risk factors, such as hyperuricaemia and hypertriglyceridaemia, also have been associated with the progression of IgAN.

Methods. In the present study we correlated clinical and metabolic risk factors with histopathological parameters in 202 patients with IgAN. Morphological changes in glomerular, tubulointerstitial and vascular tissue were semiquantitatively graded into three classes. Mesangial proliferation activity and the amount of inflammatory cells were also evaluated by immunohistochemical staining of Ki-67 (MIB-1), CD45 (LCA) and CD68 stainings. Serum uric acid, triglycerides and cholesterol, urine protein excretion (UPE), blood pressure and body mass index (BMI) were measured. Smoking habits and occurrence of diabetes mellitus also were evaluated. The independent role of serum uric acid in the development of renal morphological changes was evaluated in multivariate analysis.

Results. Serum uric acid and UPE level correlated with several histological parameters. Uric acid level showed the strongest correlation with tubulointerstitial changes and UPE with glomerulosclerosis. The level of serum triglycerides correlated with interstitial fibrosis and hyaline arteriolosclerosis. Blood pressure correlated with hyaline arteriolosclerosis, glomerulosclerosis and tubulointerstitial changes. BMI and diabetes mellitus correlated with both tubulointerstitial and vascular changes. We found no significant correlations between histopathological parameters and smoking habits or serum cholesterol level. Serum uric acid had independent associations with the presence of tubular atrophy and interstitial fibrosis and inflammation.

Conclusions. We conclude that many metabolic factors are univariately associated with renal morphological findings in IgAN. These same factors are central in the metabolic or insulin resistance syndrome and may have a pathogenetic role in the progression of IgAN. Serum uric acid may have an independent role in development of tubulointerstitial lesions as well as being associated with inflammation in renal tissue of patients with IgAN.

Keywords: body mass index; glomerulonephritis; renal pathology; serum cholesterol; serum triglycerides; serum uric acid

Introduction

The aetiology and pathogenesis of immunoglobulin-A nephropathy (IgAN) remain unclear [1]. It would appear that microbial infection in the respiratory or gastrointestinal tract may induce an abnormal systemic immunological IgA response in susceptible persons with the appropriate genes [2]. For unknown reasons, clinical and histopathological manifestations of IgAN are variable. In a previous study we found that in addition to hypertension and proteinuria, hyperuricaemia and hypertriglyceridaemia are independent risk factors for progression of IgAN [3]. Of microscopic morphological changes, severe glomerulosclerosis and, especially, tubulointerstitial damage have been reported to be the strongest risk factors for progression in IgAN [4].

Clinical risk factors for IgAN progression, for example hypertension, proteinuria, hyperuricaemia and hypertriglyceridaemia, also have been reported to be risk factors for cardiovascular diseases.
There is evidence that uric acid can promote the oxygenation of low-density lipoproteins and increase the production of free oxygen radicals [5]. In consequence of these processes, uric acid might specifically promote atherosclerosis [5]. Serum triglyceride concentration has been found to correlate with the narrowing of coronary arteries by atherosclerotic plaque. The initial triglyceride concentration may also entail a risk of progression of coronary artery disease as evaluated by angiography [6].

It is well known that hypertension, especially glomerular hypertension, can cause glomerular injury and also morphological changes in renal vascularity [7]. Proteinuria correlates mainly with tubulointerstitial but also with glomerular damage in IgAN. Much less is known of histological changes related to elevated serum levels of uric acid or triglycerides. In one experimental study, triglyceride-rich lipoproteins correlated positively with the development of glomerulosclerosis [8].

In an experimental model of hyperuricaemic nephropathy the renal injury observed was located mainly in tubulointerstitial tissue [9]. A recent rat model provided evidence that uric acid may be a true mediator of renal disease and progression [10].

In the present study we investigated the correlations between independent risk factors for progression and histopathological changes in IgAN. The focus of interest was on correlations of uric acid with renal injury. The role of serum uric acid as an independent risk factor for various histopathological changes also was investigated. In addition, other factors, such as cholesterol, triglycerides, body mass index (BMI), diabetes and smoking habits, were studied in the context of renal morphology.

**Subjects and methods**

**Patients**

The original patient population comprised all 221 adult IgAN patients diagnosed in Tampere University Hospital during a period of 11 years from January 1980 to December 1990. A renal biopsy specimen was considered representative if it contained four or more glomeruli. Of all IgAN cases, 202 fulfilled this criterion: 130 (64%) men and 72 (36%) women. IgAN was diagnosed when there was IgA as a sole or predominant glomerular immunofluorescence (IF) finding in the biopsy. The median age was 41 years (range: 16–78 years). The ethical committee of Tampere University Hospital has approved the present study.

**Renal pathological evaluation**

Paraffin sections for light microscopy were stained by the haematoxylin-eosin, periodic acid–Schiff reaction, Masson’s trichrome and periodic acid-silver methenamine methods. Mesangial cellularity, glomerulosclerosis, tubular atrophy, interstitial fibrosis and inflammation, hyaline arteriosclerosis and arterial intimal fibrosis were evaluated. The histological parameters mentioned were semiquantitatively graded into three groups (normal, mild and marked). The grading was modified mainly after the revised Banff 97 classification for renal allograft pathology [11]. The histopathological grading schema we used is presented in Table 1.

For multivariate analysis we graded aforementioned histopathological parameters into two groups: normal and abnormal (mild or marked). Also, the relative number of glomeruli with extracapillary proliferation or totally obliterated glomeruli was studied. The histopathological evaluation was done by one investigator who was not aware of the clinical data.

**Immunohistochemistry**

For light microscopic immunoperoxidase staining, 3-μm paraffin sections were cut onto ChemMate™ capillary gap microscope slides (DakoCytomation Denmark A/S, Glostrup, Denmark). Proliferation activity in the mesangial areas was investigated by staining the Ki-67 antigen (anti-Ki-67, clone MIB-1; DakoCytomation Denmark A/S) (1:100) identifying cells in all but the G0 phase of the cell cycle. Inflammatory cells were investigated by staining the CD45 [leukocyte common antigen (LCA) clone 2B11; DakoCytomation Denmark A/S] (1:2000) and CD68 (clone PG-M1; DakoCytomation Denmark A/S) (1:150) antigens identifying lymphocytes and macrophages, respectively.

Antigen retrieval was performed on rehydrated sections in a microwave oven at 850 W for two 7 min cycles using Tris–EDTA buffer (pH 9.0) as the retrieval solution. Immunostaining was carried out in a TechMate™ 500 Immunostainer (DakoCytomation Denmark A/S) using the EnVision™ polymer technique (DakoCytomation Denmark A/S). Diaminobenzidine (DAB) was used as a chromogen and haematoxylin as a nuclear stain. The specificity of immunohistochemistry was controlled by omitting the primary antibodies or replacing them with irrelevant antisera. Known positive tissue samples were also used to confirm the staining reliability of all separate staining batches.

**Quantification of immunohistochemically stained cells**

Immunoperoxidase staining results were investigated at 400× magnification with an ocular grid (0.0625 mm²). In the glomeruli, MIB-1- and LCA-positive cells were counted as the number of cells per mm² of glomerular area. The amount of glomerular CD68+ cells was graded into five groups from 1 (negative) to 5 (strong positive), because their staining pattern made it impossible to obtain exact cell numbers. All glomeruli were counted. The area of the glomeruli and tubulointerstitium were determined by point counting using a 100-point square lattice in the eyepiece. Tubulointerstitial LCA-positive cells per mm² were counted in 10 adjacent fields.

**Clinical definitions**

At the time of renal biopsy, 11 patients had purpura, two of them the typical picture of Henoch–Schönlein syndrome (abdominal pain, arthritis and purpura). Ten patients had diabetes mellitus. No patient had systemic lupus erythematosus or liver cirrhosis.
All clinical parameters were measured at the time of renal biopsy. Blood pressure was measured by sphygmomanometer after rest. Mean arterial pressure (MAP) was calculated by the formula:

\[
\text{Diastolic blood pressure} + \frac{1}{3}(\text{Systolic blood pressure} - \text{Diastolic blood pressure})
\]

Urine protein excretion (UPE) was measured in a 24h collection of urine. The serum uric acid concentration was measured in 172 (85%) patients. Two patients were using allopurinol for gout. Serum triglyceride concentrations were measured enzymatically after an overnight fast at the time of biopsy. None of the patients was on lipid-lowering medication at the time of biopsy. Serum triglyceride was measured in 172 (85%) and cholesterol in 168 (83%) patients. The BMI was calculated as weight (kg) divided by the square of height (m). Smoking habits were divided into two groups: patients who had never smoked \((n=117)\) and patients who had smoked earlier or were smokers at the time of the biopsy \((n=87)\). Also, the total numbers of cigarettes consumed was calculated.

**Statistics**

Comparison of non-normally distributed continuous variables was done by Mann–Whitney \(U\)-test or Kruskal–Wallis test when appropriate. Spearman correlation coefficients were calculated for statistically significant correlations and when studying the relationship between the relative amounts of obliterated glomeruli or glomeruli with extracapillary proliferation and clinical measurements. We also used Spearman correlations to evaluate the correlations of glomerular MIB-1, LCA and tubulointerstitial LCA with clinical parameters. The chi-square test was used to evaluate correlations of diabetes and smoking habits with histological parameters. Multivariate analysis was done by stepwise logistic regression analysis. The SPSS statistical package was used for analysis.

**Results**

**Clinical risk factors, renal biopsy and immunohistochemical findings**

Data on clinical parameters are given in Table 2. Serum uric acid levels correlated significantly with those of cholesterol, triglycerides, creatinine, UPE and MAP \((P < 0.001\) in all correlations). A summary of biopsy evaluations is presented in Table 3. The mean number of glomeruli was 12 (range: 4–56). The most frequent abnormal finding was glomerulosclerosis, which was observed in 177 (88%) samples. Mesangial hypercellularity was the second commonest abnormal biopsy finding, occurring in 139 out of 202 (69%) samples. In 12 (6%) specimens there were either normal...
glomeruli or only minimal morphological changes. Totally obliterated glomeruli were seen in 73 (36%) specimens and up to 82% of the glomeruli. Obliterated glomeruli were found mostly immediately beneath the renal capsule. Cellular extracapillary proliferation was found in 20 (10%) samples. Descriptives of immunohistochemical parameters are summarized in Table 4. In the five groups of glomerular CD68 positivity the number of patients was 60 (group 1), 54 (group 2), 26 (group 3), 15 (group 4) and 13 (group 5).

Univariate associations between clinical parameters and histopathological changes

Univariate associations between clinical factors and histopathological parameters are summarized in Tables 5–8. Serum uric acid and creatinine concentrations correlated significantly with the level of interstitial inflammation (Table 5). Of the clinical factors evaluated, serum uric acid correlated most markedly with tubular atrophy (Figure 1) and interstitial fibrosis. Mesangial cellularity was the only histopathological parameter that did not correlate with serum uric acid concentration.

The serum triglyceride concentration correlated positively only with the severity of interstitial fibrosis and hyaline arteriolosclerosis (Table 6). Patients without interstitial fibrosis had a median of serum triglyceride levels of 1.3 mmol/l, while in those with interstitial fibrosis the median was 1.7 mmol/l ($P<0.01$). There were no statistically significant correlations between serum cholesterol concentration and histopathological parameters (Table 6).

Blood pressure correlated with the severity of glomerulosclerosis, tubular atrophy, interstitial fibrosis and hyaline arteriolosclerosis, but not with mesangial cellularity or interstitial inflammation (Table 7). Systolic blood pressure and MAP, but not diastolic blood pressure, correlated statistically significantly with arterial intimafibrosis. The strongest correlation of MAP was with arteriolosclerosis.

The amount of UPE ($P<0.001$) and serum cholesterol level ($P<0.05$) correlated significantly with the relative number of glomeruli with extracapillary proliferation. The level of UPE also correlated with the severity of glomerulosclerosis, tubular atrophy, interstitial fibrosis and hyaline arteriolosclerosis (Table 5). The strongest correlation was with the level of glomerulosclerosis.

BMI correlated positively with tubular atrophy, interstitial fibrosis, hyaline arteriolosclerosis and arterial intimafibrosis (Table 8). Patients with diabetes mellitus evinced significantly more severe histopathological changes in glomerular, tubulointerstitial and also in vascular tissue (Table 8). The most prominent of the aforementioned associations were the association of diabetes with tubular atrophy ($P<0.01$) and hyaline arteriolosclerosis ($P<0.01$). There were no significant correlations between smoking habits or lifetime total cigarettes smoked and histological parameters.

The relative number of obliterated glomeruli correlated significantly with both systolic ($P<0.001$) and diastolic ($P<0.01$) blood pressure, MAP ($P<0.01$), serum triglycerides ($P<0.05$) and uric acid ($P<0.001$).

Associations of immunohistochemical measurements with clinical and histopathological parameters

Semiquantitatively graded mesangial cellularity correlated significantly with glomerular MIB-1 ($P<0.05$) and CD68 ($P<0.01$) and nearly significantly with glomerular LCA ($P=0.051$). Tubulointerstitial LCA

Table 2. Clinical parameters in 204 patients with IgAN at the time of renal biopsy

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>Mean (2 SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP (mmHg)</td>
<td>139 (42)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>86 (26)</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>103 (14)</td>
</tr>
<tr>
<td>Proteinuria (g/24 h)</td>
<td>0.97 (3.0)</td>
</tr>
<tr>
<td>Serum uric acid (mmol/l)</td>
<td>0.36 (0.21)</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>5.6 (3.0)</td>
</tr>
<tr>
<td>Serum triglycerides (mmol/l)</td>
<td>1.6 (1.9)</td>
</tr>
<tr>
<td>Serum creatinine (mmol/l)</td>
<td>106 (82)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.7 (9.6)</td>
</tr>
</tbody>
</table>

BP, blood pressure.

Table 3. Renal biopsy findings in 202 patients with IgAN

<table>
<thead>
<tr>
<th>Finding</th>
<th>No. of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glomeruli</strong></td>
<td></td>
</tr>
<tr>
<td>Normal morphology or minimal lesions</td>
<td>12 (6)</td>
</tr>
<tr>
<td>Mesangial cellularity</td>
<td></td>
</tr>
<tr>
<td>Mild hypercellularity</td>
<td>94 (47)</td>
</tr>
<tr>
<td>Marked hypercellularity</td>
<td>46 (23)</td>
</tr>
<tr>
<td>Glomerulosclerosis</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>126 (62)</td>
</tr>
<tr>
<td>Marked</td>
<td>52 (26)</td>
</tr>
<tr>
<td><strong>Tubulointerstitial tissue</strong></td>
<td></td>
</tr>
<tr>
<td>Normal morphology</td>
<td>92 (46)</td>
</tr>
<tr>
<td>Tubular atrophy*</td>
<td>80 (40)</td>
</tr>
<tr>
<td>Interstitial fibrosis*</td>
<td>61 (30)</td>
</tr>
<tr>
<td>Interstitial inflammation*</td>
<td>52 (26)</td>
</tr>
<tr>
<td><strong>Vascular tissue</strong></td>
<td></td>
</tr>
<tr>
<td>Normal morphology</td>
<td>80 (40)</td>
</tr>
<tr>
<td>Hyaline arteriolosclerosis*</td>
<td>84 (42)</td>
</tr>
<tr>
<td>Arterial intimafibrosis*</td>
<td>81 (40)</td>
</tr>
<tr>
<td>Obliterated glomeruli</td>
<td>73 (36)</td>
</tr>
<tr>
<td>Extracapillary proliferation</td>
<td>20 (10)</td>
</tr>
</tbody>
</table>

*Mild, moderate or marked.

Table 4. Glomerular MIB-1 and LCA and tubular LCA findings (cells/mm²) in 174 patients with IgAN

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean (2 SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular MIB-1</td>
<td>52.3 (155)</td>
</tr>
<tr>
<td>Glomerular LCA</td>
<td>130 (190)</td>
</tr>
<tr>
<td>Tubulointerstitial LCA</td>
<td>10.2 (22.8)</td>
</tr>
</tbody>
</table>
correlated with the presence of tubular atrophy \((P < 0.01)\) and interstitial inflammation \((P < 0.001)\).

Correlations between immunohistochemical and clinical parameters are summarized in Table 5. The amount of UPE correlated significantly with glomerular LCA \((P < 0.001)\) and tubulointerstitial LCA \((P < 0.01)\). Serum uric acid correlated with tubulointerstitial LCA \((P < 0.01)\). Patients with hyperuricaemia (males: > 0.45 mmol/l; females: > 0.32 mmol/l) had significantly higher numbers of tubulointerstitial LCA-positive cells \((P < 0.05)\). Systolic blood pressure correlated with tubular LCA \((P < 0.01)\).

Correlations of clinical and metabolic parameters with histopathological parameters in multivariate analysis

Because our aim was to investigate the independent role of serum uric acid in the development of morphological changes, all histopathological parameters correlating with uric acid in univariate analysis were studied by multivariate analysis. All clinical or metabolic factors correlating significantly with those histopathological parameters were included in the model. Of the blood pressure-measuring parameters, MAP was included. Serum uric acid was found to be correlated independently with the presence of tubular atrophy \((P < 0.01)\) when studied in a model with MAP, creatinine, UPE, BMI, diabetes and age and with interstitial fibrosis \((P < 0.05)\) when studied in a model with MAP, creatinine, UPE, triglycerides, BMI and age. MAP had independent correlations with glomerulosclerosis \((P < 0.05)\) and hyaline arteriolosclerosis \((P < 0.05)\). Age correlated independently with tubular atrophy \((P < 0.001)\), interstitial fibrosis \((P < 0.01)\), hyaline arteriolosclerosis \((P < 0.05)\) and arterial intima-fibrosis \((P < 0.001)\). Serum creatinine had independent correlation with interstitial inflammation \((P < 0.01)\).

Discussion

The present study brought out many statistically significant correlations between clinical factors and histopathological parameters in IgAN. The strongest correlations were between serum uric acid and tubular atrophy, serum triglycerides and interstitial fibrosis, blood pressure and renal vascular changes, as well as proteinuria and glomerulosclerosis. Serum uric acid seems to be an independent risk factor for the development of tubulointerstitial damage.

Numerous studies have been made of the risk factors for progression of renal insufficiency in IgAN. Hypertension, elevated serum creatinine concentration, severe proteinuria, old age, male sex and the absence of macroscopic haematuria have been found to be independent risk factors [4]. In our recent study, hyperuricaemia and hypertriglyceridaemia also emerged as independent risk factors for progression [3]. Of the histological parameters evaluated in earlier studies, marked glomerulosclerosis and, especially, tubular lesions may be the strongest risk factors for progression in IgAN [4].

Mesangial hypercellularity, observed by semiquantitative analysis in 69% of the biopsy specimens, was
further analysed by differential immunohistochemical stainings. It turned out that both inflammatory cell infiltration (lymphocytes and macrophages) and, to a lesser extent, cellular proliferation contributed to the hypercellularity. Interestingly, the number of inflammatory cells measured by quantitative immunohistochemistry, but not the number of proliferating cells, correlated with UPE.

It has been found recently that hyperuricaemia is an independent risk factor for progression in IgAN [3,12]. Kang and colleagues [10] found in experimental study that hyperuricaemic rats had more severe glomerular, vascular and interstitial changes than controls. Hyperuricaemia rats also showed also increased renin and COX-2 expression in blood vessels. Allopurinol blocked the changes in renal function and histology. They also assume that uric acid may have an independent role in the pathogenesis and progression of renal disease. In some experimental studies, hyperuricaemia and hyperuricosuria have been induced by uric acid and oxonic acid diet. The most significant light-microscopic findings were interstitial mononuclear infiltrations and fibrosis and tubular changes, while glomeruli and blood vessels were normal in morphology [9].

In the present study, the serum uric acid concentration correlated most strongly with chronic tubulointerstitial changes and less strongly with glomerulosclerosis. The serum levels of uric acid also correlated with inflammatory cell infiltration both in glomeruli and interstitium, as evaluated by immunohistochemical methods. There were independent associations between serum uric acid and tubular atrophy and interstitial fibrosis. It appears possible that high serum uric acid may independently cause progression in IgAN by damaging tubulointerstitial tissue. Because of the central role of tubulointerstitial damage in progression of IgAN, the significance of high uric acid levels may be quite important.

Recent studies have shown that serum triglycerides predict a decline in renal function in IgAN [3] and in healthy women [13], in whom high triglyceride levels are also a risk factor for proteinuria [13]. In in vitro studies it has been observed that triglyceride-rich
lipoproteins may promote mesangial cell proliferation [14]. In the present study, serum triglyceride concentrations correlated significantly with renal morphological changes. The strongest correlation was found between triglycerides and interstitial fibrosis, but it also correlated with hyaline arteriolosclerosis. Based on our multivariate analysis, serum triglycerides may not have independent associations with the presence of renal histopathological changes.

Of the cardiovascular risk factors, serum cholesterol also has been reported to be a risk factor for progression in IgAN [15]. In our previous study we, however, did not find cholesterol to be an independent risk factor for progression [3]. It has also been reported that the total cholesterol concentration does not predict the development of proteinuria or renal insufficiency in healthy persons [13]. Excluding extracapillary proliferation, the cholesterol level did not correlate with any other renal histopathological parameter in the present study.

It has been reported that obesity with hypertension or hyperlipidaemia may accelerate renal damage [16]. It was found recently that obesity might promote proteinuria in healthy men [17]. Bonnet and associates [18] found high BMI to be an independent risk factor for chronic renal failure in IgAN. In the present study, we found BMI to correlate with the level of tubulo-interstitial and vascular lesions. Strict weight control might be a good clinical practice also among IgAN patients.

Smoking has been reported to be risk factor for renal insufficiency in diabetic nephropathy and inflammatory renal diseases [19]. Tozawa and colleagues [17] found that smoking might promote proteinuria in healthy men. In our previous study, smoking was not an independent risk factor for progression in IgAN [3] and in the present study, using the same patient population, there were no significant correlations between smoking and histological parameters.

We conclude that all statistically significant clinical risk factors for progression of IgAN correlated with morphological changes in renal tissue. While serum uric acid correlated most strongly with tubulo-interstitial changes, blood pressure was the most significant factor predicting changes in vascular morphology. The level of serum triglycerides, but not total cholesterol, would seem to be associated with renal morphological changes. Factors associated with metabolic or insulin resistance syndrome, especially uric acid, triglycerides and BMI, correlated with tubulo-interstitial damage, which has been reported to be the most significant histopathological risk factor for progression in IgAN. A high level of serum uric acid seems to have an independent role in the development of tubulo-interstitial changes in IgAN. Further studies need to be performed to clarify whether uric acid-lowering therapy has an influence on the progression of IgAN.

Acknowledgements. This study was supported financially by the Medical Research Fund of Tampere University Hospital and the Finnish Kidney Foundation.

Conflict of interest statement. None declared.

References