〈Original Article〉

Variation in urinary protein components in individuals with orthostatic proteinuria as detected by lordotic load testing

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Summary Orthostatic proteinuria is diagnosed by a lordotic load test. We analyzed urinary protein levels after the lordotic load test in the context of orthostatic proteinuria. Urine test paper analysis showed that urinary protein levels after lordotic load testing for 15 min were higher than those after testing for 5 min in 6 of the 12 male students (BMI, 16.6-22.9 kg/m^2; age, 20-23 years). The excretion of glomerular proteins increased to a much greater extent after lordotic load testing for 15 min than after lordotic load testing for 5 min. In some cases, excretion of tubular proteins also increased. Individuals with orthostatic proteinuria should not only receive special attention to glomerular proteins but also to tubular proteins. Moreover, close observation may be necessary because the proteins excreted in individuals with orthostatic proteinuria are the same as those in individuals who have proteinuria with renal disease.

Key words: Orthostatic proteinuria, Lordotic load test, Glomerular proteinuria, Tubular proteinuria

1. Introduction

Orthostatic proteinuria was first reported in 1885 by Pavy, who described proteinuria with cyclic characteristics, that is, protein-free urine in the morning and at night but proteinuria during the day[1]. In 1887, Striling found that the condition was related with posture and called it postural proteinuria[2]. When only orthostatic proteinuria is present, it is believed to be a benign disorder with benign causes that would probably disappear over time. Most authoritative reports consider this condition to be benign, with renal function remaining normal after as long as 50 years of follow-up[3, 4]. However, anecdotal observations suggest that orthostatic proteinuria occasionally reflects incipient renal disease. Moreover, proteinuria is an important factor for evaluating the prognosis of nephropathy, but orthostatic proteinuria disappears quickly because of its benign nature and is therefore difficult to analyze. Lordotic load testing was intro-

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duced for the diagnosis of orthostatic proteinuria, and various methods have been reported in this regard[5-7].

In the present study, we compared the results of lordotic load testing performed for 5 min and 15 min and analyzed urinary protein levels after lordotic load testing to obtain valuable information for the diagnosis of orthostatic proteinuria.

2. Material and Methods

1. Subjects

Urine samples before and after lordotic load testing for 5 min and 15 min were obtained of 12 male students (BMI, 16.6-22.9 kg/m^2; age 20-23 years) from Bunkyo Gakuin University. The samples yielded negative results for urinary proteins before lordotic load testing. The ethical committee of Bunkyo Gakuin University approved this study, and informed consent was obtained from all the subjects.

2. Lordotic load test

The upright lordotic posture was produced by placing a bar (4 cm in diameter, 100 cm in length, and 411 g in weight) on the lumbar spine for 5 min or 15 min. The bar was held in the arms of the subjects such that it was parallel to the ground. The upper part of the body was curved at a 20-degree angle from the waist.

We collected urine samples before the lordotic load test. After the subjects were given 180 ml of water, they assumed the upright lordotic position for 5 min or 15 min. Urine was collected at 30 min after lordotic load testing for 5 min and was collected at 20 min after lordotic load testing for 15 min. The 5-min and 15-min lordotic load tests were conducted on different days.

3. Measuring Methods

1) Urine test paper method

Proteinuria was measured using Eiken Uropaper Ⅲ (Eiken Chemical, Tokyo, Japan) with a US-1000 urine analyzer (Eiken Chemical, Tokyo, Japan).

Abbreviations: Urinary total protein (TP), albumin (ALB), creatinine (CRE), transferrin (TF), retinol-binding protein (RBP), ß$_2$-microglobulin (ß$_2$-m), and α_1-microglobulin (α_1-m)

2) Assay procedure

All samples were analyzed using commercially available kits and an Accute TBA-40FR (Toshiba Medical Systems, Tokyo, Japan). Urinary total protein (TP) levels were measured using the WAKO Micro TP Test (Wako Pure Chemical Industries, Osaka, Japan). Assays for urinary albumin (ALB), creatinine (CRE), transferrin (TF), retinol-binding protein (RBP), ß$_2$-microglobulin (ß$_2$-m), and α_1-microglobulin (α_1-m) were conducted using the N-assay turbidimetric immunoassay Micro Alb, N-assay L CRE-K, N-assay LA Micro TF, N-assay LA RBP, N-assay LAß$_2$-MG-H Ⅱ (Nittobo Medical, Tokyo, Japan), and Eiken LZ test α_1-M (Eiken Chemical, Tokyo, Japan), respectively.

4. Statistical analysis

SPSS 21.0 for Windows (SPSS, Inc, USA) was used for the statistical analysis. Paired sample *t*-test was used for evaluating significant differences. Statistical differences were considered to be significant at $P < 0.05$.

3. Results

1. Urine test paper method

As shown in Table 1, urine samples were classified into 6 groups according the type of proteinuria after lordotic load testing for 5 min and 15 min. Proteinuria was negative in all samples before lordotic load test both for 5 min and 15 min. In 3 samples (25.0%) from group I, proteinuria was negative after lordotic load testing for both 5 min and 15 min. In 4 samples (33.3%) from group II, although proteinuria results were negative after lordotic load testing for 5 min, the results were (±) after lordotic load testing for 15 min. In 2 samples (16.7%) of group III, proteinuria was (±) after lordotic load testing for both 5 min and 15 min. In 1 sample (8.3%) from group IV, although proteinuria was (±) after lordotic load testing for 5 min, it was (3+) after lordotic load test for 15 min. In 1 sample (8.3%) from group V, proteinuria was (2+) after lordotic load testing for both 5 min and 15 min. In 1 sample (8.3%) from group VI, although proteinuria was (2+) after lordotic load

testing for 5 min, it was (3+) after lordotic load testing for 15 min.

2. Urinary TP level before and after lordotic load testing

The urinary protein level in the 6 groups was determined before and after lordotic load testing for 5 min and 15 min (Table 2). Similar results were obtained for the urinary protein level, relative to the levels determined by the urine test paper. Urinary protein levels in group I, II, and III were within reference intervals (<150 mg/gCRE). The urinary protein level did not considerably differ before lordotic load testing for the 5 min and 15 min tests. Although no change was observed after lordotic load testing for 5 min in group I, group II, and group III, the urinary protein level increased by approximately 2 fold after lordotic load testing for 15 min in group II and group III. However, there were no significant differences ($P > 0.05$) between before and after lordotic load testing in group I, II, or III. The urinary protein level increased by 2 fold after testing for 5 min in group IV, 21 fold after lordotic load testing for 15 min in group IV, 20 fold after testing for 5 min in group V, 8 fold after testing for 15 min in group V, 30 fold after testing for 5 min in group VI, and 173 fold after testing for 15 min in group VI. The urinary protein level tended to increase with increase in loading time. We have not performed statistical analysis between before and after lordotic load testing in group IV, V, or VI because of the small case numbers.

3. Comparison of various urinary proteins before and after lordotic load testing

The increased levels of various urinary proteins

Table 1 Classification for different loading times by using urine test paper to determine protein levels

Group	Lordotic load test for 5 min	Lordotic load test for 15 min	Number of cases
Ⅰ	－	－	3 (25.0%)
Ⅱ	－	±	4 (33.3%)
Ⅲ	±	±	2 (16.7%)
Ⅳ	±	3＋	1 (8.3%)
Ⅴ	2＋	2＋	1 (8.3%)
Ⅵ	2＋	3＋	1 (8.3%)
Total			12

Protein was not detected by urine test paper analysis in samples before lordotic load testing.

Table 2 Urinary total protein levels before and after lordotic load testing

(mg/gCRE)

Group	Lordotic load test for 5 min		Lordotic load test for 15 min	
	before	after	before	after
Ⅰ	41±36	31±11	18±7.6	27±12
Ⅱ	16±4.8	21±9.4	18±6.0	38±13
Ⅲ	16±2.2	18±9.4	17±3.0	34±12
Ⅳ	41	88	23	493
Ⅴ	9.2	187	21	172
Ⅵ	47	1422	29	5024

mean±1SD

after lordotic load testing are shown in Fig. 1. The variation in ALB and TF was similar to that in the TP levels, and these protein levels increased to a greater extent after lordotic load testing for 15 min than after lordotic load testing for 5 min in groups II, III, IV, and VI. In group VI, the ALB and TF levels after lordotic load testing for 15 min increased by 740 fold and by 160 fold.

In some cases, the excretion of tubular proteins such as α_1-m, RBP, and ß$_2$-m increased slightly. The degree of increase after lordotic load testing for 15 min was greater than that after testing for 5 min in groups II, III, IV, V, and VI for α_1-m; groups IV for RBP; and groups II, III, IV, V, and VI for ß$_2$-m.

In this comparison, we have not performed statistical analysis because of defining the ratio of each protein levels after lordotic load testing to before lordotic load testing.

4. Discussion

In this study, TP levels in orthostatic proteinuria were qualitatively and quantitatively evaluated. Urine test paper analysis showed that urinary protein levels after lordotic load testing for 15 min were higher than those after testing for 5 min in 6 of 12 cases. In particular, there was great variability between lordotic load tests performed for 5 min and 15 min in group IV. Because it was not possible to observe small changes in TP using urine test paper, the TP level after lordotic load testing was assumed to be increasing while that before testing was assumed to be 1. In group IV, the TP level after lordotic load testing for 5 min increased by 2 fold, whereas that after testing for 15 min increased by 21 fold. Therefore, high variation (approximately 11 fold) was observed between the 2 durations of the lordotic load test. Moreover, in group VI, the TP level after lordotic load testing for 5 min increased by 30 fold and that after testing for 15 min increased by 173 fold. Thus, great variation (approximately 6 fold) was observed between the 2 durations of the lordotic load test in this group as well. The differences probably occurred because a longer load time is associated with more excretion of protein in urine, which in turn arises from more pressure on the renal vein[8]. Lordotic load testing for 5 min had previously been conducted by Suzuki et al.[6], and lordotic load testing for 15 min had been conducted by Chisaki[7]. In the present study, it was considered that testing for 15 min would definitely permit the detection of orthostatic proteinuria because of the increased excretion of proteins in the urine.

Moreover, glomerular proteins such as Alb and Tf were found to be excreted to a higher extent when various proteins were analyzed, which is consistent with the results reported by Suzuki et al.[6]; however, we found that the increase in excretion of glomerular proteins was greater for the lordotic load test performed for 15 min than for the test performed for 5 min. Devaranjan proposed a hypothesis regarding the pathogenic mechanism of orthostatic proteinuria[9]; therefore, subjects in whom urinary protein excretion increased after lordotic load testing may have originally had minimal damage in the glomerulus.

Little change was observed in the excretion of α_1-m after lordotic load testing for 5 min, but the excretion generally increased, in group IV, V, and IV, after lordotic load testing for 15 min. However, the degree of increase for α_1-m was not as high as that for the glomerular proteins. The RBP level in group VI increased by approximately 16 fold after lordotic load testing for 5 min, but the increase after 15 min was similar to that in groups IV and V. Only group VI showed a great increase in ß$_2$-m levels. The levels of tubular proteins such as α_1-m, RBP, and ß$_2$-m slightly increased after lordotic load testing, which indicates that this test may influence tubular reabsorption. Subjects who have orthostatic proteinuria in which glomerular proteins are mainly excreted should be closely followed up because tubular damage is reported to be associated with proteinuria[10]. Moreover, these subjects may have to be closely observed because the proteins excreted in subjects with orthostatic proteinuria are the same as those excreted in patients with proteinuria with renal disease.

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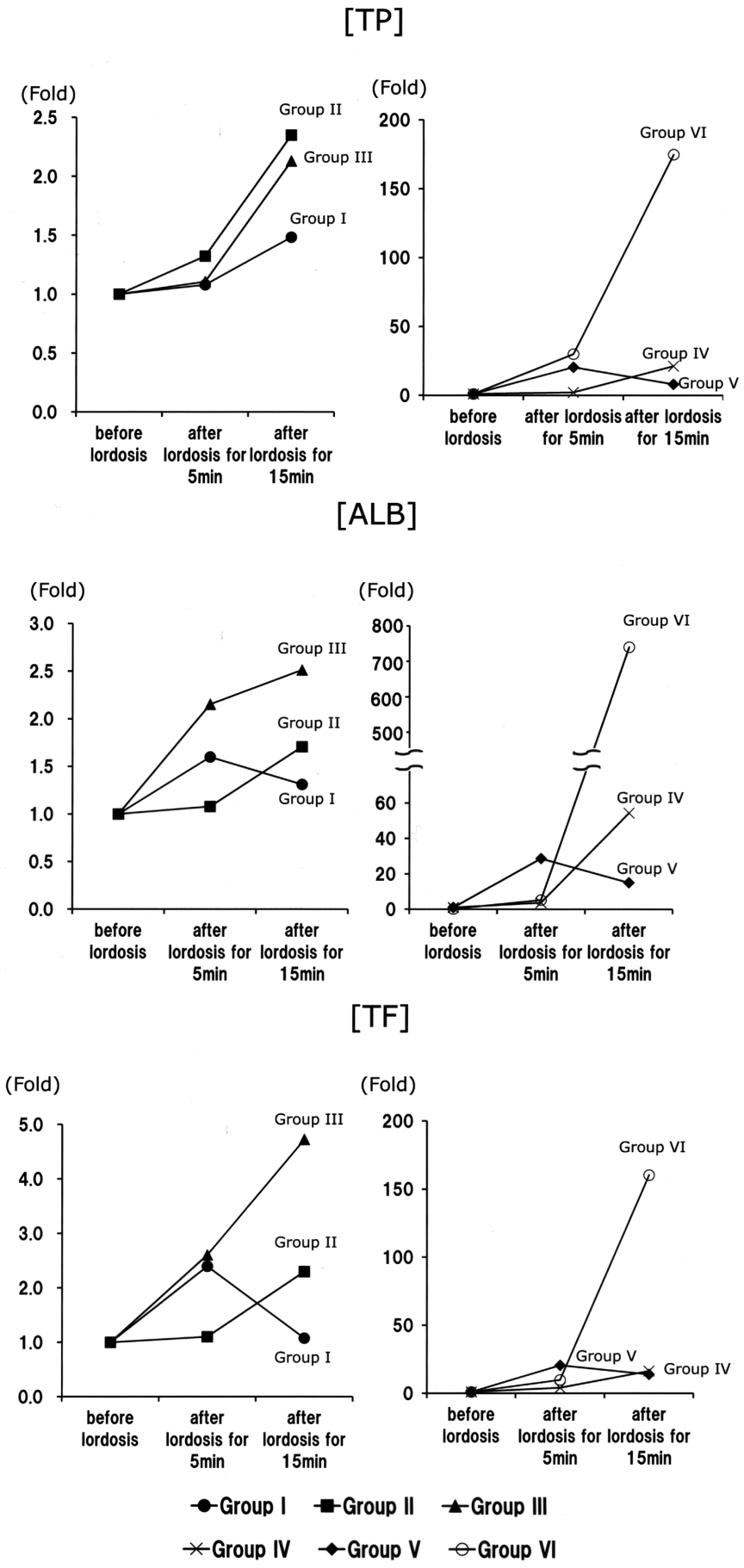

Fig. 1 Increases in the levels of various urinary proteins after lordotic load testing for 5 min and 15 min

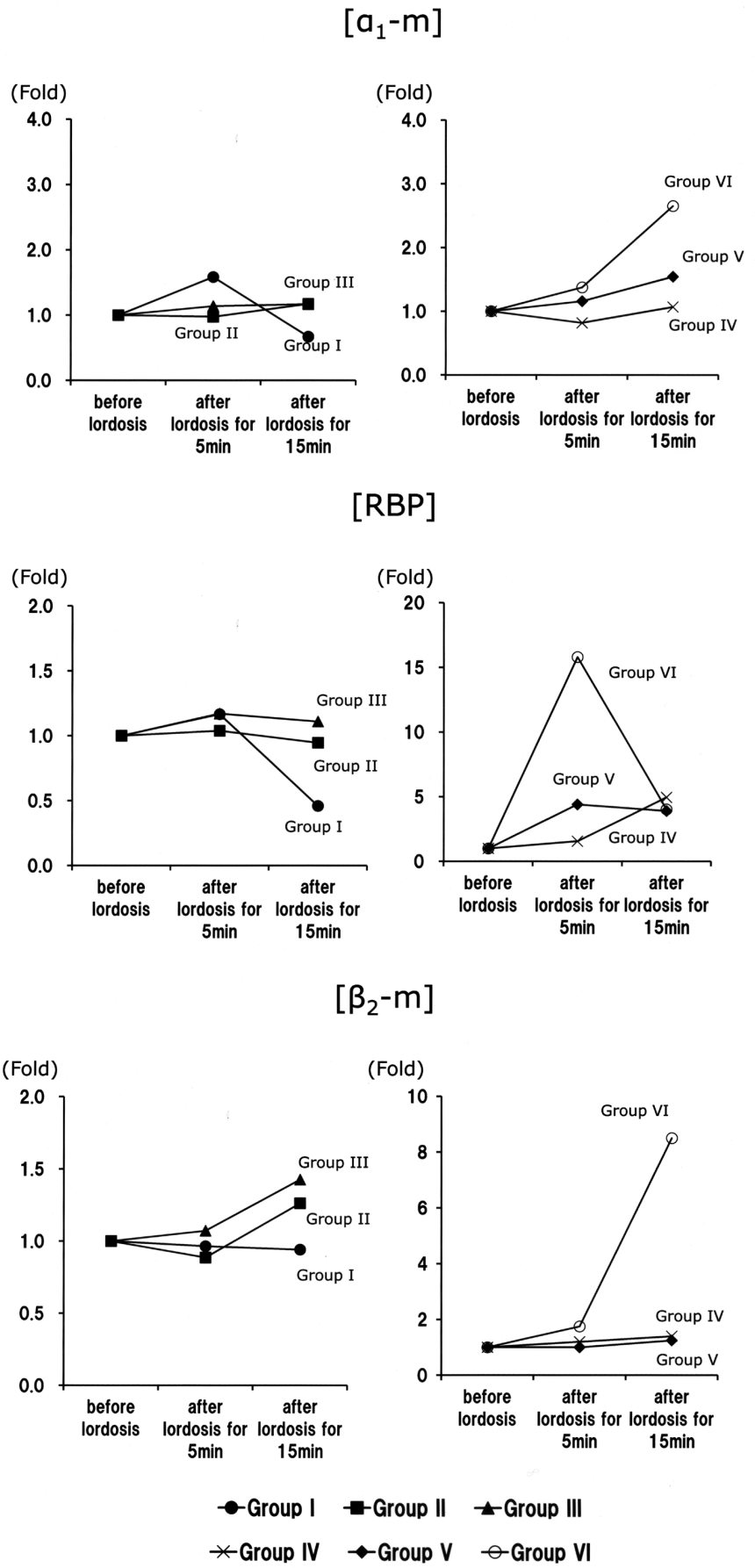

Fig 1 (continued)

Conflict of interests

The authors declare no conflict of interest.

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