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Human α_1-microglobulin levels in various body fluids

KIMITERU TAKAGI, KOHJIN KIN, YOSHIHISA ITOH, HIROMITSU ENOMOTO, AND TADASHI KAWAI

From the Department of Clinical Pathology, Jichi Medical School, Tochigi-Ken, Japan, 329-04

SUMMARY α_1-Microglobulin levels in serum and urine were estimated by using single radial immunodiffusion, resulting in the following mean values: umbilical cord blood serum, 40·6 mg/l; normal adult serum, 44·2 mg/l; and normal urine, 5·7 mg/24 h urine volume. Slightly higher levels of serum α_1-microglobulin were found in infants and the aged. Serum and urine α_1-microglobulin levels in patients with renal failure, however, were greatly increased, mean levels being 231·5 mg/l and 100·7 mg/24 h urine volume, respectively. Serum α_1-microglobulin levels in these patients correlated well with both serum creatinine and β_2-microglobulin levels. Serum α_1-microglobulin level did not correlate positively with serum levels of other plasma proteins, such as α_1-antitrypsin, haptoglobin, complement, etc. Ouchterlony immunodiffusion also revealed the presence of α_1-microglobulin in synovial fluid, ascites, pleural effusion, amniotic fluid, cyst fluid, and cerebrospinal fluid. The levels of α_1-microglobulin in these fluids were measured by single radial immunodiffusion, except that its level in cerebrospinal fluid was measured by radioimmunoassay. Mean α_1-microglobulin concentration was 20·8 mg/l in synovial fluid, 28·7 mg/l in ascites, 21·5 mg/l in pleural effusion, 2·7 mg/l in amniotic fluid, 8·2 mg/l in cyst fluid, and 42·3 ng/ml in cerebrospinal fluid.

α_1-Microglobulin (α_1-m), a low molecular weight glycoprotein, was initially isolated by Ekström *et al.*,[1] and its molecular weight was determined to be 33 000 daltons.[2] α_1-m was detected in human serum, urine, and cerebrospinal fluid; and serum and urine α_1-m levels were elevated in patients with renal tubular disorders.[1 3 4] The biological function of α_1-m, however, remains unknown, as does its site(s) of production, though it may be that blood lymphocytes are concerned.[2 5]

This paper describes the quantitative levels of α_1-m in serum and urine of normal individuals, its levels in patients with hepatic disorders and chronic renal failure, and comparative results when serum levels were correlated with other serum proteins. Further, the relationship between serum α_1-m levels and those of serum creatinine and serum β_2-microglobulin in patients with chronic renal failure were also studied as well as the distribution of α_1-m in various body fluids.

Material and methods

PREPARATION OF ANTI-α_1-M SERUM

Human α_1-m was purified from the urine of patients with renal tubular proteinuria.[2] Anti-α_1-m serum was obtained by injecting goats with purified α_1-m. Each goat was immunised in about 30 intracutaneous sites with 2·0 mg of α_1-m dissolved in 1 ml of saline and thoroughly mixed with an equal volume of complete Freund's adjuvant. A booster injection of 2·0 mg antigen without adjuvant was repeated weekly during eight consecutive weeks. The gammaglobulin fractions were separated by affinity chromatography with Sepharose 4B (Pharmacia, Inc, Uppsala, Sweden) as immunosorbent, purified α_1-m bonded to it, using a 0·1 M Tris-HCl buffer, pH 8·0, containing 0·15 M NaCl as coupling buffer. Elution was done with 0·2 M citric acid-0·4 M sodium phosphate buffer, pH 7·6. Finally, a Sephadex G-200 (Pharmacia, Inc) column chromatography was run to collect the specific anti-α_1-m goat IgG, and its monospecificity was confirmed, as shown in Figures 1 and 2.

SAMPLING OF SERA AND URINE

Normal sera were obtained from 309 healthy persons of various ages, 150 males and 159 females, and included 27 from umbilical cords. Urine was collected from seven healthy adults over a 24-hour period. Sera were also drawn from 62 adult patients with hepatic disease, including 21 patients with cirrhosis,

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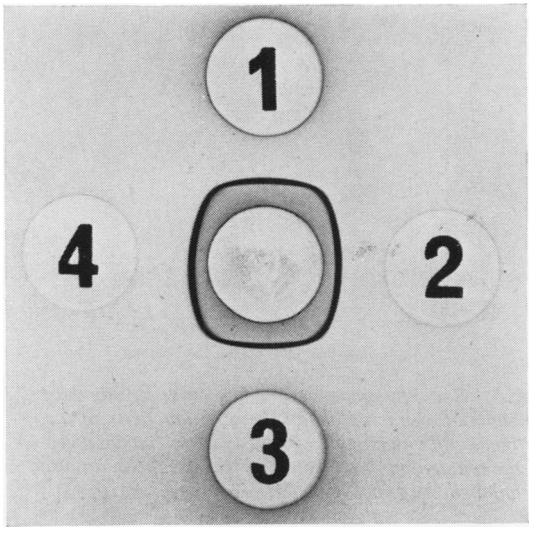

Fig. 1 *Ouchterlony immunodiffusion of purified α_1-microglobulin (1 and 3), normal serum (2), and concentrated normal urine (4). The centre well contained anti-α_1-microglobulin goat IgG fraction.*

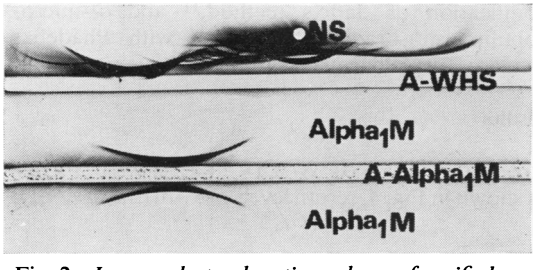

Fig. 2 *Immunoelectrophoretic analyses of purified urinary α_1-microglobulin (Alpha$_1$M). α_1-microglobulin against anti-α_1-microglobulin goat IgG (A-Alpha$_1$M) migrated, showing almost symmetric arc, at the fast α_1-region. NS and A-WHS indicate normal human serum and anti-whole human serum (purchased from Dakopatts, Copenhagen, Denmark) respectively.*

nine with acute hepatitis, 25 with chronic hepatitis, and seven with hepatoma. Sera were collected from adult patients for measurement of various serum proteins: 150 sera were tested for α_1-antitrypsin, 127 for haptoglobin, 149 for β_1C/β_1A-globulin, 148 for β_1E-globulin, 149 for C_3-proactivator, 77 for α_2-pregnancy-associated glycoprotein, 148 for CH_{50}, and 93 for C-reactive protein. In 103 cases the erythrocyte sedimentation rate was determined. Sera and urine, moreover, were obtained from 12 patients with chronic renal failure to study the relationship between their levels of α_1-m, creatinine, and β_2-microglobulin.

SAMPLING OF VARIOUS BODY FLUIDS

Cerebrospinal fluid was collected from 21 patients without abnormality in the fluid. Gastric juice was collected from 16 cases of chronic gastritis, pancreatic juice from 12 cases of chronic pancreatitis, saliva from 16 cases of Sjögren's syndrome, tear fluid from two healthy adults, nasal fluid from three cases of common cold, seminal fluid from one normal adult, synovial fluid from 42 cases of rheumatoid arthritis, cyst fluid from three cases of hepatic cyst, pus from one case of renal abscess, ascites from 15 cases of liver cirrhosis, pleural effusion from 11 cases of lung cancer, and amniotic fluid from two cases of third-trimester gestation.

QUANTITATION OF α_1-M IN VARIOUS MATERIALS

The occurrence of α_1-m in serum, urine, and other body fluids was established by Ouchterlony immunodiffusion analysis. α_1-m in various body fluids except for cerebrospinal fluid was measured by the single radial immunodiffusion (SRID) technique. Sera were used unconcentrated, but normal urine and amniotic fluids were concentrated 50 and 25 times, respectively, using a Minicon Concentrator (B15, Amicon Corp, Lexington, Mass, USA). For cerebrospinal fluid, where levels could not be determined exactly by SRID technique, α_1-m was measured by radioimmunoassay.

RADIOIODINATION AND RADIOIMMUNOASSAY OF α_1-M[6 7]

For coupling of anti-α_1-m goat IgG (3·2 mg/ml of protein concentration) was added to 200 mg of cyanogen bromide-Sepharose 4B (Pharmacia, Inc) equilibrated with 0·1 M sodium bicarbonate containing 0·5 M NaCl, and stirred for 18 hours. Thereafter, 1 M ethanolamine was added and incubated for two hours, and the reaction mixtures were washed twice alternately with 0·1 M acetate buffer, pH 4·0, containing 0·5 M NaCl and 0·1 M sodium bicarbonate containing 0·5 M NaCl. Finally, the used suspension was adjusted to 1·0 l by 1% bovine serum albumin (Sigma Chemicals Co, Saint Louis) in phosphate buffered saline (PBS).

Enzymatic iodination was used for labelling α_1-m.[8] 10 μl of α_1-m (1 mg/ml in distilled water) was mixed with 10 μl (1 mCi) of carrier-free ^{125}I-Na (New England Nuclear, Boston, Mass). To this was added 5 μl of lactoperoxidase solution (Boehringer Mannheim GmbH, West Germany; 0·1 mg/ml in 0·5 M phosphate buffer, pH 5·0) and 5 μl of hydrogen peroxide solution, which was diluted 20 000 times with the same buffer. After exactly 60 seconds, the reaction was terminated by adding 50 μl of the stopper solution (consisting of 1 g NaN_3, 8 g saccharose, 1 g KI, and 100 ml distilled water). The

iodinated α_1-m was separated from excess reagents by chromatography on a 1·0 × 15 cm column of Sephadex G-25 (Pharmacia, Inc) in 0·05 M PBS, pH 7·5, containing 0·1% bovine serum albumin.

The specific radioactivity of the protein peak was 51·6 μCi/μg, and the labelled α_1-m was diluted so as to give about 20 000 cpm per 200 μl.

Dilution of all reagents was in 0·01 M PBS, pH 7·5, containing 1% bovine serum albumin (RI-buffer) for radioimmunoassay of α_1-m. The immunoassay solution consists of 100 μl of samples or varying amounts of α_1-m standard, 200 μl of antibody-coupled Sepharose 4B, and 200 μl of labelled α_1-m. After mixing, the tubes were incubated for 18 hours at room temperature, 22°C. The precipitates were separated by centrifugation at 3000 rpm for 5 minutes, and then, after washing three times with saline, radioactivity was measured in a gamma counter (JDC-752, Aloka Auto Well Gamma System). The specificity of the assay was satisfactory, as shown in Table 1. Recovery of α_1-m added to cerebrospinal fluid was 104·6 ± 1·4% (mean ± SD). The radioimmunoassay binding curve is shown in Fig. 3, and sample levels were determined using this standard curve. The assay was sensitive enough to measure accurately 9 ng/ml of α_1-m (50% inhibition of binding).

Table 1 *Immunoreactivity in serial dilutions of cerebrospinal fluid**

Case	*Dilution*	*B/B₀ (%)*	*α₁-Microglobulin (ng/ml)*	
			Calculated	*Measured*
1	None	32	80	90
	1:2	44	40	40
	1:4	54	20	23
	1:8	65	10	12
	1:16	78	5	5
2	None	41	48	48
	1:2	53	24	24
	1:4	63	12	14
	1:8	76	6	6
3	None	43	43	43
	1:2	52	22	25
	1:4	69	11	9·6
	1:8	74	6	7

*Cerebrospinal fluid in three cases was serially diluted with RI-buffer, and α_1-microglobulin immunoreactivity was determined in each dilution: B = radioactivity of the antigen-antibody complex formed; B_0 = radioactivity of the antigen-antibody complex without the addition of unlabelled α_1-microglobulin.

QUANTITATION OF OTHER SERUM PROTEINS

Haptoglobin, α_1-antitrypsin, β_1C/β_1A-globulin, β_1E-globulin, and C_3-proactivator were measured using the SRID technique with antisera from Behringwerke A/G, West Germany. Laurell's rocket immunoelectrophoresis method[9] was used for estimating α_2-pregnancy-associated glycoprotein, and its level

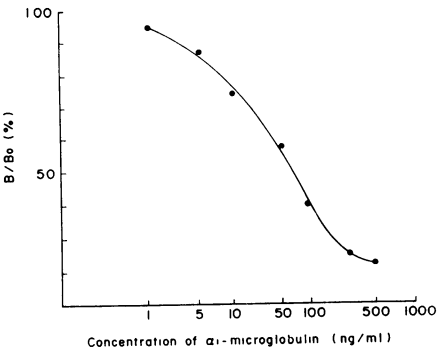

Fig. 3 *Radioimmunoassay binding curve by standard amounts of α_1-microglobulin. B = radioactivity of the antigen-antibody complex formed; B_0 = radioactivity of the antigen-antibody complex without the addition of unlabelled α_1-microglobulin.*

was expressed as a percentage of the mean value in the 30th week of gestation. CH_{50} was estimated using the modified version of Mayer's 50% technique,[10] and C-reactive protein using the capillary tube method. Serum creatinine levels were measured by a modification of Jaffe's method,[11] and β_2-microglobulin using radioimmunoassay with Phadebas β_2-micro Test (Pharmacia, Inc).

Results

NORMAL LEVELS OF α_1-M IN SERUM AND URINE

As shown in Fig. 4, serum levels of α_1-m (mean ± SD)

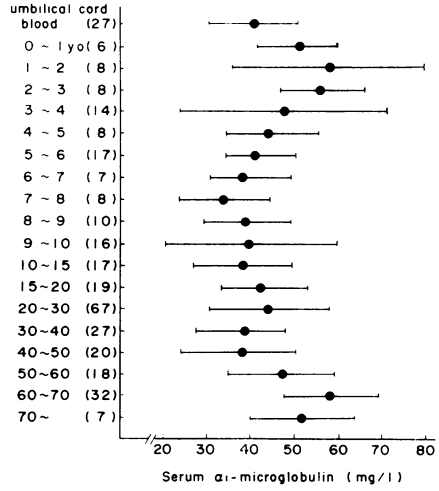

Fig. 4 *Levels of human α_1-microglobulin in umbilical cord blood and normal sera. Numbers in parentheses indicate numbers of cases tested.*

were 40·6 ± 11·3 mg/l in umbilical cord blood, and its highest level was seen during infancy, reaching 58·1 ± 23·5 mg/l. In young adults, its level ranged from 25·0 to 83·0 mg/l with a mean value of 44·2, and it increased slightly from the fifth decade on, reaching 58·6 ± 11·3 mg/l at 60-70 years of age.

There was a significant difference in its levels between the sexes, the levels in males being higher than those in females, as shown in Figure 5.

The amount of α1-m excreted in urine over a 24-hour period by seven healthy adults ranged from 3·0 to 8·8 mg, with a mean value of 5·7 mg (Table 2).

α1-M LEVELS ASSOCIATED WITH HEPATIC DISORDERS AND CHRONIC RENAL FAILURE

Serum α1-m levels were in the normal range in cirrhosis, 48·9 ± 15·0 mg/l; acute hepatitis, 46·8 ± 15·4 mg/l; chronic hepatitis, 47·2 ± 13·0 mg/l; and hepatoma, 57·6 ± 20·0 mg/l. There was no renal dysfunction in these patients.

Serum and urine α1-m levels in 12 patients with chronic renal failure were high, 231·5 ± 82·9 mg/l and 100·7 ± 48·3 mg in 24-hour volumes, respectively (Table 2).

A significant correlation was found between serum α1-m and serum creatinine levels, as can be seen in Figure 6. Furthermore, serum α1-m levels correlated significantly with serum β2-microglobulin levels, as illustrated in Figure 7. A clearly positive correlation, r = 0·977, between urine α1-m and β2-microglobulin was also observed.

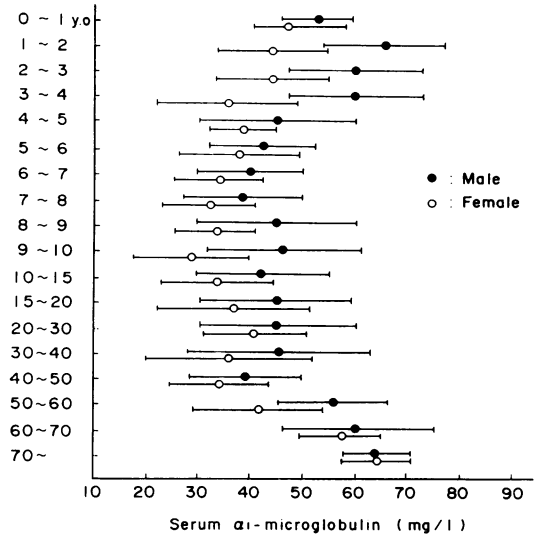

Fig. 5 *Levels of human α1-microglobulin in normal sera.*

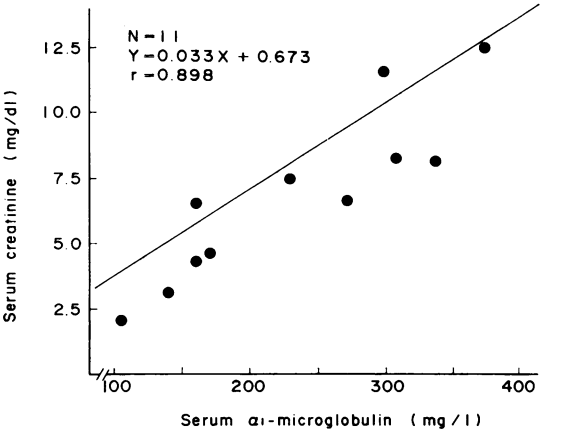

Fig. 6 *Correlation between serum α1-microglobulin and creatinine in 11 patients with chronic renal failure. A significant positive correlation was found between them.*

Table 2 *α1-Microglobulin levels in various body fluids*

Fluid	*No. of cases*	*Content**		
		Mean	*SD*	*Range*
Serum of umbilical cord blood (mg/l)	27	40·6	11·3	21-56
Serum from healthy individuals (mg/l)	67	44·2†	14·6	25-83
Serum from patients with renal failure (mg/l)	12	231·5	82·9	104-374
Urine from healthy individuals (mg/24-h volume)	7	5·7‡	1·6	3·0-8·8
Urine from patients with renal failure (mg/24-h volume)	12	100·7	48·5	42-166
Synovial fluid from patients with rheumatoid arthritis (mg/l)	42	20·8	8·5	9-35
Cyst fluid from patients with hepatic cyst (mg/l)	3	8·2	—	12, 5·1, 7·6
Ascites from patients with liver-cirrhosis (mg/l)	15	28·7	9·3	21-32
Pleural effusion from patients with lung cancer (mg/l)	11	21·5	7·9	21-26
Amniotic fluid at third trimester of pregnancy (mg/l)	2	2·7	—	2·5, 3·0
Cerebrospinal fluid showed normal laboratory findings (ng/ml)	21	42·3§	16·8	13-84

*Determined by a single radial immunodiffusion technique. †Sera obtained from 20 to 40 years of age.
‡Mean content per litre of urine 4·2 mg. §Determined by a radioimmunoassay method.

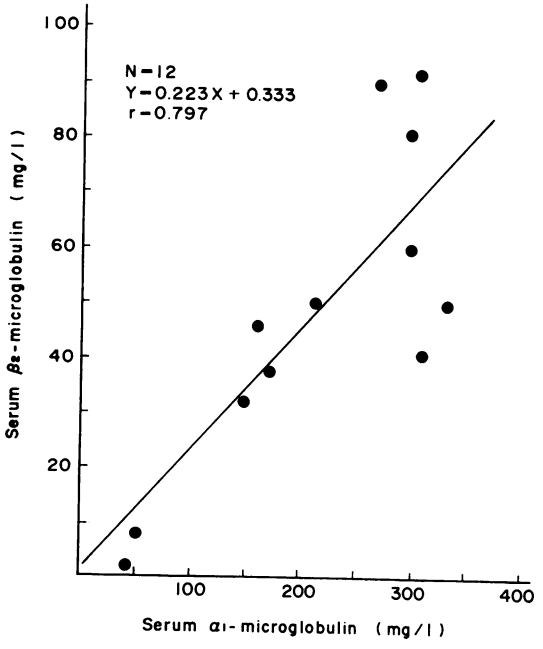

Fig. 7 *Correlation between serum α_1-microglobulin and β_2-microglobulin in the patients shown in Fig. 6. There was also a positive correlation between both proteins.*

DETERMINATION OF α_1-M IN VARIOUS BODY FLUIDS

α_1-m was found in synovial fluid, cyst fluid, ascites, pleural effusion, amniotic fluid, and cerebrospinal fluid, as noted in Table 2. The mean α_1-m values were 20·8 mg/l in synovial fluid, 8·2 mg/l in cyst fluid, 28·7 mg/l in ascites, 21·5 mg/l in pleural effusion, 2·7 mg/l in amniotic fluid, and 42·3 ng/ml in cerebrospinal fluid.

RELATIONS BETWEEN SERUM α_1-M AND OTHER SERUM PROTEINS

There was no correlation between the levels of α_1-m and various serum proteins measured in this study. Furthermore, there was no relation between the levels of α_1-m and erythrocyte sedimentation rate.

Discussion

The serum α_1-m levels in normal individuals demonstrated that they were higher in infants and the aged than in normal adults, as shown in Figure 4. It is well known that the normal blood lymphocyte count in infants is higher than in adults. Blood lymphocytes play at least a partial role in the production of serum α_1-m,[2 5] as is the case with lymphocyte production of β_2-microglobulin,[12] which may account for the higher level of serum α_1-m found in infancy.

Plasma proteins with a molecular weight of less than 50 000 seem to pass easily through the glomerular basement membrane and then be reabsorbed and catabolised by the renal tubules.[13] Only a small amount of the plasma protein such as β_2-microglobulin,[14 15] retinol-binding protein,[16] immunoglobulin light chains,[17] and lysozyme[18] are normally excreted in the urine. α_1-m, its molecular weight being 33 000,[2] is also eliminated from the blood, probably mainly in the kidney, by glomerular filtration followed by tubular reabsorption and catabolism.[13] It is suggested that a higher level of serum α_1-m in the aged may be due to latent renal dysfunction such as low glomerular filtration.

In the present study, an apparent difference of serum α_1-m levels was noted between the sexes, though no explanation is offered.

It was of interest to find that the serum α_1-m level was within the normal range in various hepatic diseases in this study, suggesting, as has already been reported by Svensson and Ravnskov,[3] and according to our previous findings,[2] that the liver is not the major site for its synthesis.

The acute-phase reactants, most of them produced by the liver, tend to increase in various inflammatory diseases. α_1-m, however, cannot be considered as one of the acute-phase reactants, since no correlation was obtained between serum levels of α_1-m and other plasma proteins tested in these experiments.

Serum α_1-m levels in 12 patients with varying degrees of chronic renal failure ranged from 104 to 374 mg/l, and its urinary excretion ranged from 42 to 166 mg/24-hour volume (Table 2). Thus, both the serum and urine from patients with renal failure contained much higher levels of α_1-m than those of healthy individuals. In addition, the serum α_1-m levels in patients with chronic renal failure correlated well with serum creatinine levels, as shown in Figure 6. Svensson and Ravnskov[3] have also reported that serum α_1-m levels correlated roughly with levels of serum creatinine. Further, serum and urine α_1-m levels in this study also correlated well with serum and urine β_2-microglobulin levels. A renal elimination of α_1-m is supported by the increased levels of the protein in the blood of patients with renal failure and in the urine of patients with tubular disorders. A close positive correlation between serum levels of creatinine and β_2-microglobulin has been reported,[15 19] and Bernier *et al.*[20] have reported a similar correlation in patients with chronic renal failure. It seems conceivable that α_1-m may be handled by the glomerular membrane in a way similar to that of creatinine and β_2-microglobulin. If so, serum and urine α_1-m levels could be used as an

indicator in estimating the extent of renal disorders.

Distribution of α_1-m in various body fluids was studied; its levels were measured in synovial fluid, cyst fluid, ascites, pleural effusion, amniotic fluid, and cerebrospinal fluid (Table 2). Clinical correlation with α_1-m levels in various body fluids is now under investigation.

Other similar or identical glycoproteins have been reported.[21 22] Tejler and Grubb[21] have isolated a similar protein, which they termed protein HC (human complex-forming glycoprotein, heterogeneous in charge) from the urine. These authors found that protein HC levels were markedly elevated in the plasma and urine of patients with advanced renal failure. They also reported that human fetal liver explants were found to secrete protein HC into the medium.[23]

Seon and Pressman[22] isolated a glycoprotein from the urine and designated it α_1-microglycoprotein. This protein was found in significant concentrations in the urine of many patients with neoplastic diseases, though they have not yet reported the details. These three glycoproteins were very similar or identical in their physicochemical properties, but further work is needed to elucidate the exact relation between them.

The biological function of α_1-m has not yet been clarified, leaving the need for further research.

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Requests for reprints to: K Takagi, Department of Clinical Pathology, Jichi Medical School, Tochigi-Ken, Japan 329-04.