CORRELATION BETWEEN PODOCYTE FOOT PROCESS EFFACEMENT AND PROTEINURIA IN HUMAN GLOMERULAR DISEASES

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ABSTRACT. The podocyte is a specialized epithelial cell with numerous interdigitating foot processes which play an important role in the regulation of the glomerular basement membrane (GBM) permeability. The nephrotic syndrome results from increased glomerular permeability to proteins and is structurally believed to be associated with podocyte foot process effacement. Despite increasing knowledge of the molecular composition of the glomerular filtration barrier, the relationship between proteinuria and foot process effacement remains unclear. The aim of this study is to analyze the relationship between podocyte foot process effacement and the level of proteinuria. Morphometric analysis was performed on electron microscopy images taken randomly from 37 patients diagnosed with various glomerular diseases. 17 of the patients had nephrotic syndrome and 20 had subnephrotic proteinuria. The mean foot process width (FPW) was quantitated for each patient and for each group and correlated with the level of proteinuria. In the non nephrotic group segmental effacement of the foot processes was present and the mean FPW was 422 ± 90 nm. In the nephrotic group the foot processes were diffusely effaced, reflected by a FPW of 1196 ± 517 nm, significantly larger than in the subnephrotic group. The level of proteinuria correlated with the FPW only when all 37 patients were analyzed together. There was no correlation when the groups were studied separately. The mean FPW was significantly larger in the nephrotic group compared to the non nephrotic group. Podocyte foot process effacement correlated with proteinuria.

Keywords: podocyte, foot process effacement, foot process width, proteinuria

INTRODUCTION

Podocytes are specialized epithelial cells that cover the glomerular basement membrane (GBM) with numerous interdigitating foot processes. These, together with the endothelial cells and the GBM are essential components of the glomerular filtration barrier against urinary loss of proteins (Bonsib, 2007). Foot process effacement is present in glomerular diseases that present with proteinuria, especially minimal change disease (MCD) – where diffuse foot process effacement is the only morphologic alteration noticed (D’Agati, 2003; van den Berg et al, 2004; Patalunan et al, 1997). Foot process effacement is also invariably present in focal segmental glomerulosclerosis (FSGS) and membranous nephropathy (MN).

Few studies approached the quantitative analysis of foot process effacement. It has been suggested that the degree of foot process effacement would depend on the underlying glomerular disease (Song et al, 2010). The mechanisms of this phenomenon remain unclear despite increasing knowledge of the podocyte structure and function (Pavenstadt et al, 2003). Furthermore, the relationship between the degree of foot process effacement and proteinuria remains controversial (van den Berg et al, 2004).

Foot process effacement can be the only ultrastructural modification in the renal biopsy specimen- typically in MCD, or it can be accompanied by other morphologic modifications of the podocyte and abnormalities characteristic for the underlying disease: the presence of immune deposits, inflammation or sclerosis/fibrosis.

In order to evaluate the relationship between the level of proteinuria and the degree of foot process effacement in human glomerular diseases we conducted a morphometric study on the degree of foot process effacement in renal biopsies from patients with various glomerular diseases and nephrotic and subnephrotic range proteinuria.

PATIENTS AND METHODS

37 patients with various glomerular diseases were included in this study in which renal biopsy was performed for diagnostic purpose between 1999 and 2008. Only the cases in which renal biopsies were examined in light, immunofluorescence and electron microscopy were taken into account.. The patients were divided in two groups: the group which presented nephrotic syndrome (glomerular proteinuria >3,5 g/24 h) (N=17) and the group with subnephrotic range proteinuria (0,16-3,4 g/24h) (N= 20) at the time of renal biopsy.

Examination of renal biopsy specimens.

For light microscopic examination, the tissue was fixed in 4% formaline and embedded in paraffin. Paraffin sections were cut at 3 μm and stained with hematoxylin and eosin, Masson’s trichrome and silver-methenamine according to standard protocols.

For direct immunofluorescence examination the tissue was frozen in liquid nitrogen. Then, 3 μm sections were cut on microscope slides and air-dried. Direct
immunofluorescence was performed using standard antisera (anti IgA, IgG, IgM, C3, C4, C1q, kappa and lambda light chains and fibrinogen) marked with fluorescein isothiocyanate (FITC) and then examined with a fluorescence microscope (Zeiss Axioplan).

For transmission electron microscopy (TEM) examination the tissue was promptly fixed in 2.7% glutaraldehyde, postfixed in 2% osmic acid, dehydrated in acetone and imbedded in Vestopal-W. Thin sections were cut on a LKB-3 ultramicrotome. Sections were stained on grids with uranyl acetate and lead citrate and examined with a Jeol Jem 1010 electron microscope.

**Foot process effacement quantitation**

Five different areas were photographed and analyzed for each case. Approximately 100 μm of GBM were measured for each case. The foot processes along the measured GBM were counted by hand. A foot process was defined as any connected epithelial segment butting on the basement membrane, between two neighbouring filtration pores or slits. For each case the arithmetic mean of the foot process width (FPW) was calculated according to the Gundersen method (Gundersen et al, 1980) as follows:

\[
FPW = \frac{\pi}{4} \cdot \frac{\Sigma \text{GBM length}}{\Sigma \text{foot process}}
\]

where \( \Sigma \text{foot process} \) is the total number of foot processes counted for each case, \( \Sigma \text{GBM length} \) is the total GBM length measured for each case, and the correction factor of \( \pi/4 \) serves to correct for presumed random variation in the angle of section relative to the long axis of the podocyte. For each patient the mean FPW was calculated and that value was used to finally calculate a mean FPW for each patient group.

**Statistical analysis**

Differences between groups were determined by the t test. Differences were considered significant when \( P<0.05 \). Correlation analysis was performed using the Spearman test.

**RESULTS**

Demographic, clinical, biochemical and clinicopathological characteristics of the studied patients at the time of renal biopsy are shown in Table 1. The levels of proteinuria and serum cholesterol were significantly higher and the levels of serum albumin were significantly lower in the nephrotic group compared to the subnephrotic group.

**Table 1.**

<table>
<thead>
<tr>
<th>Characteristics of the studied patients at biopsy</th>
<th>non SN</th>
<th>SN</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex M/F</td>
<td>N=20</td>
<td>N=17</td>
<td></td>
</tr>
<tr>
<td>Age at biopsy (years)</td>
<td>36 ± 11</td>
<td>42 ± 17</td>
<td>0.1</td>
</tr>
<tr>
<td>Serum albumin (g/dl)</td>
<td>3.6 ± 0.4</td>
<td>2.1 ± 0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Proteinuria (g/24 h)</td>
<td>1.4 ± 0.9</td>
<td>7.4 ± 3.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum cholesterol (mg/dl)</td>
<td>196 ± 48</td>
<td>359 ± 210</td>
<td>0.01</td>
</tr>
<tr>
<td>Creatinine clearance Cockcroft (ml/min)</td>
<td>77.5 ± 32</td>
<td>63.3 ± 30.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Clincopathological diagnosis (N)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alport nephritis</td>
<td>5</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Thin basement membrane nephropathy</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>MPGN</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>IgAN</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Fabry disease</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Diffuse lupus nephritis</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Poststreptococcal glomerulonephritis</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Cryoglobulinemic glomerulonephritis</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>MCD</td>
<td>0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Non IgA mesangioproliferative glomerulonephritis</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>MN</td>
<td>0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>FSGS</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Amiloidosis</td>
<td>0</td>
<td>1</td>
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</tbody>
</table>

MPGN = membranoproliferative glomerulonephritis; IgAN = IgA nephropathy; MCD = minimal change disease; MN = membranous nephropathy; FSGS = focal and segmental glomerulosclerosis.
The foot processes were well preserved in the non-nephrotic group, although segmental effacement has been observed in some cases. Mean FPW in this group was 422 ± 90 nm. In the nephrotic group extensive foot process effacement was observed. In 7 cases: MCD (N=3), MN (N=3) and GSFS (N=1) foot process effacement was diffuse. Mean FPW in the nephrotic group was 1196 ± 517 nm, significantly higher compared to the non-nephrotic group (p<0.001) (Fig. 2).

Fig. 1 Representative electron microscopy images from the studied patient groups, used for morphometric analysis of podocyte foot process effacement. (A) Well preserved foot processes; F, 46 yr, Thin basement membrane nephropathy-asymptomatic proteinuria and hematuria. (B) Complete foot process effacement - F, 20 yr, Minimal change disease – nephrotic syndrome.

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Fig. 2 Analysis of foot process effacement in the two groups, expressed as the mean of the podocyte foot process width (FPW). The error bars represent standard deviation.

Mean proteinuria in the non-nephrotic group was 1.4 ± 0.9 g/24h and 7.5 ± 3.3 g/24h in the nephrotic group. This difference was statistically significant (p<0.001). FPW was correlated to proteinuria when all patients were analyzed together (r=0.65; p<0.001). FPW did not correlate to proteinuria when the two groups were analyzed individually (non-nephrotic group: r=0.08; p=0.38 and nephrotic group: r=0.11; p=0.36) (Fig 3.).
DISCUSSION

Extensive foot process effacement was first described in 1957 in biopsies of patients with nephrotic syndrome (Farquhar et al., 1957). This phenomenon has been documented by many authors until now and has been the subject of many investigations in human and experimental glomerular diseases. Despite of intensive investigations, the pathogenesis of foot process effacement and its relation to proteinuria are still not clear. Podocyte foot process effacement seems a stereotypic reaction of podocytes to damage (Kerjaschki et al., 1994). Various ways to induce foot process effacement and proteinuria by injuring podocytes were described in experimental models: immune complex mediated injury in the Heymann nephritis model (Heymann et al., 1959; Kerjaschki et al., 1989), direct toxic podocyte injury by puromycin aminonucleoside (Michael et al., 1970; Ryan, Karnovski, 1957) or adriamycin (Kaplan et al., 1976; Bertani et al., 1982) and injury by injecting antibodies directed against distinct epitopes on podocytes, such as podoplanin (Matsui et al., 1998) and aminopeptidase A (Assmann et al., 1992). The velocity of development of foot process effacement and its severity differ between the models.

The pathogenetic mechanisms underlying podocyte foot process effacement in human glomerular diseases, such as MCN, FSGS or IgAN are still unknown. In analogy to experimental models, different pathogenetic mechanisms may underlie proteinuria and foot process effacement (van den Berg et al., 2004).

Morphometric analysis confirmed that foot process effacement was significantly more extensive in the nephrotic group compared to the subnephrotic group. Still, we observed big differences in the degree of foot process effacement in patients with comparable levels of proteinuria. The abnormal high FPW was invariably associated with proteinuria, but in many of the studied patients with massive proteinuria, foot process effacement was only segmental. This observation is in accordance with the results from other studies which show that proteinuria is not uniformly associated with foot process effacement. An experimental model has been described in which after injection of monoclonal antibodies directed against slit diaphragm components proteinuria occurs without foot process effacement (Orikasa et al., 1988; Liu et al., 2003). A familial form of human nephrotic syndrome has been reported to occur without foot process effacement (Branten et al., 2001).

In our study proteinuria did not correlate with the extent of foot process effacement in neither of the two groups. This result is in accordance with the observations of two recent studies (van den Berg et al., 2004; Deegens et al., 2008) which analyzed patients with MCD and FSGS and respectively MCD and IgAN. Other two studies (Gundersen et al., 1980; Powell HR, 1976) report the existence of a significant correlation between foot process effacement and the level of proteinuria in patients diagnosed with MCD. These studies included also patients who achieved remission and had proteinuria <1g/24h. This result shows that probably, in patients who achieve remission podocytes return to their normal architecture and that abnormal foot process width is invariably associated with proteinuria (van den Berg et al., 2004). When we analyzed all patients together we observed a weak, but significant correlation between foot process effacement and proteinuria ($r=0.65; \ p<0.001$).
Correlation between podocyte foot process effacement and proteinuria in human glomerular diseases

This result appears probably due to a practically normal FPW in patients with minimal proteinuria and due to the fact that podocytes regained their normal shape in patients with nephrotic syndrome achieving remission despite the persistence of a high level proteinuria.

One study analyzed the correlation between foot process effacement and age or treatment received prior to renal biopsy and the authors observed that foot process effacement correlated only with the mechanism of podocyte injury, more precise with the underlying disease (Deegens et al, 2008). In this regard the authors of a Chinese study affirm that foot process effacement correlates with proteinuria in patients diagnosed with IgAN (Song et al, 2010). When we analyzed the patients with IgAN included in our study, with the reticence becoming of analyzing a small number of cases (N=4), we observed that foot process effacement correlated with proteinuria (r=0.99; p=0.05). In contrast to prior studies and the present study, the Chinese group used another method for foot process effacement analysis. They estimated the percentage of the length of GBM where foot process effacement was observed. This method may seem less time consuming but is also less precise, especially for the analysis of early modifications of the foot processes compared to the Gundersen method (van den Berg et al, 2004; Gundersen et al, 1980; Fries et al, 1987) used in the prior studies.

The present study included patients with various glomerular diseases, the only inclusion criterion in one of the two groups being the level of proteinuria. The other studies analyzed a more uniform population of patients. Our results were in accordance with the results of other studies, underlying the necessity for further in depth studies of this ultrstructural modification of the podocyte with standardized methods, on larger and more uniform patient populations with glomerular diseases in order to clarify the relationship between the extent of foot process effacement and the level of proteinuria.

CONCLUSION
A significant difference between the foot process width in the nephrotic group and the subnephrotic group could be observed. The degree of foot process effacement correlated with the level of proteinuria of the patients included in the present study.

REFERENCES


