

Studies on Serum Protein Subfractions in Urine

Shigeru KAKIMOTO*, Hironobu HAKARIYA
Tomoo HOSHINO, Atsushi KONDO

*Department of Urology
Nagasaki University School of Medicine
(Director Prof. A.Kondo)*

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INTRODUCTION

There have been performed various studies on the analysis of the proteinuria, which give us some important indexes concerning screening test or diagnosis of different renal diseases. We investigated, in this study, the subfractions of the serum proteins in urine by means of antibody absorption method in renal homotransplantation and various renal diseases such as renal failure due to acute or chronic glomerulonephritis, nephrotic syndrome, SLE-nephrosis, upper and lower urinary tract diseases, chyluria, etc..

MATERIALS

1. Glomerulonephritis :

48 cases of glomerulonephritis were grouped, according to Kinoshita's classification, as follows :

subchronic glomerulonephritis29 cases (D-group)

Chronic glomerulonephritis14 cases (E-group)

2. Renal failure :

28 cases of renal failure due to chronic glomerulonephritis, who were under the hemodialysis.

3. Nephrotic syndrome :

24 cases of nephrotic syndrome under medication :

Adults12 cases

Children12 cases

*垣本 滋・計屋 紘信・星野 知生・近藤 厚

4. SLE-nephrosis :

10 cases who present the reaction positive in urinary protein test among patients with SLE-nephrosis.

5. Chyluria :

10 cases in pre-treatment phase.

6. Diseases of the upper urinary tract (14 cases) :

Renaltuberculosis 3 cases
 Wandering kidney3 cases
 Pyelonephritis5 cases
 Renal calculus2 cases
 Hydronephrosis.....1 case

7. Diseases of the lower urinary tract (22 cases) :

Prostatic hypertrophy8 cases
 Cystitis5 cases
 Chronic prostatitis3 cases
 Vesical tumor1 case
 Prostatic cancer1 case
 Vesical calculus1 case
 Urethral stricture.....1 case

8. Renal homotransplantation :

This is a subject (man, 23 years old) whose kidney was homotransplanted with kidney from a death by reason of the renal failure due to chronic glomerulonephritis. we found on the day 60 after transplantation a syndrom which seems a rejection :

At that time, BUN :15-20mg/dl, creatinine:1. 0-2.0 mg/dl, creatinine clearance : 85.89-109.62, quantative reduction of urine, slight lowering of the urinary osmotic pressure, slight tenderness at the transplanted part and mild pyrexia.

Besides, since the day 330, the renal function began to be lowering ; BUN : 30-40 mg/dl, creatinine 2.0-2.5mg/dl, creatinine clearance : around 44.65ml/min. That seemed to be a chronic rejection. Since then, the renal function became more lowering. 4 years and 4 months after operation, the transplanted kidney was excises and this patient have been treated under the hemodialysis up to today.

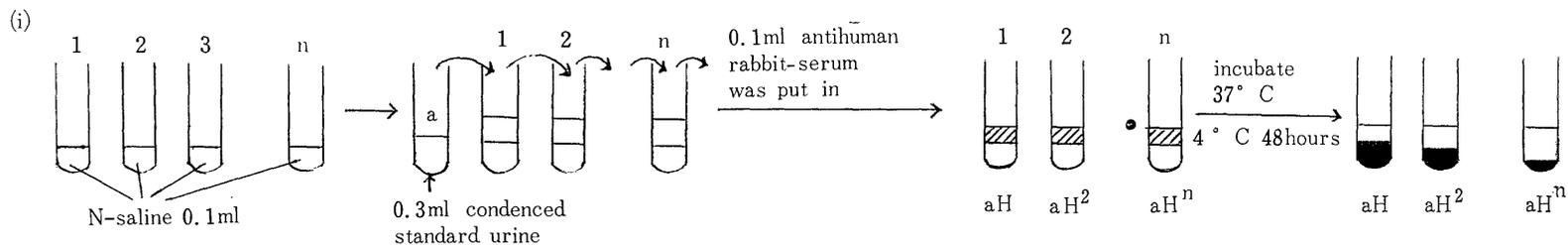
METHODS

The serum protein subfractions in urine was determined according to the method of Hoshino's¹⁾ Antibody Absorption.

1. Determination of the equivalent point of the standard urine. Urine 10 ml of a normal subject and 0.1 ml of human standard serum (Behring-werke) were introduced into a Visking-tube 18/32. This specimen dialysed for 24 hours in a cold chamber (4°C), and,

Fig. 1 ANTIBODY ABSORPTION METHOD (A. A. M.)

1. Determination of the equivalent point of standard urine

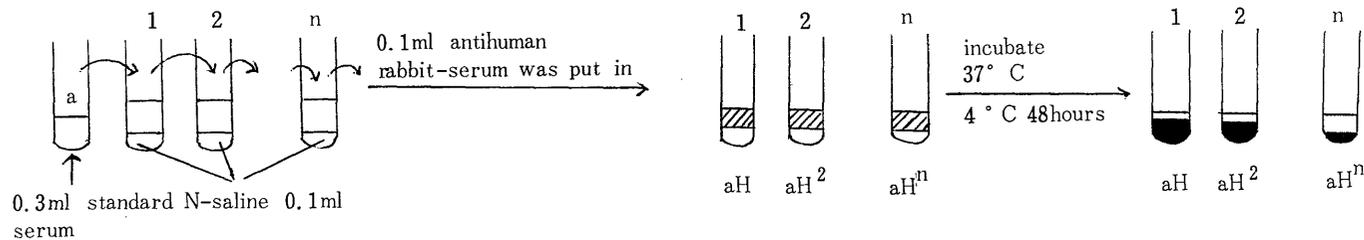


(ii) Immunoelectrophoresis



the equivalent point can be obtained as the appearing point of the precipitating line of subfraction.

2. Determination of equivalent point of standard serum



→ Immunoelectrophoresis

after dialysis, it was concentrated with polyethylene glycol 4000 up to 0.3ml ($\times 33$). Preparing a series of dilutions in preparation of diluting ratio $H=3/4$ from this concentrated standard urine, the equivalent point of serum protein was determined by means of the antibody absorption. (Fig. 1) Electrophoresis was performed for 1 and a half hour at 200 V.

2. Determination of equivalent point of the standard serum. Following addition of 0.1 ml of N-saline to 0.3 ml of standard human serum (Behring-Werke), a series of dilutions was prepared in preparation of diluting ratio $H=3/4$. Adding 0.1 ml anti-rabbit-serum (prepared by us), to each of dilutions, the obtained supernatant fluid of each dilution was subjected to the immuno-electrophoresis with pooled serum of normal subject, and the equivalent point was determined.

3. Determination of the equivalent point of the specimen urine. The equivalent point was determined following the operation under the same condition as one used for the standard urine.

4. Calculation of serum protein level in standard urine. Under the condition 1 and 2, both level of the equivalent point of standard urine and one of standard serum must be equal. Therefore the following formula should be established:

$$aH^n = b \frac{1}{100} \times 33H.^m \text{ and } a = b$$

a = protein level of the standard serum

n = equivalent point of the standard serum

b = protein level of standard serum in standard urine

m = equivalent point of the standard urine i. e. As to α_2 -M

$$a(3/4)^{10} \longrightarrow b. \frac{1}{100} 33. \left(\frac{3}{4}\right)^6$$

$$0.96 \longrightarrow b. a \approx b$$

Also, as to other protein levels, the equation of $a \approx b$ can be established.

5. Calculation of serum proteins in urine. The calculation level in urine can be calculated with the following formula:

$$aH^n = X. 33H^m$$

$$X = \frac{aH^n}{33H^m}$$

a = protein level of the standard serum

n = equivalent point of the standard serum

x = protein level in urine

m = equivalent point of the urine

6. Determinable minimum level of serum proteins in urine. As the protein level in standard serum is unclear, α_1 -lipoprotein (shortened as α_1 -Lp hereunder) and α_2 -Lp are expressed in % of equivalent point of the urine to that of pooled serum of normal subject

Determinable minimum levels are as follows:

α_1 -Lp0.006%

α_2 -Lp0.24%

α_2 -M0.216mg/dl

Tr.....	0.08mg/dl
$\beta_{1C}/1_A$	0.04mg/dl
Ig-M	0.108mg/dl
Ig-G.....	0.02mg/dl

RESULTS

1. Glomerulonephritis.

It was Ig-G which showed the highest amount among all serum proteins in urine in both groups D and E, and its average was of 1.737 ± 1.602 mg/dl in D-group and of 3.271 ± 3.181 mg/dl in E-group, followed by Tr level which were 0.626 ± 0.975 mg/dl in D-group and 1.642 ± 2.199 mg/dl in E-group. α_1 -Lp was of $0.03 \pm 0.054\%$ in D-group and of $0.153 \pm 0.289\%$ in E-group; same as Tr, E-group presented a value superior to D-group. $\beta_{1C}/1_A$, α_2 -M and Ig-M were found respectively only in 3, 1 and 4 subjects in D-group. However α_2 -Lp level was inferior to the determinable minimum level commonly in all the cases of both groups D and E. (see table 1).

Table 1 Urinary protein in SLE nephrosis, glomerulonephritis and nephrotic syndrome

	α_1 -Lp %	Tr mg/dl	$\beta_{1C}/1_A$ mg/dl	Ig-M mg/dl	Ig-G mg/dl
SLE-nephrosis	1.113 ± 0.665	1.201 ± 1.127	0.127 ± 0.129	2.644 ± 3.390	22.429 ± 34.291
Glomerulonephritis D-group	0.031 ± 0.054	0.626 ± 0.957	3cases positive	4cases potive	1.737 ± 1.602
Glomerulonephritis E-group	0.153 ± 0.289	1.642 ± 2.199	0.095 ± 0.144	0.126 ± 0.216	3.271 ± 3.181
Nephrotic syndrome Adult	0.309 ± 0.492	3.950 ± 5.462	0.431 ± 0.823	0.142 ± 0.268	9.110 ± 12.865
Nephrotic syndroem Pediatic	0.168 ± 0.087	0.935 ± 0.944	0.065 ± 0.120	0.421 ± 0.263	6.873 ± 9.131

2. Nephrotic syndrome.

Comparing between adult subjects (12 cases) and infant subjects (12 cases), the average of Tr was of 3.950 ± 5.462 mg/dl in adults and of 0.935 ± 0.944 mg/dl in children. Ig-G: 9.110 ± 12.865 mg/dl in adults, 6.873 ± 9.131 mg/dl in children. These facts tell that the proteins of relatively low molecular weight were observed more in adult subjects than infant subjects, on the contrary Ig-M was higher in infant subjects; 0.142 ± 0.268 mg/dl in adults and 0.421 ± 0.263 mg/dl in children. However, there were no marked differences for other proteins between these two categories of subjects.

3. SLE-nephrosis

Concerning 10 cases of SLE nephrosis presenting a reaction positive for urinary

proteins, the averages were of 2.644 ± 3.390 mg/dl for Ig-M and of 22.49 ± 34.291 mg/dl for Ig-G. That means the superiority of the immunoglobulins to other categories of diseases.

4. Results in other diseases

In Fig 2-a, b, c, d shown are the results obtained in 28 cases of renal failure due to chronic glomerulonephritis, 10 cases of chyluria, 14 cases of upper urinary tract diseases and 22 cases of lower urinary tract diseases. The marked increase of the serum proteins in urine characterize the renal failure due to chronic glomerulonephritis, and above all the following points are characteristic:

- 1) α_2 -Lp seen in 5 subjects
- 2) α_2 -M seen in 8 subjects
- 3) High value of $\beta_{IC}/1_A$; average = 0.25 ± 0.46 mg/dl

In chyluria, all fractions of serum proteins were markedly increases in urine:

- 1) High level of α_2 -Lp; average = 0.71 ± 0.41 %
- 2) High level of Ig-M; average = 2.60 ± 1.90 mg/dl

There were no marked differences between the upper urinary tract diseases and the lower urinary tract diseases, and the level of serum proteins in urine was low in common.

- 1) α_2 -Lp not seen
- 2) $\beta_{IC}/1_A$ not seen
- 3) Low level of Ig-M: seen only in 10 subjects

5. Results in the case of renal homotransplantation

Clinical course and determination of serum proteins in urine. Among all serum proteins in urine except albumine, Ig-G level was the highest throughout whole clinical course. Its maximum reached approximately 30mg/dl, followed by Tr, of which the level-max reached 9mg/dl., on the other hand, Ig-M and $\beta_{IC}/1_A$ being respectively of 0.7mg/dl and of 0.2mg/dl, both were much low in comparison with Ig-G and Tr. Throughout whole course, the level of α_2 -M was inferior to 0.216mg/dl and α_2 -Lp was less than 0.24%. The Fig.3 shows the determined value following transplantation.

a. Changes in 3 weeks after transplantation.

α_1 -Lp, Tr, Ig-M and Ig-G abruptly increased soon after transplantation. The peak of α_1 -Lp (=0.24%) and one of Tr (=8.7mg/dl) were found on the 14th day following transplantation. The peak of Ig-M (=0.4mg/dl) appeared on the days 4 and 9, and the peak of Ig-G (=27.7mg/dl) on the day 4. Since then, the levels rapidly decreased, and they became almost normal in 3 weeks after transplantation.

b. Changes in 200 days after 3rd week.

Between the day 60 and the day 69, a reaction of rejection-like was observed. At that time, α_1 -Lp increased to 0.13%, Tr to 1.7mg/dl and Ig-M to 11.6mg/dl, but α_2 -M, α_2 -Lp and $\beta_{IC}/1_A$ did not appear.

c. Changes between the days 200 and 300 after transplantation.

Increase was observed for the levels of α_1 -Lp, Tr and Ig-G, which were respectively 0.01%, 1.2mg/dl and 8.3mg/dl, and each of them reached the peak on the day 276. on the other hand, α_2 -Lp, α_2 -M, $\beta_{IC}/1_A$ and Ig-M did not come out at that time. And, in

Fig. 2a Renal failure due to chronic glomerulonephritis

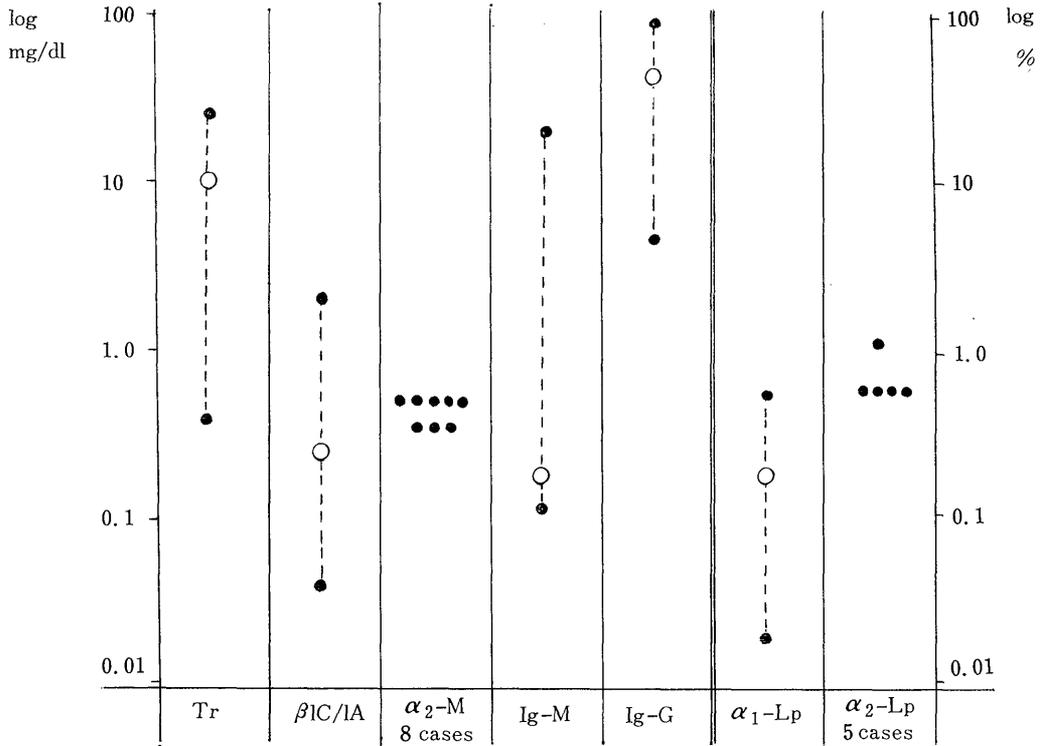


Fig. 2b Chyluria

○ shows average

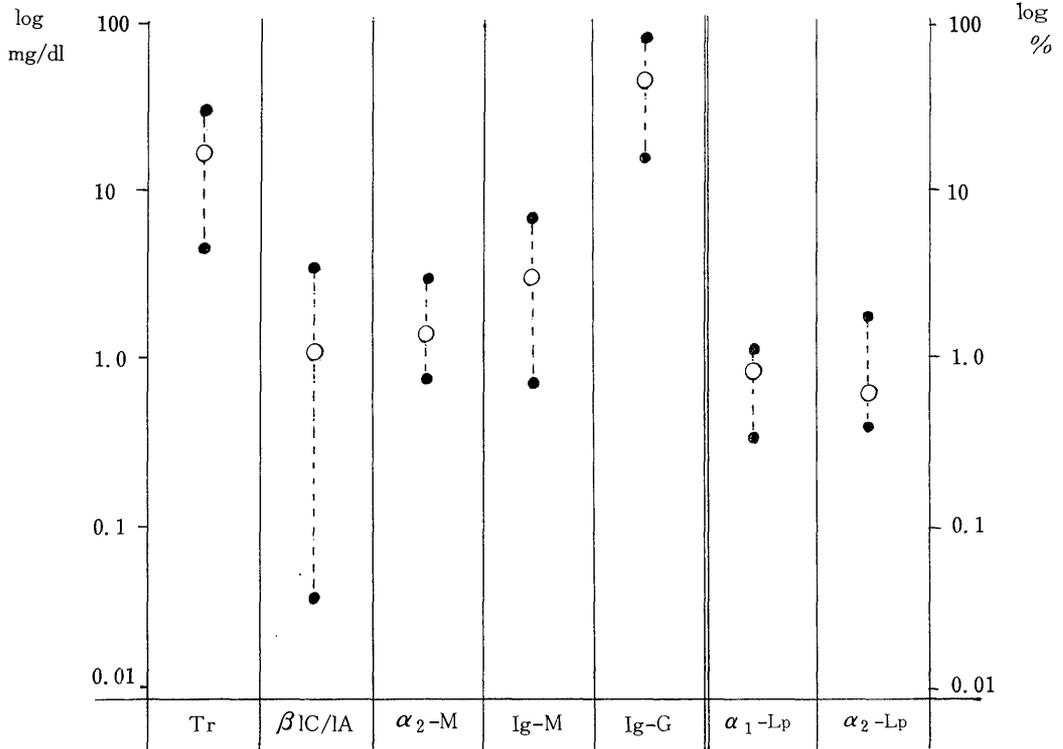


Fig. 2c Upper urinary disease

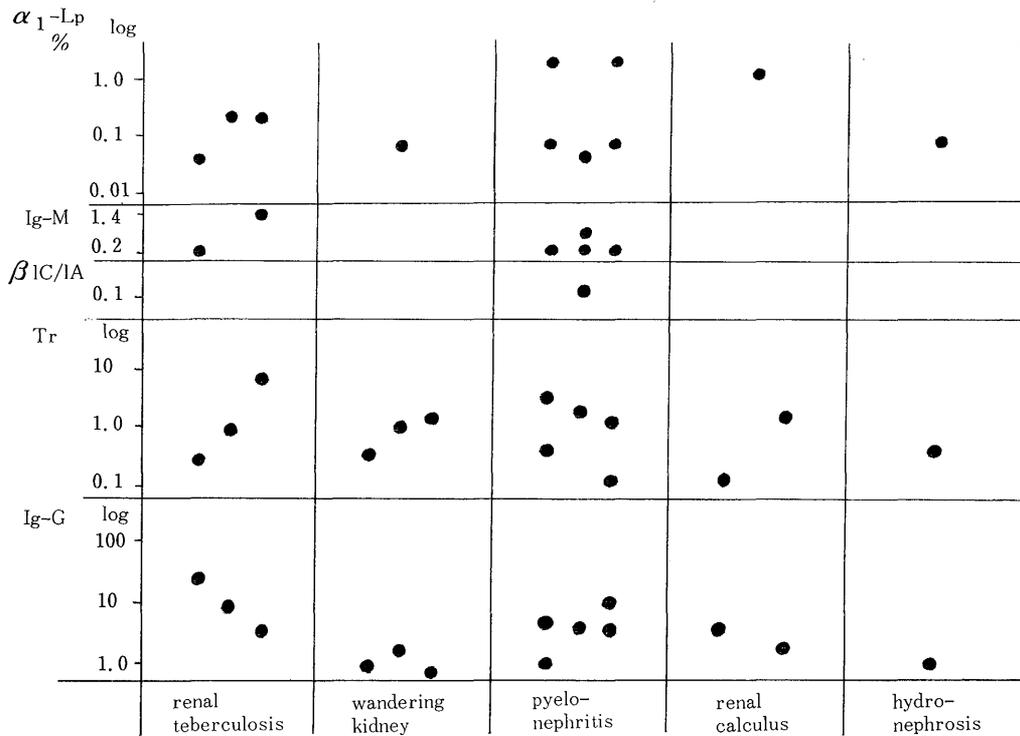


Fig. 2d Lower urinary disease

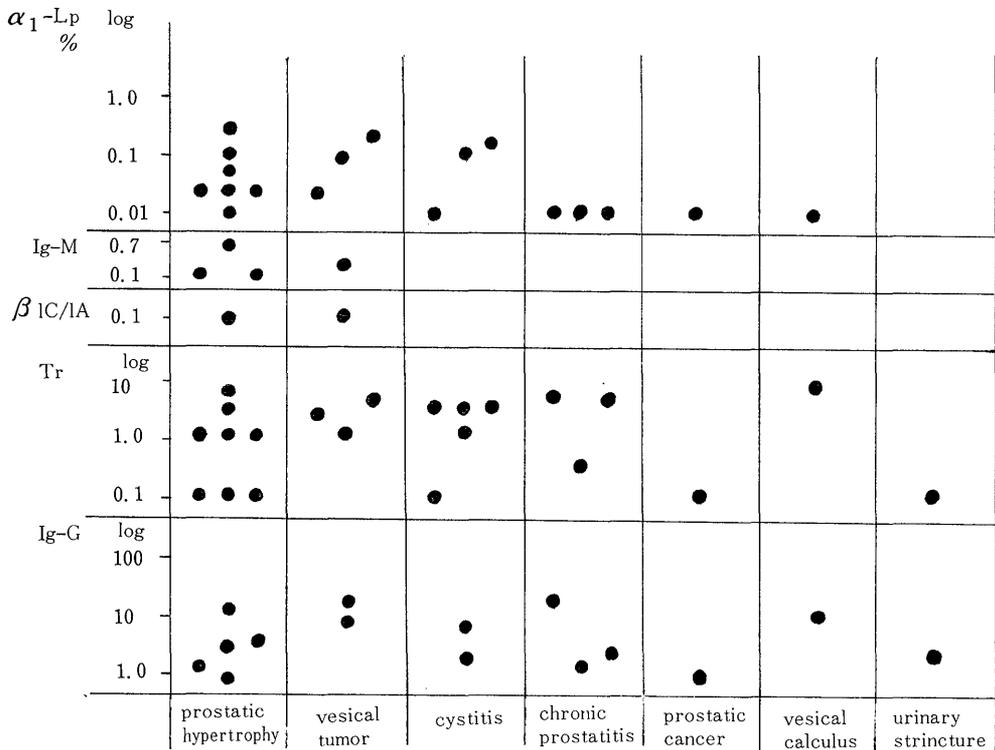
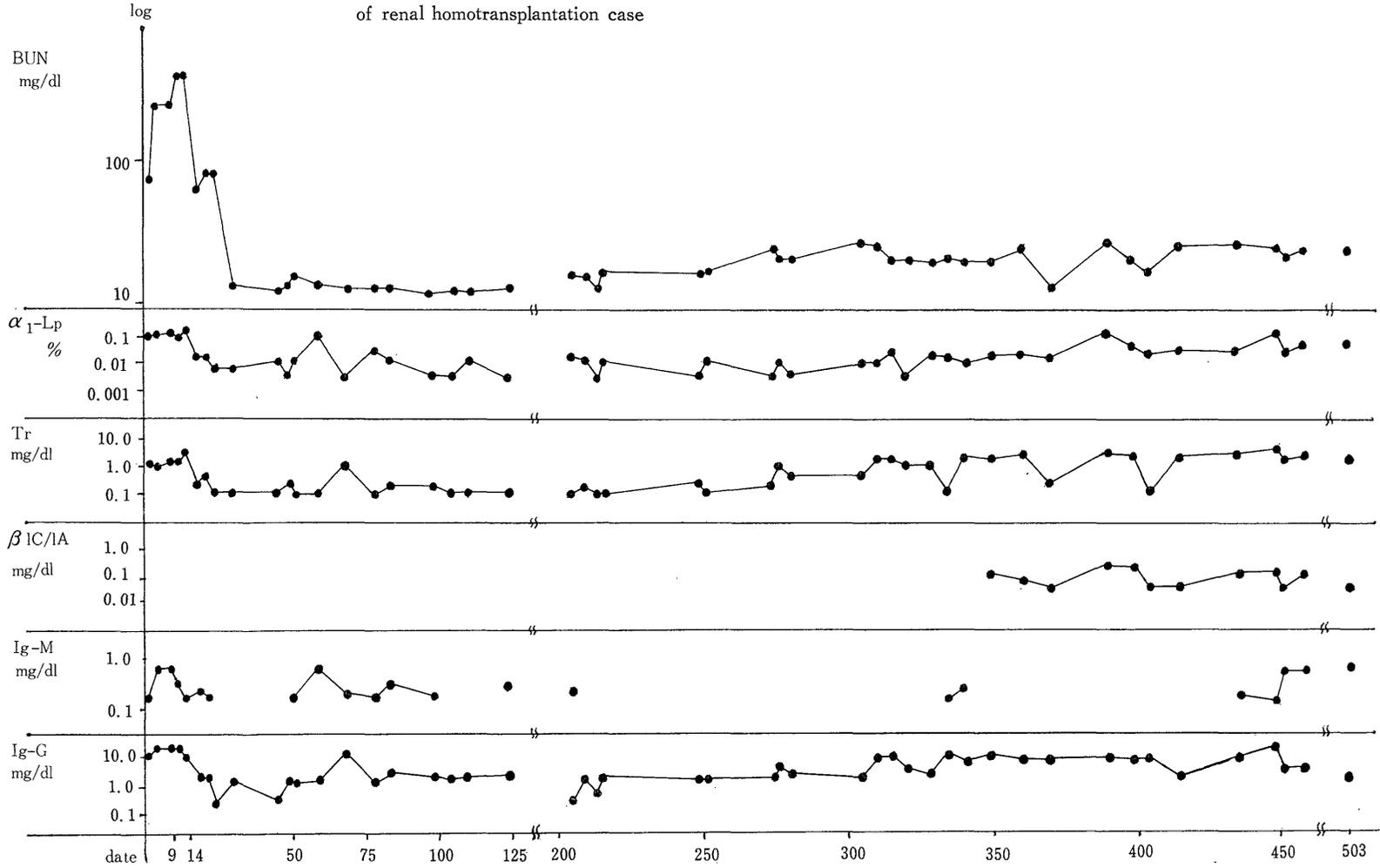


Fig. 3 Relationship between BUN and serum including urinary protein in clinical course of renal homotransplantation case



the same period, the reaction of rejection was clinically unclear.

d. Changes in 480 days after the day 330.

The levels of α_1 -Lp, Tr and Ig-G increased gradually; respectively from 0.01 to 0.18% from 0.1 to 9.3mg/dl and from 11.6 to 30.0mg/dl. Especially, an increase of Ig-G was remarked. Besides, $\beta_{IC/1A}$, which had not been seen, began to appear and continuously increased from 0.04 to 0.3mg/dl. The appearance of $\beta_{IC/1A}$ was coincided with the period in which a chronic rejection was clinically doubted. Further, Ig-M, which was not seen since the day 110 up to the day 330, increased continuously from 0.12 to 0.6mg/dl. However, α_2 -M and α_2 -Lp were not observed.

DISCUSSION

1. Characteristics of serum protein in urine of patients with nephritis, nephrotic syndrome, SLE-nephrosis and renal failure due to chronic glomerulonephritis. In glomerulonephritis, the levels of α_1 -Lp, Tr, $\beta_{IC/1A}$, Ig-M and Ig-G were higher in E-group than in D-group this fact means that the basement membrane of the glomeruli is more degenerated in E-group than in D-group. It coincided with the aggravation degree confirmed in renal biopsy.

Moreover, in renal failure due to chronic glomerulonephritis, the characteristic patterns of urinary proteins were very similar to that of E-group. This fact indicated that the diseases of E-group is just in a pre-stage of renal insufficiency and they are going to evolve to the renal insufficiency.

Comparing between adult subjects and infant subjects in the category of nephrotic syndrome in view of proteins in urine, the proteins of high molecular weight are apt to appear more in children, on the contrary that of relatively low molecular weight were more seen in adults. However, it is premature to conclude from this fact that the renal lesions are more developed in children, and it is necessary to take account of treatment term, starting of treatment, difference of administrated drugs, etc. and to study more in detail before conclusion.

In SLE-nephrosis, α_1 -Lp, Ig-M, and Ig-G were much observed, especially the immunoglobulin was very remarked in comparison with other diseases. This fact permit to guess the existence of various stages of glomerular lesions in SLE-nephrosis.

2. Patterns of fractions of urinary proteins

For patterns of protein fractions in renal proteinuria, Friberg (1950)² and Kerwick (1955)³ have distinguished glomerular proteinuria from tubular proteinuria by zone-electrophoresis. As for glomerular proteinuria, Hardwicke (1959)⁴ et al. introduced a concept of selectivity in order to define the selective permeability of the basement membrane of glomeruli. Cameron (1966)⁵ and Manuel (1965)⁶ classified, according to the selectivity index, the glomerular proteinuria into 3 types as follows:

- 1) Non selective proteinuria
Selectivity index (TransR-IgG) > 0.50
- 2) Selective proteinuria
Selectivity index < 0.10
- 3) Intermediate type proteinuria
Selectivity index 0.10-0.50

a. Proteinuria after renal homotransplantation.

Debray-Sachs (1966)⁷, Hulme (1970)⁸, Laterre (1970)⁹ and Revillard (1965)¹⁰ reported, according to changes of the starch gel electrophoretic patterns, that the glomerulotubular pattern appears immediately after homotransplantation and that this tubular pattern is neither due to the lesions of the ren proprius nor the immune phenomena, but is related to the cellular troubles of the tubule due to ischemia at the moment of the renal transplantation, and, also, the glomerular proteinuria is related to permeability of the glomerular basement membrane. This type of glomerulotubular proteinuria, which appears soon after transplantation, changes itself gradually into the tubular type. This fact suggests that the glomerular basement membrane is ameliorated before the tubule is recovered.

This study was made in view of the fractions of the proteinuria in a case of renal homotransplantation according to Cameron Manuel's criterion. In other word, we investigated the relation between the proteinuria and the clinical changes according to various types of subfractions of urinary proteins appearing immediately after transplantation and to their molecular weight.

The peaks of changes is seen between the days 4 and 14 after transplantation. In this period appear the proteinuria of relatively low molecular weight such as α_1 -Lp, Tr, Ig-G, which suggest the selective proteinuria and also the participation of the tubular proteinuria. Even in case of unclearness of the reaction of rejection in clinical point of view, the appearance or the increase of the proteins of high molecular weight, such as Ig-M, $\beta_{1C}/1_A$, α_2 -M and α_2 -Lp, can be regarded as witness of the existence of the rejection. In case of chronic rejection, the continuous appearance of very slightly over 0.1mg/dl of $\beta_{1C}/1_A$ and the increase of Ig-M can be considered as index. It can be safely said that the type proteinuria in chronic rejection is rather the intermediate type proteinuria than non selective proteinuria as α_2 -Lp and α_2 -M don't appear.

It is not useful to analyse the serum proteins in urine for an early diagnosis of the rejection, as it takes much time, but a good method for a diagnosis of a chronic rejection, of which the clinical signs are not clear, and for a prognosis estimation.

b. Proteinuria in various renal diseases.

The common finding in renal homotransplantation, nephritis, renal failure due to chronic glomerulonephritis, chyluria and lower urinary tract diseases is the fact that Ig-G of molecular weight $1.56 \times 10^5 \sim 1.61 \times 10^5$ appears the most frequently, followed by Tr (mol = 0.9×10^5).

The quantity of the proteins of high molecular weight such as α_2 -Lp, $\beta_{1C}/1_A$ and α_2 -M is very low in comparison with Ig-G and Ig-M. Therefore, it can be through that the urinary proteins, in renal homotransplantation, nephritis and all cases of renal failure,

are influenced by the selectivity of the glomerular basement membrane. However, in SLE-nephrosis and nephrotic syndrome, Ig-M appears more, next to Ig-G, than Tr, and, in nephrotic syndrome, $\beta_{1C}/1A$, the third complement, is seen more than Tr. These facts are very interesting because they endorse that the urinary proteins are not influenced merely by the selectivity of the glomerular basement membrane, and they reveal the diversity of lesions.

On the other hand, it is estimated that in case of nephritis appears the intermediate type proteinuria which transfers itself to non selective proteinuria following the progress of nephritis. In nephrotic syndrome and SLE-nephrosis it is difficult to judge the degree of the lesion only from findings of proteins in urine because of the diversity of its lesions.

The patterns in chronic rejection are clearly different from that in upper and lower urinary tract diseases. So it should be easy to recognize the former from the later even if the patient has a complication of infection after renal homotransplantation.

3. Relation between renal function and urinary proteins.

Dividing 38 cases of renal homotransplantation into 2 groups : good renal function group and unfavorable renal function group, Debray Sachs tried to define the relation between protein quantity in urine and renal function, but he reported the absence of the significant relation between the two.

We also investigated the relation between BUN and subfraction of serum proteins in urine in 28 subjects with renal failure due to chronic glomerulonephritis, yet we could not find any significant relation.

However, we analysed the relation between BUN and proteins with 48 specimens throughout the clinical observation of the case of renal homotransplantation.

As shown in Fig. 3 :

α_1 -Lp : $r=0.217$, $p>0.05$

Tr : $r=0.428$, $p<0.05$

Ig-G : $r=0.554$, $p<0.001$

From these facts, while the significant relation of α_1 -Lp,-BUN was not established, Tr and Ig-G showed a significant relation with BUN. Regarding Ig-M inferior to 0.108 mg/dl as zero (0), the relation of BUN-urinary proteins was studied, then it was confirmed when $r=0.456$ and $p<0.05$.

As the urinary proteins should be originated from the transplant crisis and glomerular damage after homotransplantation of kidney, the relation between renal function and serum proteins in urine must be significant; in this subject Tr, Ig-G and Ig-M showed a significant relation. However, no relation with α_1 -Lp; it can be thought, as reason, that α_1 -Lp could not pass the glomerular basement membrane even when its permeability was stimulated by the reaction of rejection, as α_1 -Lp's molecular weight higher than Ig-G and Tr.

CONCLUSION

We determined the subfraction of serum proteins in urine by means of antibody absor-

ption in 43 cases of nephritis, 28 cases of renal failure due to chronic glomerulonephritis, 10 cases of SLE-nephrosis, 24 cases of nephrotic syndrome, 10 cases of chyluria, 14 cases of upper urinary tract diseases, 22 cases of lower urinary tract diseases and 1 case of renal homotransplantation. Subjected protein subfractions were α_1 -Lp, α_2 -Lp, α_2 -M, $\beta_{1C/1A}$, Tr, Ig-M and Ig-G.

1. In glomerulonephritis appeared Ig-G, Tr and α_1 -Lp, especially Ig-M and $\beta_{1C/1A}$ were seen more in E-group than D-Group.
2. Nephrotic syndrome: Each of subfractions appeared. Ig-M was seen more in infant subjects, and, Tr as well as Ig-G, in adult subjects.
3. In SLE-nephrosis more appeared α_1 -Lp, Ig-M and Ig-G, above all the appearance of immunoglobulin was remarked.
4. In chyluria the serum proteins directly came out in urine. The levels of α_2 -Lp and Ig-M were remarkably high. In renal failure due to chronic glomerulonephritis, the appearance of α_2 -Lp, as well as high levels of α_2 -M and $\beta_{1C/1A}$ was characteristic.
5. In upper and lower urinary tract diseases, the level of fractions low, especially low level of Ig-M and absence of α_2 -Lp and $\beta_{1C/1A}$ were notable.
6. Clinical observation and variation of urinary proteins in renal homotransplantation.
 - 1) α_1 -Lp, Tr and Ig-G increased remarkably after transplantation, but they became normal in 3 weeks. No appearance of proteins of high molecular weight such as $\beta_{1C/1A}$, α_2 -M, α_2 -Lp, etc.
 - 2) α_1 -Lp, Tr, Ig-M and Ig-G increased on the day 60 after transplantation, when the rejection seemed to have occurred, however, α_2 -Lp, α_2 -M and $\beta_{1C/1A}$ were not seen.
 - 3) Since the day 330, when the chronic rejection began to be suspected, $\beta_{1C/1A}$, which had not been until then, continuously appeared and Ig-M increased.
 - 4) Concerning the relation between urinary proteins and BUN, no significant relation was confirmed with α_1 -Lp, but with Tr and Ig-G.

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