Clinical significance of urinary fibrin/fibrinogen degradation products (FDP) as measured by latex photometric immunoassay in renal diseases

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Abstract

It is possible that abnormalities of intraglomerular coagulation and fibrinolysis are involved in the exacerbation of kidney diseases. Urinary fibrin/fibrinogen degradation products (FDP) are regarded as an index of the intraglomerular coagulation and fibrinolysis. Although the conventional latex agglutination method for detecting urinary FDP has disadvantages such as a poor sensitivity and is of the semiassay type, latex photometric immunoassay (LPIA), a recently developed technique, is an assay with a high sensitivity. The present study was undertaken in an attempt to clarify the significance of urinary FDP as measured by latex photometric immunoassay in renal diseases. The subjects comprised were 60 patients with 15 kinds of renal diseases. Occasional urine samples and blood samples withdrawn at the time of urinary collection were examined. The FDP and FDP-E fractions (FDP-E) were measured by LPIA, and the FDP-D fraction (FDP-D) and fibrinopeptide A (FPA) were measured by enzyme immunoassay. The highest level of urinary FDP was seen in cases with diabetic nephropathy, followed by renal amyloidosis and chronic glomerulonephritis. While no correlation was noted between the urinary FDP levels and blood FDP levels, positive correlations were observed among the urinary protein, urinary FDP-E, FDP-D and FPA. The urinary FDP also revealed an inverse correlation with the 1/serum creatinine. All cases with high levels of urinary FDP displayed renal dysfunction. These findings suggest that quantitative assay of the urinary FDP using LPIA is important for determining the degree of abnormality of intraglomerular coagulation and fibrinolysis in renal diseases.

Introduction

We reported previously that the intraglomerular coagulation and fibrinolysis system is involved in the exacerbation of renal diseases, and that urinary fibrin/fibrinogen degradation products (FDP) can be utilized as an index [1, 2]. In these reports, we indicated that the urinary FDP/urinary protein ratio is clinically more important than the amount of urinary FDP itself. However, because the method of urinary FDP measurement used at that time was tanned red cell hemagglutination inhibi-

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be measured with a high sensitivity and accuracy. The purpose of the present was to evaluate the clinical significance of urinary FDP by LPIA.

**Method and subjects**

   The basic principle is to measure the agglutination between latex sensitized with FDP antibody and the FDP in the sample by rate turbidimetry in which a luminous diode with a central wavelength of 940 nm is used as a light source.

2. Method of urinary FDP measurement by LPIA
   The standard FDP was preparation diluted so as to adjust the concentration to 0, 10, 20, 40, and 80 µg/ml, and a working curve was prepared in advance. Five µl of the sample was placed in a plastic cell and 200 µl of stabilizer was added. Following automatic infusion and mixing of latex reagent, a 32-sec preincubation was carried out and the turbidity was measured 10 times at 3-sec intervals. The mean degree of change was then estimated, and the reaction speed was calculated. The turbidity was obtained from the working curve. LPIA-100 (MITSUBISHI KASEI Corp.) was used for the measurement.

3. Determination of FDP-E products (FDP-E), FDP-D products (FDP-D), and fibrinopeptide A (FPA)
   The urinary FDP-E was measured by the LPIA method using LPIA-100 (MITSUBISHI KASEI Corp.), similarly to the case of the urinary FDP. The urinary FDP-D products (FDP-D) and fibrinopeptide A (FPA) were measured by enzyme immunoassay (EIA). A Testzym FDP kit (DAIICHI PURE CHEMICALS) [5] and an Asserachrom FPA kit (Boehringer Mannheim Co.) [6] were employed for the former and latter determinations, respectively.

4. Subjects
   The subjects comprised 60 patients (28 males and 32 females), including 8 cases with minimal change, 4 cases with IgA nephropathy, 16 cases with chronic glomerulonephritis, 2 cases with membranous nephropathy, 1 case with focal glomerulosclerosis, 5 cases with chronic renal failure (non-hemodialysis), 7 cases with diabetic nephropathy, 3 cases with renal amyloidosis, 1 case with polycystic kidney disease, 1 case with gouty kidney, 5 cases with toxemia of pregnancy, 1 case with hydronephrosis, 1 case with progressive systemic sclerosis, 1 case with purpura nephritis, and 4 cases with lupus nephritis. The mean age of the group was 47.5 ± 16.7 years. Occasional urine samples were collected when the patients appeared at the outpatient clinic. Each urine sample was centrifuged at 1500 rpm for 10 min, and the supernatant was stored at -20°C for use as the sample. Concomitantly, 2 ml of blood was withdrawn into a vacuum tube for FDP estimation (with addition of aprotinin and thrombin), and centrifuged at 3000 rpm for 10 min to separate the serum for the FDP sample. The plasma was used for the measurement of FPA.

**Results**

1. Relationship between blood FDP and urinary FDP
   Fig. 1 illustrates the relationship between the serum FDP and urinary FDP. No correlation was observed between them.

2. Urinary FDP levels in various renal diseases
   Fig. 2 plots the urinary FDP concentrations in individuals with various renal diseases. The levels were 23.3 ± 34.3 µg/ml in the cases with diabetic nephropathy (the highest value, followed by 8.1 ± 7.3 µg/ml in the cases with renal amyloidosis, 0.4 ± 0.3 µg/ml in the cases with lupus nephritis, and 0.4 ± 0.3 µg/ml in the cases with chronic renal

![Fig. 1. Relationship between fibrin/fibrinogen degradation products (FDP) in the blood and urine. B-FDP, Blood FDP; U-FDP, Urinary FDP. The shaded area indicates the normal range of blood and urinary FDP.](image-url)
failure. The level of urinary FDP was thus generally low even in chronic renal failure.

3. Relationship between urinary FDP and urinary protein

As shown in Fig. 3, a strong positive correlation was noted between the urinary FDP and urinary protein ($r = 0.78$, $p<0.001$). The mean level of urinary protein was $248.6 \pm 391.0$ mg/dl.

4. Relationship between urinary FDP and urinary FDP-E

Fig. 4 illustrates the relationship between the urinary FDP and urinary FDP-E. A clear positive correlation was observed ($r = 0.78$, $p<0.01$). The mean level of urinary FDP-E was $85.9 \pm 232.1$ ng/ml.

5. Relationship between urinary FDP and urinary FDP-D

Fig. 5 shows the relationship between the urinary FDP and the urinary FDP-D. A clear positive correlation was recognized between them ($r = 0.86$, $p<0.01$). The number of cases which showed abnormally high levels of urinary FDP-D amounted to 21 out of 57.

6. Relationship between urinary FDP and urinary FPA

Fig. 6 illustrates the relationship between the urinary FDP and urinary FPA. No correlation was observed between them. The mean level of urinary
Fig. 5. Relationship between urinary fibrin/fibrinogen degradation products (FDP) and FDP-D in the urine. U-FDP, Urinary fibrin/fibrinogen degradation products; U-FDP-D, Urinary fibrin/fibrinogen degradation product-D.

Fig. 6. Relationship between urinary fibrin/fibrinogen degradation products (FDP) and fibrinopeptide A in the urine. U-FDP, Urinary fibrin/fibrinogen degradation products; U-FPA, Urinary fibrin/fibrinogen degradation product-A.

FPA was 2.8 ± 2.1 ng/ml.

7. Relationship between urinary FDP and 1/serum creatinine

Fig. 7 shows the relationship between the urinary FDP and 1/serum creatinine. A negative correlation was evident between them (r = 0.40, p < 0.02). Cases of renal insufficiency with increased levels of serum creatinine did not always reveal high levels of urinary FDP. Conversely, the serum creatinine levels were increased in all cases with high levels of urinary FDP. High levels of urinary FDP were recognized only in cases with a reduced renal function.

Discussion

It is known that FDP are excreted into the urine of patients with various renal diseases [1, 2, 7-15]. The available methods for FDP measurement include the precipitation method [16], tanned red cell hemagglutination inhibition immunoassay (TRCHII) [17], the staphylococcal clumping test [18], the latex agglutination test [19], and radioimmunoassay [20]. Although radioimmunoassay represents the most sensitive technique, it is not used widely because special equipment is required,
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it is time-consuming, and the measurement are costly. TRCHII, which detects FDP ranging from low to high molecular weight with a relatively good sensitivity, previously enjoyed wide application. Subsequently, the latex agglutination test became widely used because of its simplicity, although the method dose have disadvantages such as a poor sensitivity and is of the semia assay type. The problematic points in the measurement of urinary FDP include: (1) lack of quantitativeness, (2) poor sensitivity, (3) no discrimination between primary fibrinolysis and secondary fibrinolysis, and (4) no information on the FDP-producing sites. Thus, a new method, which eliminates some of the disadvantages of the latex agglutination test, has recently been developed. This technique is latex photometric immunoassay (LPIA). LPIA enables disadvantages (1) and (2) to be overcome. In fact, the measurement sensitivity for urinary FDP with LPIA is 5 pg/ml. The normal level obtained by us was 3330 ± 7950 ng/day [21]. The blood FDP was not correlated with the urinary FDP (Fig. 1). We have reported that among cases of kidney diseases, high FDP levels by the TRCHII method [1] were observed patients with acute glomerulonephritis, lupus nephritis, membranoproliferative glomerulonephritis or glomerulonephritis with severe proliferative changes. In addition, the present study revealed that cases of diabetic nephropathy and renal amyloidosis also had high FDP levels among renal diseases. The patients with diabetic nephropathy were found to lack any increase in urinary FDP levels because they were not at the active stage. The 5 cases with chronic renal failure did not display any increase in urinary FDP. This suggests that high level of urinary FDP are not always observed in cases of severe renal functional disturbance complicated by a damaged glomerular basement membrane and low selective proteinuria. The opinion expressed by Hall et al. [10], that the urinary FDP reflects the selectivity of the glomerular basement membrane, is therefore not appropriate. As we have already proposed, there are two origins for urinary FDP: first, the coagulation dominant type, due mainly to an increase in intraglomerular coagulation and fibrinolysis; and second, the permeability dominant type, due to an increase in permeability of the glomerular basement membrane [1]. However, one point of difference from acute glomerulonephritis of the typical coagulation dominant type is that the levels of urinary protein in diabetic nephropathy and renal amyloidosis are also high when the urinary FDP levels are high. The reason that, in the present study, a positive correlation was noted between the urinary FDP and urinary protein, may have been the fact that a total of 10 cases of renal amyloidosis and diabetic nephropathy (a relatively large proportion were included as subjects. The results may have arisen because among the 60 cases, FDP positivity was evident in 16 cases originating from the urinary FDP, including 3 cases of diabetic nephropathy and 3 cases of renal amyloidosis (total, 9 cases; 56.25%). The finding that the urinary FDP showed a good positive correlation with the urinary FDP-E and FPA suggests that these positive cases for urinary FDP were of the coagulation dominant type. The relationship between the urinary FDP and 1/serum creatinine in the 60 cases with 15 diseases proved to be a significantly inverse correlation. When the relationship between the urinary FDP and 1/serum creatinine was investigated from the standpoint of its distribution, it was clear that the renal function in cases with high levels of urinary FDP had already decreased, but all cases with a reduced renal function did not always display high levels of urinary FDP. In other words, activation of the intraglomerular coagulation and fibrinolysis is frequently induced in cases with a reduced renal function. This suggests that renal such dysfunction may be an essential condition for the activation of intraglomerular coagulation and fibrinolysis. Since the urinary FDP can be measured by LPIA, a quantitative diagnosis of renal intraglomerular blood coagulation could be possible. Further studies are needed in order to clarify the frequency and degree of intraglomerular coagulation and fibrinolysis occurring in diabetic nephropathy and renal amyloidosis.

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References