Morphometric analysis of the glomerular basement membrane withminimal change disease

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Abstract:

Objective To investigate the thickness of glomerular basement membrane (GBM) in patients withminimal change disease (MCD). **Methods** Select MCD patients aged 41 to 50 years without hema-turia, hypertension, diabetes, or hereditary kidney disease, and initially treated. Renal biopsy specimens were fixed as routine, embedded ultrathin sectioned, and observed under transmission electron microscope. For each sample, we took 10 to 15 photos according to the principle of equidistant curve movement. Test lines were randomly set. The vertical lines through the points where the test lines intersect with the podocyte side of GBM were made. The distance between the inner and outer intersections of the basement membrane was the section width of the GBM which represents the thickness of the **GBM. Results** The thickness of the GBM for 30 samples was 314.21±42.22 (256.97~393.51) nm, among which male was 360.30±47.0 (256.97~452.43) nm and female was 314.21±42.22 (256.97 ~393.51) nm. There was significant difference between the two groups (P = 0.009). The correlation be-tween GBM thickness and age was not significant. **Conclusions** The thickness of the GBM in newly diag-nosed MCD patients aged 41 to 50 years is constant, thicker in males than in females.

Keywords: Electron microscopy; Glomerular basement membrane; Stereology; Minimal change disease

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0 Introduction

The changes of glomerular basement membrane (GBM) thickness are of great significance for the diagnosis of some clinical diseases. For example, thin basement membrane nephropathy is a disease with GBM thinning as the main pathological manifestation, and its clinical manifestation is hematuria. The diagnosis must rely on the measurement of GBM thickness by electron microscope; Diabetes nephropathy is characterized by diffuse homogeneous thickening of GBM, especially in the early stage. GBM thickening may be the only histological feature of patients and the key basis for diagnosis. At present, the diagnostic criteria for GBM thickness related nephropathy are implemented with reference to foreign recommendations [1-2]. These standards are based on the GBM thickness of the normal population. In clinical work, we often find that the clinical manifestations of some patients do

not coincide with the morphological characteristics to a considerable extent when they are diagnosed according to international standards. The main reason is that the reference value is not suitable. At present, there are different reports on normal GBM thickness. It is inappropriate to use various diseased renal tissues, previous research data of others, or GBM thickness without gender and age matching as a control. There is no systematic research result for reference in China. As it is difficult to obtain normal kidney tissue, we hope to have more alternative tissues for research and analysis. Previous studies have suggested that minimal change disease (MCD) may be one of the alternatives [3]. Therefore, we need to know the GBM thickness of MCD patients. In order to reduce the influence of age factors, we selected patients aged 41~50 as the subjects.

1 Materials and methods

1.1 Selection of samples

Patients aged 41 to 50 who were hospitalized in the first hospital of Peking University and diagnosed with MCD by renal puncture from 2015 to 2021 were selected as the research objects. All the enrolled samples met the following conditions: there were no diseases that may affect the thickness of GBM, such as hematuria, hypertension, diabetes and hereditary kidney disease, and the patients were not treated with relevant drugs; All the tissues were negative by immunofluorescence staining, or only a small amount of nonspecific igm was deposited; Under light microscope, the morphology of glomerulus was normal or only mild segmental proliferation of mesangial cells and matrix was observed, with or without acute injury of renal tubules and renal interstitium; Transmission electron microscopy showed that GBM had no delamination, no obvious shrinkage, and extensive fusion of epithelial foot processes. A total of 30 samples were eligible for inclusion, including 13 males and 17 females; there were 5 obese patients (BMI ≥ 28) (1 male, 4 female), 14 non obese patients (bmi<28) (7 males and 7 females respectively), and the BMI of the remaining 11 patients was unknown; there were 9 patients (8 males and 1 female) with smoking history (more than 10 cigarettes per day for more than 10 years).

1.2 Tissue treatment and photographic methods

Fresh renal puncture specimens were fixed with 2.5% glutaraldehyde for 2 hours, 1% osmic acid for 1 hour, dehydrated with gradient alcohol and embedded with epon812, 3 μm semi thin sections were stained with toluidine blue. A glomerulus with good structure was selected from each sample for ultra-thin section. After double staining with uranium acetate and lead citrate, it was observed under jeol 1230 transmission electron microscope. The transmission electron microscope conditions are uniformly set at 60 kv, the filament saturation is set at 75%, and the digital image is taken at a magnification of 15000 times. Follow the principle of equidistant movement when taking photos. Take 10~15 photos of glomeruli on each section.

1.3 GBM section width measurement method

The photos taken are randomly set with the test grid under the scandium test system (Olympus). The intersection of the test line and GBM podocyte side is taken as the starting point of the measurement, and a lengthening line is made in the direction perpendicular to GBM to reach the intersection of endothelial cells. The length of the line segment is the section width of GBM at this point. GBM in the following areas shall not be measured: vascular pole, mesangial area, widened subendothelial space, GBM

shrinkage or poor opening of capillary cavity.

1.4 Statistical analysis

SPSS 13.0 software was used for statistical processing, and GBM thickness was expressed as mean ± standard deviation. T-test was used to compare the difference of GBM thickness among MCD patients with different gender, obesity or without smoking history. Pearson test was used to analyze the correlation between age and GBM thickness. All tests were bilateral tests, with significant difference using $P < 0.05$.

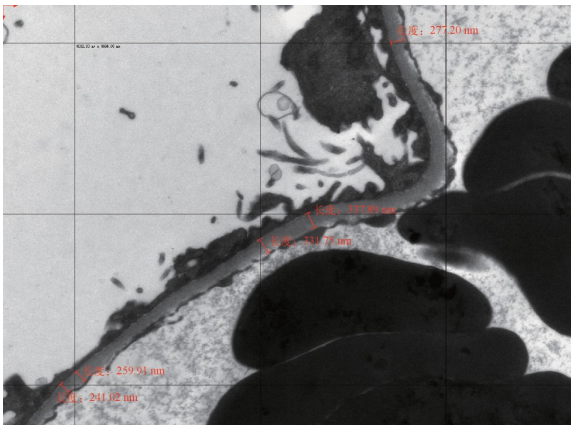

Figure 1 Measurement method of GBM section width (TEM, × 15 000)

There are 5 Intersections between randomly set grid lines and GBM podocyte side, and 5 Effective measurements.

2 Results

The number of effective intersections between randomly set grid lines and GBM in each photo is 1~12, and the number of effective section widths measured in each case is 71.93 ± 17.81 (38~114). The measurement is shown in Figure 1. The average section width of GBM in all 30 samples was 314.21 ± 42.22 (256.97~393.51) nm, including 360.30 ± 47.0 (256.97~452.43) nm for men and 314.21 ± 42.22 (256.97~393.51) nm for women. There was a significant difference between the two groups (P = 0.009); there was no significant correlation between GBM thickness and age increase in different genders (p>0.05). At the same time, no significant difference was found between the average cross-sectional width of GBM in patients of different gender groups and whether they were obese or long-term smokers (p>0.05). In this study, the standard deviation of the measured values at each point of each sample was analyzed. It was found that there were significant differences in the standard deviation of the measured values of patients of different sexes (P = 0.009), and men were higher than women.

3 Discussion

Glomerulus is the basic structure of hemofiltration, in which the pore membranes of capillary endothelial cells, GBM and podocytes together constitute the glomerular filtration barrier. Any structural change may lead to glomerular disease, including the change of GBM thickness. The common diseases related to GBM thinning are thin basement membrane nephropathy, Alport syndrome and benign familial hematuria. GBM thickening is the most common in diabetes nephropathy, and GBM thickness is related to glomerular filtration rate, diagnosis and prognosis of diabetes nephropathy [4-5]. To judge whether GBM is thickened or thinned, it is necessary to compare it with its normal thickness. The research data of normal GBM thickness mainly come from a few groups of foreign data in the early years [6-9]. In terms of influencing factors of GBM, a few studies believe that it has nothing to do with gender [10-11], but most studies show that GBM thickness is related to gender, age, measurement method and tissue treatment [11-12], and whether it is related to race has not been reported. There are very few data on GBM thickness in the normal population in China. Liulinchang et al. [13] mainly focused on gender differences in their research. Due to the small number of cases and large age span, it

is impossible to systematically analyze different age factors. Due to the different thickness of GBM in different age groups, it is necessary to narrow the age group for analysis. However, it is difficult to obtain a sufficient number of normal human renal tissues in the actual work. If the samples in the existing sample bank of renal puncture patients can be replaced, these works can be completed. There is evidence of GBM thickening in MCD patients after long-term hormone therapy, but there is no evidence of GBM thickness changes in untreated MCD patients compared with normal renal tissue.

Danilewicz et al. [3] showed that there was no significant difference in GBM thickness between MCD patients and normal people, suggesting the possibility of using such patient samples to analyze GBM thickness of normal people. The purpose of this study is to understand the GBM thickness of MCD patients who meet the inclusion conditions, so as to analyze whether it can be used as the data of normal GBM thickness in the later stage. When selecting MCD cases, we excluded the known relevant factors that may affect the thickness of GBM from the clinical and histopathological levels, including diabetes, hematuria, hereditary kidney disease, hormone therapy and other factors. During the measurement, we also excluded areas that may not represent the normal thickness of GBM, such as mesangial area, vascular pole, widening of subendothelial space, GBM shrinkage or poor opening.

The most commonly used method for GBM measurement is the orthogonal intercept method, which was first published by Jensen et al. [14] in 1979. They suggested that this method be used for GBM measurement. In short, a grid of equidistant intersecting lines is placed on the micrograph, and a line on the grid intercepts each point on the GBM interface of endothelial cells for GBM measurement. In order to reduce the inaccuracy of GBM thickness due to section, gun dersen et al. [15] suggested that the harmonic average thickness of GBM be calculated by correction to represent the true thickness of GBM. However, the calculation of harmonic mean thickness is complex and not suitable for clinical work. Therefore, it is more feasible to directly measure the GBM section width in practical use. If the method is used properly, the two measurements are completely comparable [16]. In order to facilitate the implementation and data comparison in clinical work, this study measured the GBM section width, and expressed the GBM thickness by the arithmetic mean value without calculating the harmonic mean thickness.

The results of this study show that the average section width of GBM is significantly different in patients of different genders ($P = 0.009$), and men are higher than women, which is the same as most previous reports [8, 13, 17]. It is further proved that there is a gender difference in GBM thickness. At the same time, it is also proved that the average section width of GBM is not related to age in the age range of 41~50 years, and the thickness of GBM is relatively constant. When analyzing the clinical data of patients, it was found that the measured value of the average cross-sectional width of GBM had nothing to do with whether the patients were obese or had smoking history. In view of the small total sample size in further grouping and the large difference in sample size between different groups, this result may not represent the actual situation. In the future, the sample size will be expanded for analysis to confirm the relevant conclusions. On this basis, the measured values of each sample were analyzed. It was found that the standard deviation of the measured values of male patients was significantly different from that of female patients ($P = 0.009$), and the variation of male

patients was more obvious, that is, the measured values of male patients were more uneven in the same case. The influencing factors need to be further analyzed.

In conclusion, in the analysis of GBM cross-section width of untreated MCD patients aged 41-50 years, it was found that the GBM thickness of patients at this age was constant, and men were thicker than women.

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