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Can we use serum and urine immunoglobulin levels as biomarkers in patients with glomerulonephritis?

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Abstract

Introduction: Non-invasive biomarkers for assessing disease activity and progression are continuously being sought, but difficult to validate. For glomerulonephritis (GN), various molecules in both blood and urine are undergoing stringent research. Immunoglobulin (Ig) levels have also been sought as potential biomarkers with variable results.
Objectives: We aimed at determining the utility of serum and urine Ig levels to ascertain severity of proteinuria and renal functions in GN patients.
Materials and Methods: Blood and urine of 25 GN patients and 13 healthy controls were tested for Ig levels including IgG, IgM, IgA and IgE. The degree of proteinuria, renal functions and histopathological features were recorded from the case files of these patients.
Results: The mean serum IgM and IgA levels were significantly high in GN patients compared to controls. However, two patients had high IgM and one had high IgA levels. Three patients had low IgG levels but did not correlate with urinary loss. Moreover IgGuria was not different in patients with or without severe disease. In patients with acute GN IgMuria was more prevalent. While in chronic GN, IgAuria was more common. Mean serum IgE levels were more in healthy controls and did not correlate with renal dysfunction. However, mean IgEuria was more pronounced in patients with renal dysfunction
Conclusion: The utility of serum and urine Ig as biomarkers of disease activity and progression in GN patients is still debatable and require further studies. Abnormal levels of these proteins in blood of GN patients require further workup to rule out any concomitant pathology.

Introduction

In patients with glomerulonephritis (GN), several serum and urinary biomarkers have been evaluated for the assessment of disease activity. However, proteinuria remains an important therapeutic target (1-5). Serum immunoglobulin levels have also been investigated as the potential biomarkers to predict disease activity or progression in these patients. A high serum IgA is often seen in IgA nephropathy. Low IgG in nephrotic syndrome (NS) may be due to urinary loss or impaired B cell maturation for IgG production. This immaturity may also relate to increased IgE production in these patients (6,7). High IgE levels have been documented in various GN with or without atopy (6,8,9). There are reports that IgE levels correlate with disease activity or response to treatment. However, its direct role in the pathogenesis of GN is not clear (9). Similarly high IgM levels have been implicated in steroid resistant nephrotic syndrome (SRNS), but their exact role in the pathogenesis is not known (7,10).

Key point

There is a need to elucidate non-invasive biomarkers for diagnosis and assessing disease progression in patients with GN. Serum and urinary proteins have promising role. However judicial use of these biomarkers such as measurement of serum and urine immunoglobulin levels should be ensured.

Urinary biomarkers can be a very useful tool in predicting renal inflammation. In this regard, Zhang et al studied 61 patients with lupus nephritis (LN) and found that urinary monocyte chemoattractant protein (MCP) along with serum creatinine (SCr)

can be a modestly useful predictor of disease progression (11). Urinary IgG is found to correlate with severity of proteinuria in GN patients (12). However, data on urinary IgE levels in GN patients is scarce. On the other hand, IgE antibodies in urine may be found in very small quantities in healthy individuals. Locally residing plasma cells are responsible for the presence of IgE in urine (13).

Objectives

In the present study, we have investigated the utility of serum and urine immunoglobulin levels for predicting disease activity in our GN patients

Materials and Methods

It was a descriptive and cross-sectional study conducted at the department of immunology, Sindh Institute of Urology and Transplantation (SIUT), Karachi, Pakistan from September 2012 to January 2013.

Blood and urine of 25 patients with GN and 13 healthy controls were tested for immunoglobulin (Ig) levels including IgG, IgM, IgA and IgE after taking informed consent. The reference ranges of serum immunoglobulins are as follows: IgG: 7.51-15.6 g/L, IgM: 0.46-3.04 g/L, IgA: 0.82-4.53 g/L and IgE: up to 3 years = ≤ 10 IU/mL, 3 to 4 years = ≤ 25 IU/mL, 4 to 7 years = ≤ 50 IU/mL, 7 to 14 years = ≤100 IU/mL and 15 years or above = ≤150 IU/mL

The GN patients were recruited from nephrology outpatient department. The patients were divided into two groups of acute and chronic GN. The results of other laboratory parameters that were recorded from the case files included SCr levels, 24 hours urinary protein, results of urinary dipsticks (showing presence of proteins, red blood cells [RBCs] and pH) and renal biopsy reports. Taking SCr value of 1.5 mg/dL as the upper normal reference range, we classified the patients into two; (*a*) with normal renal functions and (*b*) with renal dysfunction. Proteinuria was divided into mild (≤ 300 mg/d), moderate (> 300 mg/d to ≤ 1000 mg/d) and severe (> 1000 mg/d).

Ethics issues

The research followed the tenets of the Declaration of Helsinki. The research was approved by the ethical committee of Sindh Institute of Urology and Transplantation, Karachi, Pakistan.

Statistical analysis

The data was analyzed using SPSS software version 20 (IBM SPSS Statistics 20). Numerical data was expressed as mean ± SD and categorical data was expressed in percentages. Student's *t* test and χ^2 tests were applied for numerical and categorical variables respectively. *P* value less than 0.05 were taken as significant difference between the variables.

Results

Mean age of the patients was 31.6 ± 12.7 years which was not significantly different from the control group (*P* = 0.874). Male to female ratio was 3:2.

Histopathological classification of the disease is given in Figure 1. The most frequent diagnosis was of focal segmental glomerulosclerosis (FSGS) followed by membranous GN.

Of all patients, seven (28%) presented with acute GN while 18 (72%) had chronic renal disease. Proteinuria was mild in 8 patients, while moderate to severe in 14 patients. In three patients there was no proteinuria. Renal dysfunction was present in 15 (60%) patients. The mean serum and urine immunoglobulins in patients and controls are shown in Table 1. Only serum IgM and serum and urine IgA were significantly higher in GN patients compared to controls.

Of 25 patients, serum IgG was high in 5 (20%) and low in 3 (12%) patients. While none of the patients had low serum IgM, IgA or IgE levels. However IgM was high in 2 (8%), IgA was high in 1 (4%) and IgE was high in 6 (24%) patients. All these 6 patients were males and 4 of them had IgE levels > 1000 IU/mL in blood. All of these patients did not give history of allergies and were non-smokers.

In urine of control group, IgG, IgM and IgA were absent while IgE was present. Moreover, mean IgE was high in urine of control group compared to patients (Table 1). The relation of immunoglobulinuria to serum Ig levels, acute versus chronic GN, degree of proteinuria and renal function status are shown in Table 2. Mean urine IgG was high in patients with normal serum IgG levels (Table 3). Similarly all patients with IgEuria had normal serum IgE levels (Tables 2 and 3).

Of 3 patients with low serum IgG levels, one had no proteinuria, while one each had mild and moderate to severe proteinuria. Serum IgE levels in patients with renal dysfunction were normal except one but with levels less than 1000 IU/mL.

Discussion

Investigators are continually trying to elucidate biomarkers which can predict disease progression or activity non-invasively (14-16). However, validation of these markers is cumbersome and difficult to achieve (17). For renal diseases, not only serum but urinary biomarkers have been extensively studied and evaluated. Moreover urine is an

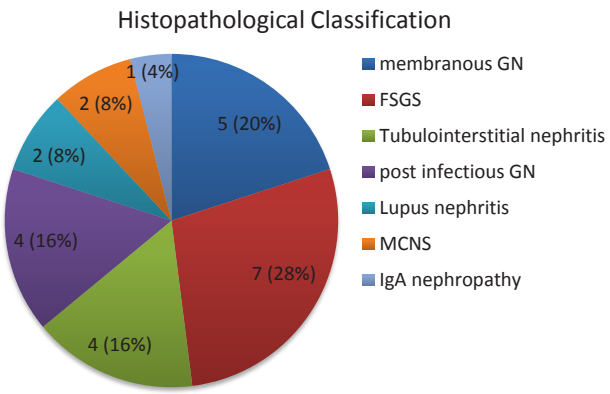

Figure 1. Number of patients with various histopathological diagnoses. FSGS = focal segmental glomerulosclerosis; GN = glomerulonephritis; MCNS = minimal change nephrotic syndrome.

Table 1. Serum and urine immunoglobulins in patients with glomerulonephritis

	Serum (G/L)				Urine (G/L)			
	IgG	IgM	IgA	IgE	IgG	IgM	IgA	IgE
Controls (n = 13)	14.8 ± 2.1	1.2 ± 0.5	1.9 ± 0.6	328 ± 15	0	0	0	5.7 ± 3.4
Patients (n = 25)	13.2 ± 5.6	2.0 ± 1.2	2.7 ± 1.14	505.3 ± 68	1.14 ± 2.1	0.08 ± 0.4	0.14 ± 0.2	2.7 ± 7.5
P values	0.333	0.047*	0.025*	0.361	0.065	0.421	0.023*	0.179
Acute GN (n = 7)	14.5 ± 6.0	1.9 ± 1.4	2.1 ± 1.2	596 ± 796	0.24 ± 0.3	0.23 ± 0.04	0.07 ± 0.1	6.2 ± 14.0
Chronic GN (n = 18)	12.7 ± 5.5	2.0 ± 1.2	2.9 ± 1.04	470 ± 648	1.5 ± 2.5	0.1 ± 0.4	0.2 ± 0.23	1.4 ± 2.6
P values	0.479	0.916	0.085	0.686	0.200	0.628	0.319	0.159
Proteinuria								
Nil to mild (n = 11)	14.0 ± 3.7	1.7 ± 1.0	2.3 ± 1.1	354 ± 46	0.3 ± 1.03	0.003 ± 0.01	0.02 ± 0.05	3.9 ± 3.7
Moderate to severe (n=14)	13.4 ± 6.3	1.8 ± 1.2	2.6 ± 1.0	600 ± 69	1.5 ±2.6	0.14 ± 0.5	0.22 ± 0.3	3.5 ± 4.8
P values	0.745	0.752	0.358	0.194	0.046*	0.169	0.001*	0.845
Renal functions								
Dysfunction (n = 15)	14.3 ± 6.1	1.8 ± 1.3	3.0 ± 1.3	297 ± 300	0.8 ± 1.3	0.13 ± 0.5	0.11 ± 0.11	4.2 ± 9.5
Normal (n=10)	11.5 ± 4.5	2.3 ± 1.0	2.3 ± 0.8	818 ± 950	1.6 ± 3.0	0.004 ± 0.1	0.25 ± 0.31	0.5 ± 1.7
P values	0.211	0.307	0.155	0.057	0.362	0.391	0.431	0.246

Abbreviation: GN, glomerulonephritis.

Table 2. Urinary immunoglobulinuria in relation to serum immunoglobulins and renal function status

	Presence of urinary IgG (n= 23)	Presence of urinary IgM (n= 7)	Presence of urinary IgA (n= 17)	Presence of urinary IgE (n= 7)
Serum immunoglobulins	Serum IgG	Serum IgM	Serum IgA	Serum IgE
	Low (n=3): 3	Low: 0	Low: 0	Low: 0
	Normal (n= 17): 16	Normal (n=23): 6	Normal(n= 24): 16	Normal (n= 19): 7
	High (n= 5): 4	High (n= 2): 1	High (n= 1): 1	High (n= 6): 0
P value	0.511	0.490	1.000	0.137
Acute GN (n= 7)	7	4	4	2
Chronic GN (n= 18)	16	3	13	5
P value	1.000	0.066	0.640	1.000
Degree of proteinuria				
Nil (n= 3)	2	0	1	1
Mild (n= 8)	7	3	4	2
Moderate to severe (n= 14)	14	4	12	4
P value	0.132	0.466	0.088	0.961
Renal dysfunction (+) (n= 15)	14	5	10	6
Renal dysfunction (-) (n= 10)	9	2	7	1
P value	1.000	0.659	1.000	0.179

appropriate sample for the identification or determination of these markers non-invasively due to its easy accessibility in large amounts and less complex proteome than serum or plasma (18).

These biomarkers have been categorized according to the information they reveal based on the type of injury, stage of disease activity and disease progression (19). For instance a high degree of proteinuria signifies a higher degree of interstitial inflammation in the kidneys (11).

Serum and urine immunoglobulins have been investigated as potential biomarkers for prediction of disease activity in GN patients with variable results (20). Samavat et al have shown differential expression of various proteins in urine after an extensive proteomic evaluation including C region of IgG molecules in IgA nephropathy (4). In a study by Roy et al, low serum IgG and high IgM with reduced IgG/IgM ration has been claimed as a predictor of SRNS (7). Tofik et al investigated the utility of measuring urinary IgG in GN patients (21). In their prospective study they showed that patients with high IgGuria are more prone to develop end stage renal disease (ESRD). While Li et al showed that urinary IgG is not a good predictor of severe GN with sclerosis and ESRD (20). In our study most of the patients had IgGuria with a significantly high mean urinary IgG levels with increasing degree of proteinuria but we did not find a correlation with poor renal functions. Another

Table 3. Mean urine immunoglobulins in relation to serum immunoglobulin levels

Serum IgG levels (n)	Urine IgG (g/L)
Low (3)	0.4 ± 0.5
Normal (17)	1.5 ± 2.5
High (5)	0.4 ± 0.6
P value	0.519
Serum IgM levels (n)	**Urine IgM (g/L)**
Low (0)	-
Normal (23)	0.9 ± 0.4
High (2)	0.007 ± 0.01
P value	0.767
Serum IgA levels (n)	**Urine IgA (g/L)**
Low (0)	-
Normal (24)	0.14 ± 0.2
High (1)	0.1
P value	0.836
Serum IgE levels (n)	**Urine IgE (g/L)**
Low (0)	-
Normal (19)	3.6 ± 8.5
High (6)	0
P value	0.324

important finding is patients with low serum IgG levels did not show high mean urinary IgG levels. This indicate that low serum IgG levels should not be ignored in GN patients as a result of increased protein loss and they should be further evaluated for its etiology especially in patients with associated recurrent infections.

IgM antibodies in blood have a major role not only fighting infectious agents but also in the development of tolerance and clearance of immune complexes preventing autoimmunity. IgM deficient mice and human are more prone to develop severe GN (10,22). We did not find any difference in mean serum IgM levels in patients with high degree of proteinuria or renal dysfunction compared to less severe disease. However the mean concentration of urinary IgM was higher (though not significantly) in our patients with severe proteinuria and renal dysfunction as reported earlier in a group of transplant patients (5) or in anti-neutrophil cytoplasmic antibodies (ANCA) associated acute GN (23). Nonetheless, there was no difference in the number of patients with or without renal dysfunction or severity of proteinuria showing presence of IgM in urine. Hence utility of measuring urinary IgM for prediction of renal damage or prognosis remains controversial in GN patients and require further studies.

Significantly high IgA levels in both serum and urines of patients especially with a higher degree of proteinuria were seen in this study as reported earlier (24). However as with IgG and IgM, IgA levels did not correlate with renal dysfunction in our study.

IgE antibodies are produced as a result of TH2 response. These are associated with type 1 hypersensitivity reactions and confer immunity against parasitic infections (25,26). IgE levels are determined in conditions exemplified by very high levels of these antibodies as an aid in the diagnosis. These include atopy, hyper IgE syndrome, allergic bronchopulmonary asthma and multiple myeloma (13). However these antibodies have been linked with disease pathogenesis in LN (27-29). In our patient population with various GN, serum IgE levels were not found significantly high in patients compared to controls. Only few patients had very high IgE levels in GN with no relation to severity of proteinuria or renal dysfunction. Moreover, IgEuria was more pronounced in healthy controls compared to GN patients. Nonetheless there was more proportion of patients with renal dysfunction who had IgEuria compared to normal renal functions and mean urinary IgE levels was also high in these patients.

Conclusion

In conclusion, utility of measuring serum and urine immunoglobulins in GN patients for prediction of disease activity or prognosis remains debatable. Therefore these tests should be ordered with clear indication and not routinely. However, an abnormal result such as low serum IgG levels or high serum IgE levels should not be attributed only to immune dysregulation in GN patients rather these patients should be further evaluated for the associated diseases besides GN.

Limitations of the study

The limitation of this study is that these patients were not followed prospectively. Therefore, there is a need to do serial measurements of serum and urine immunoglobulin levels prospectively on a larger patient population. This might help to deduce the utility of these antibodies in patients with various GN.

Authors' contribution

All authors engaged in the design of the research and acquisition of information and contributed equally to the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

Ethical considerations

Ethical matters such as (plagiarism, misconduct, data fabrication, falsification, and double publication or submission) have been thoroughly controlling by all authors.

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