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The role of glomerular morphometric features in pediatric podocytopathies – a single center study

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Abstract

Podocytopathies represent a well-studied subgroup of glomerulopathies, being characterized by proteinuria due to damage or dysfunction of podocytes. Glomerular size in podocytopathies has been studied in different population, but only a few studies take in consideration the pediatric population. There are different methods to assess the glomerular size, but most of the studies report the maximal profile area as being the most accurate one. The aim of this study is to determine the range values of glomeruli in pediatric population with glomerulopathies and to establish a correlation between the measured size and several laboratory features. The patients that undergo renal biopsy in the Department of Nephrology, "Maria Skłodowska Curie" Clinical Emergency Hospital for Children, Bucharest, Romania, were divided into two groups: control *vs.* affected/patient group. The control group included children that require renal biopsy for renal impairments other than high-range proteinuria (most of them recurrent microscopic asymptomatic hematuria), while the affected group had nephrotic-range proteinuria. Thirty patients were selected to be part of the control group and 30 patients in the affected group. In control group, the mean value diameter was 166.23±13.04 μm, and the area of the glomerulus had a mean value of 19 126.86±3070.83 μm^2. In the affected group, we obtained the following results: the mean value diameter was 192.42±28.15 μm, while the glomerular cross-sectional area had a mean value of 23 535.55± 6456.57 μm^2. Using the linear regression, we concluded that all the cases with increased-size glomeruli had more urinary protein loss compared with the ones that had small-size glomeruli and low-range proteinuria.

Keywords: glomerular diameter, glomerular area, proteinuria, pediatric population, podocytopathies.

Introduction

Glomerulopathies are defined by a specific relationship between clinical features and laboratory impaired tests – nephritic or nephrotic syndrome, arterial hypertension, renal insufficiency accompanied by histopathological lesions. According to the *Romanian Renal Registry* [1], the most frequent diagnosis of glomerulopathy was immunoglobulin A (IgA) nephropathy (28%), followed by membranous nephropathy (25%), minimal change disease (20%) and focal segmental glomerulosclerosis (15%). However, these data are reported in the adult population, while in the Romanian children population the incidence of glomerulopathies is unknown due to lack of data. Also, the reported incidences are influenced by the indication of renal biopsy and the frequency of performing them. In "Maria Skłodowska Curie" Clinical Emergency Hospital for Children, Bucharest, Romania, the most frequent indication of renal biopsy in children was steroid-resistant nephrotic syndrome (64.8%) and from this group, 62.1% had minimal change disease. Nowadays, this diagnosis is part of a bigger entity called podocytopathies [2, 3].

Podocytopathies represent a well-study subgroup of glomerulopathies, being characterized by proteinuria due to damage or dysfunction of podocytes [4–6]. According to Barisoni *et al.* [7], the podocytopathies include minimal-change nephropathy, focal segmental glomerulosclerosis, diffuse mesangial sclerosis, and collapsing glomerulopathy. Lupus podocytopathy has also been described [8], even though it is not included in the revised *International Society of Nephrology* (ISN)/*Renal Pathology Society* (RPS) classification [9].

Glomerular size in podocytopathies has been studied in different population [10–12], but only a few studies take in consideration the pediatric population [13–16]. It is well known that glomerular size varies between different races [17, 18]; therefore, we cannot anticipate that the normal values will have the same range with the ones reported in other populations.

There are different methods to assess the glomerular size, but most of the studies report the maximal profile area as being the most accurate one [19, 20].

The aim of this study is to determine the range values of glomeruli in pediatric population with glomerulopathies and to establish a correlation between the measured size and several laboratory features.

Materials and Methods

The cases were selected from the Department of Nephrology, "Maria Skłodowska Curie" Clinical Emergency

Hospital for Children in Bucharest. The patients that undergo renal biopsy in the Department of Nephrology were divided into two groups: control *vs.* affected/patient group. The control group included children that require renal biopsy for renal impairments other than high-range proteinuria (most of them recurrent microscopic asymptomatic hematuria), while the affected group had nephrotic-range proteinuria. Patients were assigned to the control group following the age and gender criteria (for each patient in the affected group, a same age and gender patient was assigned in the control group; the most important difference between the patients from the two groups was that the one in the control group had the indication of renal biopsy due to recurrent hematuria, while the one in the affected group had his indication usually for steroid-resistant nephrotic syndrome). Moreover, a patient was included in the control group if he did not have clinical signs or symptoms (such as edema, arterial hypertension) or low glomerular filtration rate. The most common histopathological diagnosis in the control group was thin basement membrane disease. On the other hand, a patient was included in the affected group if he had high-range proteinuria and one of the following diagnosis: minimal change disease, membranous nephropathy, lupus nephritis, proliferative extracapillary glomerulonephritis or focal segmental glomerulosclerosis. All the children that were selected had a normal weight for age and gender (between 10–90th percentile).

After the kidney biopsy was obtained with a cutting-needle biopsy "gun" under ultrasound guidance, the tissue was prepared for each of the required procedures: immunofluorescence, light and electron microscopy examination. This study aims to focus on optic microscopy examination of the described cases. The tissues were processed according to the standard protocol for electron microscopy examination (glutaraldehyde-fixed) and after that, sectioned at 1 μm thickness. The semithin sections were stained with Toluidine Blue and examined at light microscope. Moreover, the slides were digital scanned with Leica Aperio AT2 and the measurements were performed using the software provided by Leica (Digital Image Hub version 4.0.6).

Two methods were used to determine the exact values of glomerular size: the measurement of diameter and its area by free-hand drawing tool. All the cases included in this study had 10–20 glomeruli per biopsy. We selected all glomeruli on the slide with almost a spherical shape, the vascular pole included and no signs of sclerosis; the measurements were expressed as mean value. From the overall 60 cases, only two cases did not have the vascular pole included in the glomerular cross-section; therefore, we selected the glomeruli with the biggest diameter and area, assuming that the section was made near its center. First, we measured several diameters of the glomerulus and we used the mean value as corresponding diameter for each case (Figure 1, a and b). For instance, the glomerulus shown in Figure 1 (a and b) had a corresponding diameter of 159.79 μm.

Furthermore, we measured the area by drawing a free-hand shape, using the above-mentioned software (Figure 2).

The statistical analysis was completed with Statistical Package for the Social Sciences (SPSS) and Microsoft Excel software. The results were expressed as mean value ± standard deviation. The following tests were required in data analysis: Student's *t*-test, one-way analysis of variance (ANOVA), Mann–Whitney–Wilcoxon test. To assess the correlation between two variables, we used the linear regression analysis. For all the aforementioned statistical tests, we set p-value <0.05 to be statistically significant.

Results

Thirty patients were selected to be part of the control group and 30 patients in the affected group (in each group, 13 males and 17 females). The mean value of age for the overall group was 10.77±3.23 years; the mean age in the female group was 10±3.53 years, while the mean age in the males group was 11±2.76 years. The affected group had the following distribution according to histo-pathological diagnosis (Figure 3).

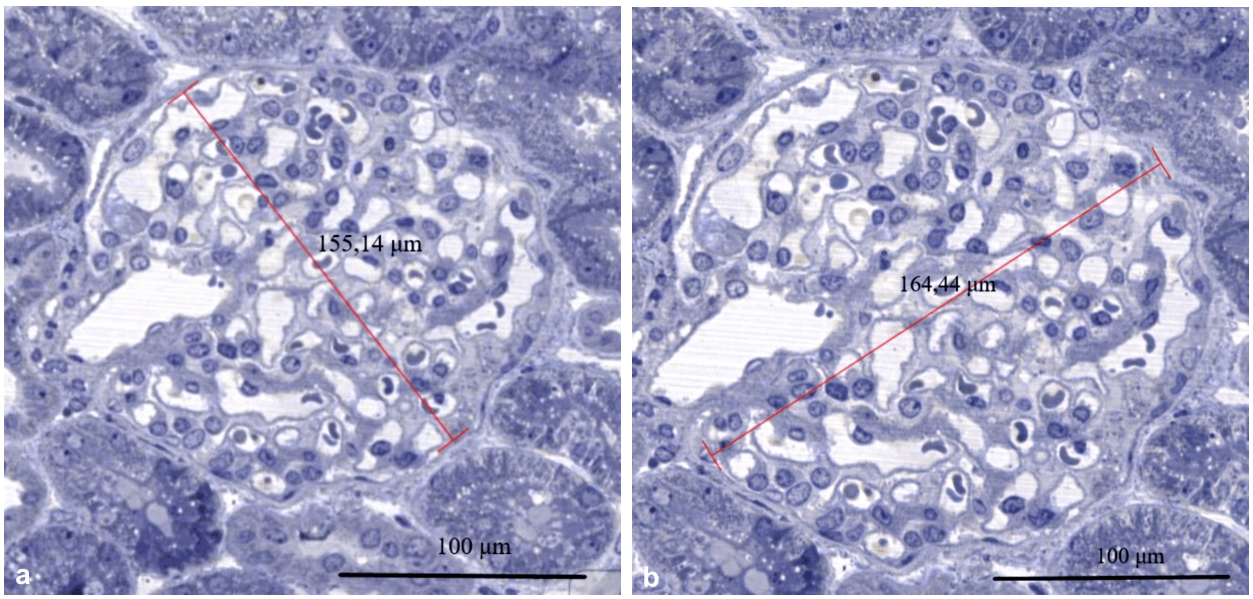

Figure 1 – ***(a and b) Measuring several diameters of the glomerulus. Toluidine Blue staining, ×200.***

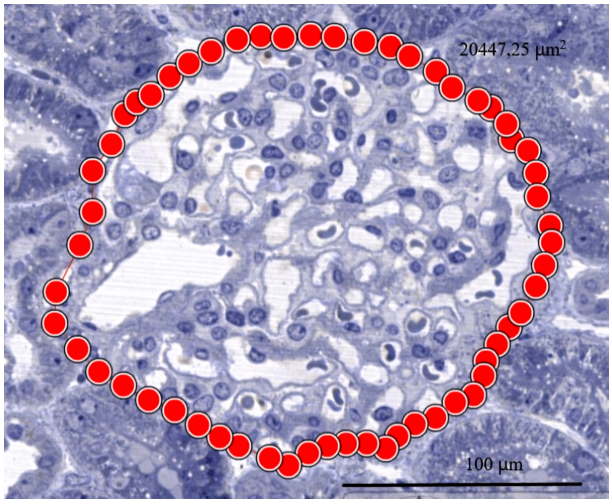

Figure 2 – ***Glomerular area measured by free-hand drawing. Toluidine Blue staining, ×200.***

We wanted to see if the results we obtained by the two aforementioned methods (measuring the diameter *vs.* area) are similar to describe the size of the same glomerulus. After we measured the diameter, we used the classic formula to determine the surface of a sphere (πr^2). We compared the values we obtained using the area formula with the ones measured by free-hand drawing in the software provided by Leica.

We run a paired two-tailed Student's *t*-test to determine if there is a difference between the two types of measurement, and the obtained *p*-value was 0.8467 ($p>0.05$). Because of this, we concluded that no matter through which method we are measuring the glomerulus (just the diameter or by drawing a free-hand shape to approximate the area), they both provide the same results, revealing no statistically significant difference between these methods. Having these methods validated in our study, we proceed by applying them in the control *vs.* patient group.

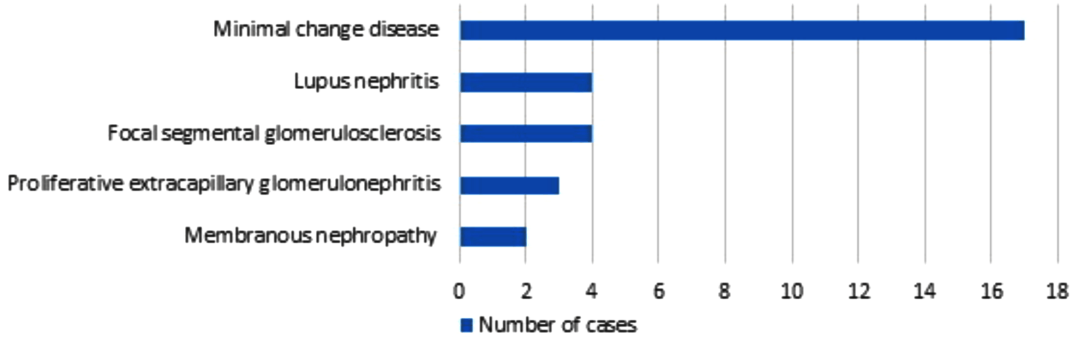

Figure 3 – ***Case distribution according to histopathological diagnosis.***

In control group, the diameter range was 150–195.48 µm, with a mean value of 166.23±13.04 µm, and the area of the glomerulus ranged between 15 356.43–24 320.34 µm², with a mean value of 19 126.86±3070.83 µm². In the affected group, we obtained the following results: the diameter range was 121.15–272.99 µm, with a mean value of 192.42±28.15 µm, while the glomerular cross-sectional area range was 8547.11–42696.5 µm², with a mean value of 23 535.55±6456.57 µm². A paired sample two-tailed Student's *t*-test was run and the results were statistically significant – *p*-value was 0.022 (<0.05). However, due to lack of data regarding the normal values of glomerular diameter and surface and their normal distribution in pediatric population, we preferred not to settle with the *t*-test, but also to run a Mann–Whitney–Wilcoxon test. After this test, the *p*-value was 0.00528904 (<0.05), which also confirmed that there is a statistically significant difference between the two groups.

After this finding, we wanted to see if there is a correlation in the affected group between the size of the glomerulus (measured by its diameter and area) and the values of proteinuria that the patient had when he underwent the renal biopsy. The following hypothesis was stated: the bigger the glomerulus, the more loss of urinary proteins in a specific case. Linear regression was used to compare on the one hand the size of the glomerulus with the loss of urinary proteins and on the other hand the area of the glomerulus with the proteinuria. In both cases, a strong correlation was obtained (*p*-value <0.05) between these two variables (Tables 1 and 2; Figures 4 and 5). All the cases with increased-size glomeruli had more urinary protein loss compared with the ones that had small-size glomeruli and low-range proteinuria.

Discussions

Due to lack of data in pediatric population, it was difficult to establish the normal size of glomeruli in Romanian children population. All the measurements reported in the scientific studies are mostly adult or mice-related [21–26].

According to the method of measurement, we used the method described by Kambham *et al.* [19] involving the glomerulus diameter, as a reliable marker to express the overall glomerulus size. However, Kambham *et al.* used a light microscope with a built-in micrometer to perform all the required measurements, while in our study we prefer to scan all the slides and determine the exact size of diameter and area in a digitalized manner for a better accuracy. Also, by measuring in two different ways (the diameter and the free-hand drawing area), we increased the probability to obtain precise results with a high degree of reliability.

One of the disadvantages of the presented study is the small number of cases included (60 total – 30 in the control group and 30 in the affected group). This group was designed through a 7-year period of performing renal biopsies in children (2010–2017). This may be because the indication to perform a renal biopsy in children is under strict specifications of *Kidney Disease Improving Global Outcomes* (KDIGO) guidelines [27]; therefore, only a few renal biopsies are taken every year (most of them for steroid-resistant nephrotic syndrome).

Table 1 – ***Regression statistics: diameter of the glomerulus* vs. *level of proteinuria***

Regression statistics						
Multiple *R*	0.907090296					
R^2	0.822812806					
Adjusted R^2	0.816484691					
Standard error	0.992780209					
Observations	30					
		ANOVA				
	df	***SS***	***MS***	***F***	***Significance F***	
Regression	1	128.1542354	128.1542	130.0249639	0	
Residual	28	27.59715123	0.985613			
Total	29	155.7513867				
	Coefficients	***Standard error***	***t Statistics***	***p-value***	***Lower 95%***	***Upper 95%***
Intercept	-7.518095697	1.064876272	-7.06007	0.0000001113	-9.699395857	-5.336795536
Diameter	0.060280859	0.005286474	11.40285	0	0.049452009	0.071109709

ANOVA: Analysis of variance; df: Degree of freedom; SS: Sum of squares; MS: Mean squares.

Table 2 – ***Regression statistics: area of the glomerulus* vs. *level of proteinuria***

Regression statistics						
Multiple *R*	0.848951297					
R^2	0.720718304					
Adjusted R^2	0.710743958					
Standard error	1.246402128					
Observations	30					
		ANOVA				
	df	***SS***	***MS***	***F***	***Significance F***	
Regression	1	112.2528752	112.2529	72.2571969	0.0000000031	
Residual	28	43.49851144	1.553518			
Total	29	155.7513867				
	Coefficients	***Standard error***	***t Statistics***	***p-value***	***Lower 95%***	***Upper 95%***
Intercept	-2.963425615	0.9010200321	-3.28897	0.002715929	-4.809082075	-1.117769154
Area	0.000304718	3.58474E-05	8.500423	0.0000000031	0.000231288	0.000378148

ANOVA: Analysis of variance; df: Degree of freedom; SS: Sum of squares; MS: Mean squares.

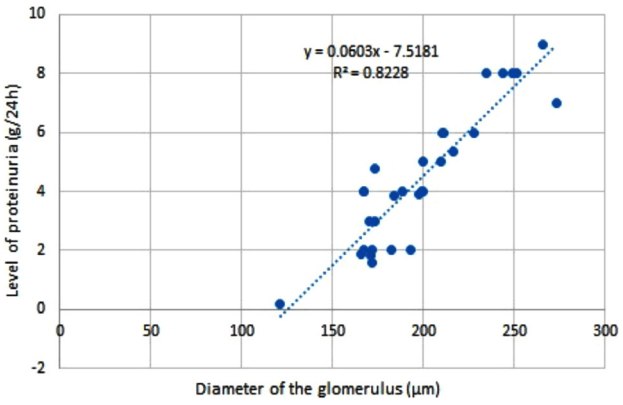

Figure 4 – ***Regression line: diameter of the glomerulus* vs. *level of proteinuria.***

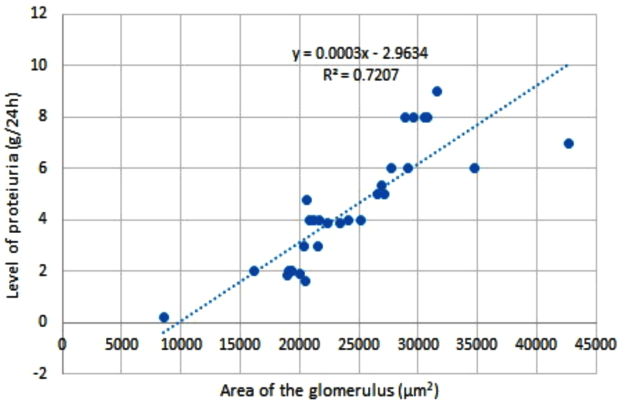

Figure 5 – ***Regression line: area of the glomerulus* vs. *level of proteinuria.***

In the selected cases, we found a correlation between proteinuria level and the size of the glomerulus, but we did not include in our measurements the sclerotic glomeruli from the slide. This correlation may be due to inflammation (overall swelling of the glomeruli) keeping in mind the fact that the biopsy was usually performed in patients with multiple relapses or being steroid resistant. Therefore, the probability to perform a renal biopsy soon after a relapse or to a steroid-resistant child is very high. On the other hand, most of the children were under steroid treatment when the renal biopsy was performed; this treatment should have decreased the local inflammation

and size of the glomeruli should have been similar with the one in the control group, but most of them did not respond to steroid treatment.

Even if in other studies shown no difference between the control and study group regarding the glomeruli size [21], this study was run on pediatric population, with different characteristics than the adult one (no obesity, arterial hypertension or type 2 diabetes mellitus). All the children included in the study had a normal body mass index, with a weight range between 10–90th percentile. Some of them developed arterial hypertension because of steroid-resistant nephrotic syndrome, but this happen after the biopsy was performed (1–2 years later).

There are several studies which take under consideration the difference between superficial *vs.* juxtamedullary glomeruli, showing that in 51–69 years old adults, the superficial glomeruli are larger than the juxtamedullary ones [28]. In our study, it was impossible to distinguish between superficial or juxtamedullary glomeruli because on each slide there were 3–4 semithin sections, each with one or two glomeruli. Even if per case we had 10–20 glomeruli, we could not state from where exactly the sections were taken, according to renal cortex.

Conclusions

Although we obtain statistically significant difference between the control group and the affected group, as well as a strong correlation between the size of the glomeruli and the level of proteinuria, more studies need to be performed specifically on children, at a bigger scale, to validate these results. These studies need to be designed and conducted in pediatric population by distinguishing first the specific characteristics of this population and after that, by applying them in an unbiased study. Therefore, being the most common diagnosis in children after kidney biopsy, podocytopathies are still under research, especially related to podocyte foot process effacement.

Conflict of interests

The authors declare that they have no conflict of interests.

Compliance with ethical standards

We undersign, certificate that the procedures and the experiments we have done respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2000 (5), as well as the national law.

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