Original article

Pathological influence of obesity on renal structural changes in chronic kidney disease

Shigeko Kato[1,2], Alifa Nazneen[1], Yumiko Nakajima[1], Mohammed S. Razzaque[1], Tomoya Nishino[3], Akira Furusu[3], Noriaki Yorioka[4] and Takashi Taguchi[1]

1. Department of Pathology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

2. Department of Nutrition, Faculty of Nursing and Nutrition, University of Nagasaki, Nagasaki, Japan.

3. Second Department of Internal Medicine, Nagasaki University Hospital of Medicine and Dentistry, Nagasaki, Japan

4. Department of Advanced Nephrology, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan

Running headline: Renal pathological changes in obesity

The name and address for Correspondence: Takashi Taguchi,
Department of Pathology, Nagasaki University Graduate School of Biomedical Sciences,
1-12-4, Sakamoto, Nagasaki 852-8523, Japan.
Tel.: +81-95-819-7055, Fax: +81-95-819-7056, E-mail: taguchi@nagasaki-u.ac.jp

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Abstract

Background: Role of obesity on renal pathological and structural changes is not completely understood, and this study was designed to examine the pathological effects of obesity on renal structural components in the patients with and without chronic kidney diseases (CKD).

Methods: The study included 66 obese patients (BMI ≥25 kg/m^2) of four types of renal diseases; non-glomerulonephritis (Non-GN), IgA nephropathy (IgAN), benign nephrosclerosis (BNS) and thin basement membrane disease (TMD). The patients were evaluated by glomerular lesions (including mesangial proliferation and focal segmental or global glomerulosclerosis), glomerular size and the thickness of glomerular basement membrane (GBM), and the obtained data were compared with the similar glomerular parameters of 65 non-obese patients.

Results: Urinary protein excretion was increased in obese patients of Non-GN, IgAN and BNS. All obese patients showed FSGS lesions. The glomerular size was larger in obese patients than non-obese patients in Non-GN and IgAN groups. In non-obese patients, TMD and BNS patients showed significantly larger glomerular size than Non-GN patients. Obese patients had thicker GBM than non-obese patients in all the groups.

Conclusion: In the patients of IgAN, BNS and TMD, obese condition influences an increase of proteinuria and structural changes such as glomerulomegaly and GBM thickening, similar to the obese Non-GN patients (obesity-related nephropathy). Obesity appears be a promoting factor of the progression of CKD.

Introduction

Recently, a number of studies suggested a possible association with obesity and renal dysfunction [1-5]. Obesity should be put high on the list of potentially preventable causes of chronic kidney disease (CKD) [6]. Clinical features of obese patient without systemic disorders or apparent renal disorders, so-called obesity-related glomerulopathy (ORG), are associated with slowly progressive proteinuria. Various structural changes, including glomerulomegaly, focal segmental glomerulosclerosis (FSGS) and glomerular basement membrane (GBM) thickening can be seen in biopsy sections of ORG [2,3,7].

Glomerulomegaly is also observed in the kidneys of diabetic nephropathy and hypertensive kidney [1,6,8]. An increase in glomerular volume is usually the consequence of glomerular hyperfiltration, and can be associated with expansion of capillary loops [8,9,10]. Obesity can accelerate renal dysfunction in patients with glomerulonephritis such as IgA nephropathy (IgAN). In fact, in obese patients complicated with IgAN, the renal pathological changes and urinary protein are often severe [4]. However, histopathological studies, especially ultrastructural studies in CKD patients with obesity are not fully investigated.

The aim of this study is to examine the clinical and pathological influence of obesity in IgAN, benign nephrosclerosis (BNS) and thin basement membrane disease (TMD)

Materials and methods

Patients and Clinical Parameters

The clinical and pathological information available in 3908 renal biopsy samples collected from 1997 to 2007 at the Department of Pathology, Nagasaki University

Graduate School of Biomedical Sciences, Nagasaki, Japan, was reviewed. The selected subjects were older than 15 years old. All the renal biopsies of all were examined by light microscopy (LM), electron microscopy (EM) and immunofluorencence. The intended cases were selected from four patient groups: Non-glomerulonephritis (Non-GN), IgAN, BNS and TMD. The patients of Non-GN group have no evidence of systemic diseases other than obesity, and are considered as ORG. In IgAN group, the patients with mild mesangial proliferation were selected for evaluation. BNS was defined as renal alteration accompanied with essential hypertension. As for TMD patients, there was no evidence of clinical renal diseases except for micro/macro hematuria and defined as thin GBM (<250nm) involving at least 50% of GBM [11]. The patients with systemic disorders, including diabetes mellitus, were excluded.

Obesity was defined as body mass index (BMI) ≥25 kg/m^2, BMI was calculated as body weight (kg) / height^2 (m^2). According to the definition by the Japan Society for the Study of Obesity [12], classification was assigned as follows; class 1 obesity, 25≤BMI<30 kg/m^2; class 2 obesity, 30≤BMI<35 kg/m^2; class 3 obesity, 35≤BMI<40 kg/m^2; class 4 obesity, 40 kg/m^2≤BMI. The obese patients were divided into two obese groups; mild obesity (class 1 obesity) and severe obesity (class 2, 3 and 4 obesity). Age-matched non-obese (BMI < 25) patients were also examined as control in each group. The laboratory data were selected at the time of renal biopsy. This study was approved by the Ethics committee of Nagasaki University Graduate School of Biomedical Sciences.

Histopathological Analysis

Histopathological lesions of the glomeruli were evaluated on LM sections stained with periodic acid-schiff (PAS) and periodic acid silver-methenamin (PAM). Histopathological analysis was performed on the index of glomerular lesion (IGL), incidence of FSGS and sclerotic glomeruli (SG) as described previously [13]. IGL took into account both proliferative and sclerotic changes.

Measurement of glomerular diameter was performed using LM sections by WinRoof ver5.03 (Mitani Corp,Tokyo ,Japan). The mean glomerular size was calculated from average of top 5 glomerular diameters (μm) per case to approximate glomerular equatorial section[2]. The size (μm^2) was calculated as: $[(\text{diameter}/2)^2 \times 3.14]$.

EM photographs at original magnification of ×3,000 were used to determine the GBM thickness using the technique described by Osawa et al [14].

Statistical Analysis

All data were expressed as mean±SD. SPSS 16.0 J for Windows was used to perform the analysis (SPSS Inc, Chicago, IL). Differences were performed by one-way analysis of variance (ANOVA), non-paired Student`s *t*-test and Fisher`s exact test. Statistical significance was defined as $p<0.05$.

Results

Clinical features

The clinical and laboratory findings of the patients were shown in Table 1. All the patients both of obese and non-obese groups had maintained renal function. Urinary protein was greater in obese patients of Non-GN, IgAN and BNS than non-obese patients, respectively. But it was not depending on the severity. There were no patients with severe obesity (30≤BMI) in TMD group. The titers of serum creatinine and BUN were not significantly different between obese and non-obese patients. In Non-GN patients, blood pressure, triglyceride and total cholesterol were significantly higher in obese patients than non-obese patients. Also in IgAN and TMD, triglyceride was significantly higher in obese patients. In TMD patients, blood pressure was higher in obese patients than non-obese patients.

Renal histopathological findings

Tables 2 and 3 showed the scores assigned to histological features of the patients.

Non-GN group: FSGS lesions were not seen in non-obese (Fig.1.A) or mild obese patients. Two of severe obese patients (7%) showed FSGS lesion (Fig1.B). The IGL and FSGS score were higher in severe obese patients than non-obese and mild obese patients (Table 2).

IgAN group: The IGL was significantly higher in severe obese patients than non-obese and mild obesity patients. There were no differences in FSGS score and SG score among non-obese and obese patients. All patients of IgAN showed FSGS lesion and sclerotic glomeruli (SG) (Table2). This result may reflect the characteristic pathological feature of IgA nephropathy.

BNS group: The IGL, FSGS and SG lesions were not different among obese and non-obese patients. Although most of BNS patients showed high incidence of SG lesion, FSGS lesion was noted in one non-obese patient and one severe obese patient (Fig.1 E,F, Table3).

TMD group: No severe obese patients were noted in TMD group. There were no significant differences in the IGL lesion, FSGS or SG scores between obese and non-obese patients (Table 3). Only one obese patient had FSGS lesion and the other one had two sclerotic glomeruli.

Glomerular size

The glomerular sizes of all groups were shown in Fig.2. In Non-GN, the glomerular size in both mild and severe obesity patients was significantly larger than non-obese patients. Mild obese patients had a tendency to contain larger glomeruli than severe obesity, but not statistically different.

In IgAN, the glomerular size of obese patients was significantly greater than non-obese patients, depending on the severity of obesity (Fig.1 C,D). In BNS patients, glomerular size was larger in obesity than non-obese, but it was not significant difference. On the other hand, glomerular size of non-obese BNS patients was significantly greater than non-obese Non-GN (Fig.2, Table.3).

TMD Group had only mild obesity patients. The glomerular size was slightly larger in obese than non-obese patients, having no significant difference. When comparing in non-obese patients, the glomerular size was significantly greater in TMD than Non-GN, in spite of same blood pressure levels (Fig.1 G,H and Fig.2).

Thickness of glomerular basement membrane (GBM)

The results of measurement of GBM by EM observation were shown in Fig.4. In Non-GN Group, the thickness of GBM became greater in obese patients, depending on the severity of obesity (Fig. 3 A, B). The significant difference of GBM thickness was present between non-obese patients and severe obesity ($p<0.05$) or whole obesity patients ($p<0.01$). In IgAN, the BGM thickness of obese patients was slightly thicker than non-obese patients, but no significant difference was detected. The patients of IgAN occasionally show thin GBM in various distribution, associated with thick GBM (Fig.3 C,D).

In BNS, the GBM was significantly more thickened in all obesity patients, compared with non-obese patients ($p<0.05$) (Fig.3 G, H). In TMD, there was no significant difference in GBM thickness between obese and non-obese patients. Thin GBM was diffusely seen in TMD patients, as definition (Figs.3 G, H).

Discussion

In the present study, to observe the influence of obesity in CKD, we selected the patients with mild structural changes; IgAN of mild form, BNS and TMD. The obese patients in non-glomerulonephritis group of our study were compatible to obesity-related glomerulopathy (ORG). To our knowledge, this is the first report of clinocopathological study of the influence of obesity in CKD, focusing on GBM thickness and obesity level.

We observed glomerulomegaly and FSGS lesion in ORG patients, as seen in previous reports [4]. In particular, FSGS lesion was observed in severe obesity patients, and IGL core was increased depending on obesity levels. The GBM thickness might

depend on obesity level in ORG patients. Kambham et al reported focal GBM thickening in ORG patients [2]. The recent ultrastructural study of ORG showed widened foot processes and enlargement of podocytes in extremely obese patients, in addition to marked thickening of GBM [15]. These reports and our study suggest that obese condition might influence on the GBM width in ORG probably depending on the severity of obesity. Hypertension is thought to be an important factor for progression of the disease in ORG [1]. In our study, systemic blood pressure and the thickness of GBM became greater depending on the severity of obesity. Increase of intraglomerular pressure may contribute to thickening of GBM.

A few studies of obesity and IgAN have been reported [4, 16]. When obesity accompanies IgAN, the pathological changes were more severe and proteinuria was grater [4]. Ultrastructural study of IgAN with obesity has seldom reported. Tanaka et al reported that obese IgAN patients showed greater GBM thickening and larger total glomerular tuft areas, compared with the lean IgAN patients [16]. Our data also showed significantly larger glomerular size in obese IgAN than non-obese IgAN. Although the thickness of GBM in obesity was slightly larger than that non-obesity, no significant difference was noted. Thin GBM is variably encountered in most of IgAN. It may be one reason not to detect significant difference between obesity and non-obese groups in the thickness of GBM. IGL was higher in severe obesity patients. These results indicated that glomerular alterations might be accelerated in IgAN by obese condition, same as ORG patients.

Many studies investigated that BMI and blood pressure was associated with glomerular lesions in ORG patients [5,6,17,18]. In hypertensive patients, increasing renal tubular sodium reabsouption and glomerular hyperfiltration may cause

glomerulomegaly [1]. In hypertensive patients, the glomerular number was fewer and glomerular volume was larger than non-hypertensive people [19,20]. In our study, glomerular size of non-obese BNS patients was larger than non-obese patients of Non-GN and IgAN groups. It may be caused by intraglomerular hypertension and compensatory hypertrophy of fewer glomerular numbers. The GBM thickness was significantly larger in obese BNS patients than non-obese patients. Although glomerular injury was caused by hemodynamic abnormality in BNS, obese condition may accelerate the glomerular lesions.

TMD is known as excellent prognosis, and thin GBM is considered to be reflected abnormality of collagen type IV of the GBM [11, 21]. Interestingly, glomerular size of non-obese TMD patients was significantly larger than non-obese Non-GN and IgAN patients. Due to abnormal constitution of GBM, glomerular capillary lumina may easily expand against intraglomerular pressure. Severe obesity patients are not present in TMD group. When the GBM may become thicker in severe obesity patients with TMD, they are not included in the group in TMD as definition. Further examination is necessary for the behavior of GBM thickening in obesity TMD patients, in association with analysis of collagen type IV [11].

Conclusions

Our findings suggest that urinary protein excretion and glomerular damage was accelerated by obesity in patients with IgA nephropathy, benign nephrosclerosis and thin basement membrane disease. In addition, hypertension might be an important factor to precipitate renal disease in obesity-related nephropathy. In the patients of CKD, obese condition might influence an increase of proteinuria and structural

changes, as in obesity-related nephropathy. That is, obesity might be a promoting factor of the progression of CKD.

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Figure legends

Fig.1: Histopathological findings with non-obese [A, C, E, G] and obesity [B, D, F, H] patients. (A,B) non-glomerulonephritis, (C,D) IgA nephropathy, (E,F) benign nephrosclerosis and (G,H) thin basement membrane disease. PAM stain, scale bars = 50 μm. Inset of picture B and D were each serial section with PAS stain.

Fig. 2: Average glomerular size of non-obese patients, mild obesity and severe obesity patients with Non-GN (non-glomerulonephritis), IgAN (IgA nephropathy), BNS (benign nephrosclerosis) and TMD (thin basement membrane disease). Bars are expressed as mean+SD, a, $p<0.01$ vs non-obese Non-GN ; b, $p<0.05$ vs non-obese patients in each group; c, $p<0.01$ non-obese BNS or non-obese TMD vs non-obese Non-GN ; d, $p<0.05$ non-obese BNS or non-obese TMD vs non-obese IgAN.

Fig.3 Features of electron microscopy with non-obese [A, C, E, G] and obese [B, D, F, H] patients. (A,B) non-glomerulonephritis, (C,D) IgA nephropathy, (E,F) benign nephrosclerosis and (G,H) thin basement membrane disease. Original magnification: ×3000. Inset of picture D showed focal thinning of GBM.

Fig. 4 The GBM thickness of in Non-GN (non-glomerulonephritis), IgAN (IgA nephropathy), BNS (benign nephrosclerosis) and TMD (thin basement membrane disease). Bars are expressed as mean+SD, a, $p<0.05$ vs non-obese patients in each group; b, $p<0.01$ vs non-obese Non-GN.