Original paper

Patterns of glomerular disease based on 4-year kidney biopsy material analyzed by light microscopy and immunofluorescence: a retrospective single-center analysis in Poland

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The aim of this study was to assess the epidemiology of different patterns of chronic glomerular diseases based on clinical, histopathological and immunofluorescent findings of glomerulonephritis patients hospitalized in the Department of Nephrology, Transplantology and Internal Diseases in Poznan between January 2009 and December 2012.

We retrospectively studied 418 patients who had been subjected to renal biopsies. Data on serum creatinine concentration, 24 h proteinuria, arterial hypertension, diabetes mellitus, and histological and immunofluorescent findings were collected. The patients' mean age was 42 ±15. The male sex prevailed (53.1%). Immunoglobulin A nephropathy was the most common finding (18.9%), followed by focal segmental glomerulosclerosis (16.3%), membranous glomerulonephritis (10.1%), lupus nephritis (8.4%), extracapillary glomerulonephritis (3.3%) and membranoproliferative glomerulonephritis (2.6%). In 69 (16.5%) patients the biopsy was non-informative or non-diagnostic. Patients with membranous nephropathy presented the highest frequency of nephrotic syndrome (71.4%), followed by membranoproliferative glomerulonephritis and focal segmental glomerulosclerosis.

Combined analysis of the clinical, histopathological and immunofluorescent findings in glomerulonephritis patients based on a single center's data can provide important epidemiological findings.

Key words: renal biopsy, glomerulonephritis, epidemiology.

Introduction

Glomerulonephritis (GN) is a relatively infrequent disease of incidence varying according to the racial and socioeconomic factors, with numerous clinical and histopathological subtypes [1]. However, glomerular diseases account for a substantial number of chronic kidney disease (CKD) cases, and the preva-

lence of CKD, with more than 10% of people affected, reaches the prevalence of diabetes [2].

A kidney biopsy is an important diagnostic procedure used in GN patients. It inestimably aids in determining the proper diagnosis and treatment and helps to predict medical prognosis for these subjects. It also enlightens the epidemiology of glomerular changes. The data from existing national registries and databases revealed different patterns of adult glomerulopathies in various countries, changing over the years. Of note, in North and South America (data from centers in the United States, Uruguay and Brazil) focal and segmental glomerulosclerosis (FSGS) is the most frequent GN [3, 4, 5]. In contrast, data from European registries point to IgA nephropathy (IgAN) as the most common GN [6, 7, 8], although there are reports suggesting significant differences in the epidemiology of GN between Eastern and Western Europe, with the higher prevalence of other than IgAN causes of GN in the former region [9]. A recent study from central Poland showed the results of retrospective analysis of 746 consecutive native kidney biopsies, with mesangioproliferative GN (MesGN) including IgAN remaining the most frequent type of GN [10]. However, considering the number of renal biopsies in relation to the general Polish population, there are still scarce data regarding the frequency and distribution pattern of different forms of GN.

The aim of the present retrospective study was to identify the occurrence of different patterns of chronic glomerular disease based on 4-year kidney biopsy material collected at our center and analyzed by light microscopy and immunofluorescence.

Material and methods

A retrospective analysis of 418 kidney biopsies investigated at Heliodor Swiecicki Clinical Hospital during the period 2009-2012 was carried out. The study was approved by the Institutional Review Board at Poznan University of Medical Sciences (No. 616/13). All of the kidney tissue specimens were obtained by performing a percutaneous needle biopsy (automatic needle Tru-Cut 16G) during hospitalization at the Department of Nephrology, Transplantology and Internal Diseases. Most of the kidney biopsy specimens were taken from the left kidney lower pole. They were embedded in paraffin and cut into 2-3 μm sections and evaluated with light microscopy using standard histology techniques (staining with hematoxylin–eosin, Periodic Acid Schiff, Congo red). Direct immunofluorescence (DIF) technique identifying immunoglobulin IgA, IgG and IgM, complement components C1q, C3, C4 and fibrinogen was performed on frozen sections made from separate kidney tissue blocks, cut in cryostat [11]. In selected cases, where necessary, the specimens were investigated by electron microscopy. Each kidney biopsy report was evaluated together with clinical data of a patient and was assigned to a particular group according to the diagnosis of a kidney/glomerular disease (excluding non-informative and non-diagnostic biopsy specimens): primary GN, defined as no evidence of multisystem disease at the time of investigation (membranous GN (MGN), IgAN, FSGS, MesGN, membranoproliferative glomerulonephritis (MPGN), minimal change disease (MCD), crescentic glomerulonephritis (CGN) without coexisting systemic disease), IgM nephropathy and sclerosing GN (SGN), secondary GN (associated with systemic lupus erythematosus (SLEN), amyloidosis (AMN), systemic vasculitis (VAN) and Alport syndrome). Other conditions included hypertensive arteriolar nephrosclerosis (HAN), diabetic nephropathy (DMN), thin basement membrane disease (TBMD) and BK virus–associated nephropathy. Non-informative specimens comprised material without glomeruli and/or damaged tissue.

All data were stored in a standard Excel database. The calculations were carried out with Microsoft Excel 2010, STATISTICA version 10 software (StatSoft Inc.) or StatXact 9.0 (Cytel Inc., Cambridge, MA, USA). Patients' demographic data were analyzed using descriptive statistics. Analyzed data are presented as means and standard deviations or percentage, as appropriate. One-way ANOVA was used to analyze the data with normal distribution and homogeneous variances. Normality of the distribution was tested with the Shapiro-Wilk test, and the equality of variances was checked with Levene's test. Data that did not follow a Gaussian distribution were analyzed with the Mann-Whitney U test or the Kruskal-Wallis test and Dunn's *post-hoc* test. Categorical data were analyzed with the χ^2 test or the Fisher-Freeman-Halton test. All results were considered significant at $p < 0.05$.

Results

All 418 kidney biopsies were obtained from adults (42 ±15 years, range 18-80 years, 196 women, 222 men) hospitalized during 2009-2012. The frequency of kidney biopsy was highest in 2010 (n = 126), when compared with 2009 (n = 99), 2011 (n = 97) and 2012 (n = 96).

The majority of biopsies were performed in patients between 18 and 40 years old (51.4%) in comparison to the age range 41-60 years (35%) and older than 60 years (13.6%). The average number of glomeruli in a biopsy specimen evaluated with light microscopy was 11.02 ±6.9, and evaluated with DIF was 4.6 ±3.0. The number of non-informative and non-diagnostic kidney biopsies in the case of histological examination was 69 (16.5%), while tissues assessed with DIF non-informative samples accounted for 85 specimens (20.3%). Eighteen kidney biopsies (4.3%) were eval-

uated by electron microscopy. Primary GN was found in 58.6% of all biopsies, secondary GN in 10.8% and 14.1% of biopsies were assigned as other conditions. Among primary GN, IgAN was responsible for the most cases of glomerular involvement (32.2%). Six out of 245 primary GN patients (2.4%), in whom DIF samples were non-informative, were diagnosed as Mes-GN. In the case of secondary GN, SLEN dominated as a reason for glomerular damage (77.8%). The most frequent reason for renal biopsy was nephrotic syndrome (NS), defined as urine protein excretion greater than 3.5 g/24 hours/1.73 m^2, hypoalbuminemia less than 3 g/dl, peripheral edema and/or hyperlipidemia; it accounted for 38.8% of biopsies (Fig. 1).

In particular, the highest frequencies of NS were observed in patients with primary GN: MGN (71.4%), MPGN (45.5%) and FSGS (41.2%). The majority of biopsies were performed in patients with stages 1 and 2 of chronic kidney disease (CKD), 42.1% and 31.1%, respectively (Fig. 2).

The majority of patients in stages 1 and 2 of CKD had IgAN (44% and 35%, respectively), and the majority of patients in stages 3 and 4 of CKD had FSGS (33% and 21%, respectively) (Fig. 3).

Patients with IgAN comprised the youngest subjects and the frequency of IgAN diminished with age; in the case of MGN the tendency was exactly the opposite (Fig. 4).

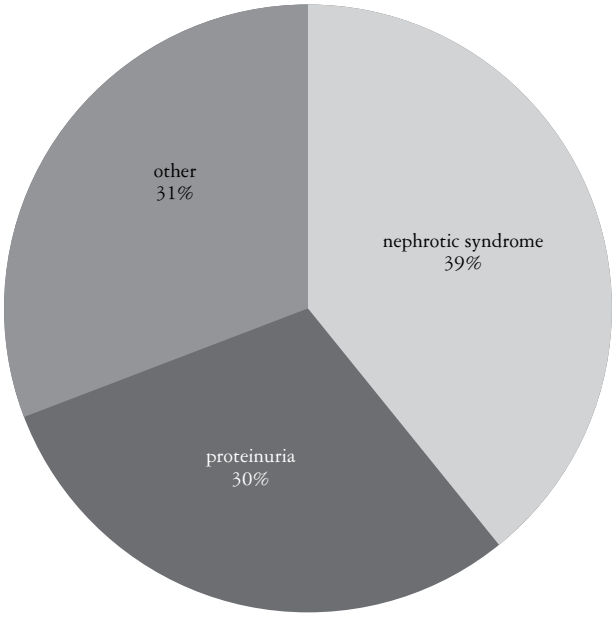

Fig. 1. Clinical indications for renal biopsy

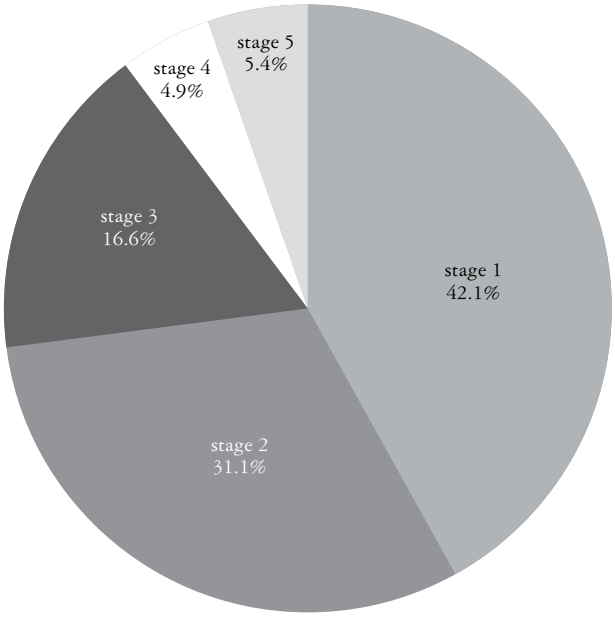

Fig. 2. Percentage of different stages of chronic kidney disease

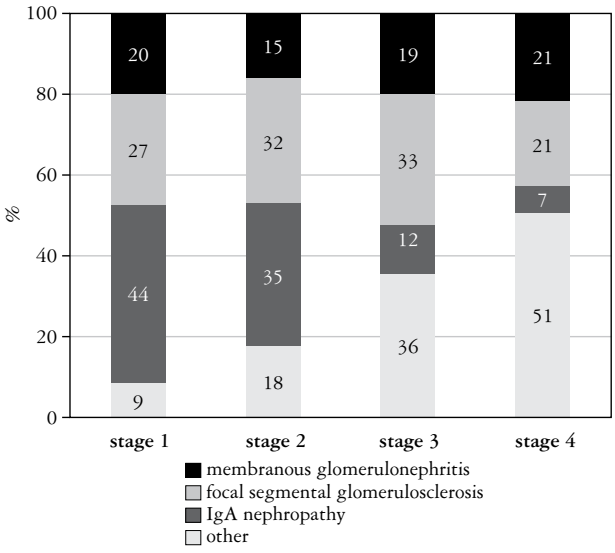

Fig. 3. Percentage of patients with the most frequent primary glomerulonephritis classified according to the stage of chronic kidney disease (CKD)

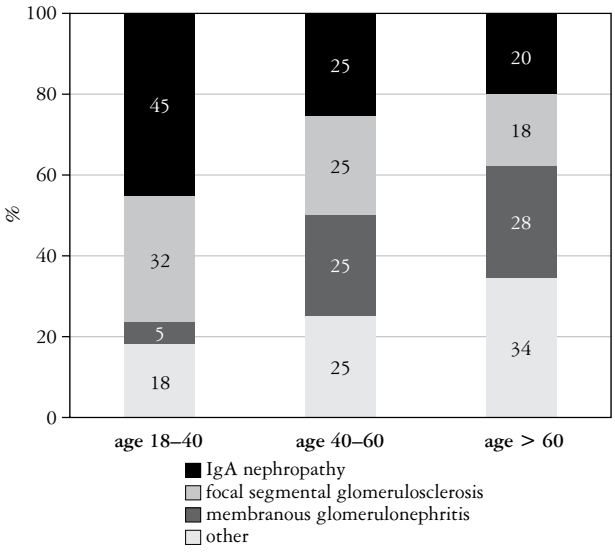

Fig. 4. Percentage of patients with the most frequent primary glomerulonephritis classified according to age

Information about the distribution of glomerular diseases is summarized in Table I.

In all performed renal biopsies there was a predominance of male patients over females (53.1% vs. 46.9%). The mean age of the primary GN patients was 44 ±14.5 years. Patterns and frequency of primary GN in relation to gender are summarized in Table II. Comparison between female and male cases in the primary GN group revealed a statistically significant difference in their frequency ($p = 0.01$). Except CGN there was a predominance of male patients in all primary GN groups.

In the group of secondary GN the most frequent GN was SLEN (77.8%), with the predominance of females (71.4%).

We found statistically significant differences in the proteinuria between MGN and the other types of primary GN ($p < 0.05$) and in serum creatinine level between CGN and the other types of primary GN ($p < 0.05$). The values of serum creatinine and proteinuria in primary GN with the number of patients equal to or more than 10 are summarized in Table III.

Discussion

Our analysis provided information about the prevalence of biopsy-proven GN during a period of 4 years in a single University Hospital center located in west-central Poland. According to this retrospective study, IgAN was the most common glomerular

Table I. Distribution of glomerular diseases

Group of diseases	n (%)
Primary glomerulonephritis (GN)	245 (58.6)
Membranous GN	42 (10.1)
Immunoglobulin A nephropathy	79 (18.9)
Focal and segmental glomerulosclerosis	68 (16.3)
Mesangioproliferative glomerulonephritis	6 (1.4)
Membranoproliferative glomerulonephritis	11 (2.6)
Minimal change disease	4 (1)
Crescentic glomerulonephritis	14 (3.3)
Sclerosing GN	20 (4.8)
IgM nephropathy	1 (0.2)
Secondary glomerulonephritis	45 (10.8)
Lupus nephritis	35 (8.4)
Henoch–Schönlein purpura	5 (1.2)
Alport syndrome	1 (0.2)
Systemic vasculitis	1 (0.2)
Renal amyloidosis	3 (0.7)
Other conditions	59 (14.1)
Non-diagnostic/non-informative	69 (16.5)
Total	418 (100)

Table II. Frequency of primary glomerulopathies in relation to gender

Primary glomerulonephritis (GN)	n (%)	Gender	
		Female	Male
Membranous GN	42 (17.1)	12	30
IgA nephropathy	79 (32.2)	35	44
Focal and segmental glomerulosclerosis	68 (27.8)	29	39
Mesangioproliferative glomerulonephritis	6 (2.5)	1	5
Membranoproliferative glomerulonephritis	11 (4.5)	5	6
Minimal change disease	4 (1.6)	1	3
Crescentic glomerulonephritis	14 (5.7)	8	6
Sclerosing GN	20 (8.2)	8	12
IgM nephropathy	1 (0.4)	0	1
Total	245 (100)	99	146

Table III. Serum creatinine and proteinuria in primary glomerulonephritis

	IgAN	FSGS	MGN	MPGN	SGN	CGN
Serum creatinine, mg/dl	1.0 ±0.6	1.0 ±0.4	1.0 ±0.5	2.2 ±1.7	1.5 ±0.8	4.1 ±2.0*
Proteinuria, g/day	2.9 ±4.1	3.2 ±2.8	5.6 ±3.4*	3.1 ±2.6	3.6 ±2.7	2.3 ±1.9

*IgAN – immunoglobulin A nephropathy; FSGS – focal segmental glomerulosclerosis; MGN – membranous nephropathy; MPGN – membranoproliferative glomerulonephritis; SGN – sclerosing glomerulonephritis; CGN – crescentic glomerulonephritis. Data expressed as mean ± SD, * $p < 0.05$*

disease in the studied population (18.9%), but was closely followed by FSGS (16.3%). In many European countries (Czech Republic, Spain, Italy, Estonia, France), similar to data from our center, IgAN occurrence has been the most common, ranging from 16 to 35% of all GN [7, 8, 12, 13, 14]. Also the results of a 30-year renal biopsy study conducted in the predominantly white residents of Olmsted County, USA, point to IgAN as the most frequent type of GN (25%) [15]. However, data from other European countries, Serbia and Romania showed a different picture of GN frequency, with the dominance of non-IgA MesGN and MPGN, respectively, what is probably the reflection of socioeconomic conditions [9, 16]. It is also believed that the frequency of IgAN is often linked to ethnic and genetic factors, i.e. is less frequent in some American and Asian countries [4, 17]. However, it should be noted that according to data from Central Poland the number of cases of IgAN diagnosed in kidney biopsy specimens increased systematically together with an increase of performing DIF and eventually reached 100% of MesGN diagnoses [10]. Therefore, MesGN is currently rarely diagnosed and hardly encountered in recent literature data.

Compared to other types of GN, we also noted high frequency of non-inflammatory glomerulopathy, FSGS. The occurrence of FSGS in our center (16.3%) is comparable with the results of other Polish centers (17.3%) and the USA (17%), but higher than in studies from other European countries, such as Estonia (7.3%) or the Czech Republic (10.8%) [7, 10, 13, 15]. The reasons for the different epidemiologic patterns in renal biopsy are not completely clear. However, there are reports on increasing occurrence of FSGS in Europe and USA [10, 15]. In our data, like in the study within Caucasian populations in the USA, FSGS was second only to IgAN as the leading cause of GN in white adults [15].

Another finding in this study was that the occurrence of MPGN in our region was not high (4.5%). Similar low prevalence of MPGN was observed in developed European countries [7, 13], in contrast to Romania, where the MPGN frequency reached 29.3% of primary GN [9]. The differences are probably associated with worse control of chronic viral and bacterial infections in poor economic conditions.

In general, primary GN accounted for more than a half (58.6%) of all renal biopsies and were more common in males that in females, which is in agreement with previous epidemiological observations [8, 12, 14].

A higher frequency of secondary GN was observed in females and is related to higher incidence of systemic lupus erythematosus in this gender, as GN induced by immunological factors (SLEN) was the most frequent among secondary GN. This is similar to other epidemiological studies from the majority of European and non-European countries [4, 7, 12, 13].

Regarding clinical syndromes associated with GN, our data also confirmed MGN as the main cause of NS [12, 16]. MGN for years was regarded as a primary cause of NS globally, but some recent reports showed distinct data pointing to MPGN or FSGS as a leading cause of NS [4, 9]. The reasons for these discrepancies may be increasing frequency of FSGS in different parts of a world and local epidemiological disparities in the occurrence of GN patterns. In addition, more than 70% of patients with MGN presented with NS, followed by about 45% of patients with MPGN. These results are concordant with the recently presented data estimating that NS is present in 60-80% of MGN patients [18] and with the data from a multicenter Korean study on MPGN, where NS was present in 45.6% of MPGN patients [19].

We should also mention the main limitation of the present study, which is the lack of calculation of prevalence of GN per million population, because the study was single-center/regional and not nationwide. Another disadvantage was the presence of non-informative DIF samples, which precluded complete tissue evaluation and correct diagnosis, e.g. could result in underestimation of IgAN.

To conclude, there exists considerable diversity in the different categories of primary GN in various parts of the world. We believe that our data represent an important contribution to the epidemiology of GN in Poland. Further studies to create a national registry should be undertaken.

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The authors declare no conflicts of interest.

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