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Alpha-1-microglobulin: an indicator protein for renal tubular function

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SUMMARY A comparison of urinary α_1-microglobulin concentrations to the behaviour of other indicators of renal tubular disorders, β_2-microglobulin, retinol-binding protein and N-acetyl-β-D-glucosaminidase (NAG) has been made. In acute tubular disorders the concentrations of urinary β_2M and RBP are highly correlated (r = 0·89) but this is less marked for α_1M and β_2M (r = 0·55) and α_1M and RBP r = 0·48. NAG tends to run a parallel course to α_1M concentrations but lags behind the recovery of low molecular weight protein reabsorption following injury of the tubular cells.

The concentrations of α_1M, and in particular its stability at low pH suggest that this protein may be useful in screening for tubular abnormalities and detecting chronic asymptomatic renal tubular dysfunction.

Urinary α_1M >15 mg/g creatinine is strongly suspicious of a proximal tubular dysfunction. The distinction between pure tubular proteinuria and mixed glomerular and tubular proteinuria requires further analysis.

The studies of low molecular weight proteins isolated from the urine of patients with renal tubular damage by Berggård and his colleagues have resulted in a purification of β_2-microglobulin (β_2M),[1] free light chains,[2] retinol-binding protein (RBP)[3] and α_1-microglobulin (α_1M).[4] These low molecular weight proteins (<33 000 daltons) share the property of being readily filtered by the glomerulus and reabsorbed and catabolised by the proximal tubular cells.[5] The measurement of urinary β_2M has been widely advocated as an indicator of tubular proteinuria, mainly as the result of the performance of this analyte as a sensitive marker of tubular damage in cadmium[6] and mercury poisoning[7] and more recently in following the nephrotoxic action of drugs.[8,9] However, β_2M loses its antigenicity at pH <6·0 and in routine clinical practice can lead to an underestimate of the intensity of low molecular weight proteinuria. Due to its stability in urine, RBP has been suggested to be used as a marker of tubular dysfunction.[10] There is considerable literature on the increased urinary excretion of enzymes of tubular origin, especially N-acetyl-β-D-glucosaminidase (NAG)[12,13] and alanine aminopeptidase (AAP)[14] as indicators of tubular disorders especially after exposure to aminoglycosides. However, it is still uncertain which of these indicators of tubular function is most useful for routine use or whether their selection depends on the clinical condition being investigated.

It is evident that urinary α_1M concentrations can be high in cadmium poisoning[1] and in renal failure[15–17] but so far there appears to be little information on how it relates to the spectrum of urinary analytes whose concentration can be increased in tubular disorders. α_1M is a glycosylated protein of molecular weight estimated to be between 26 000 and 33 000 daltons according to the type of measurement[1,18] containing 167 amino acids.[19] The liver is probably the main site of synthesis.[20] Apart from severe liver disease[20] when α_1M can be low and in renal failure where the levels are raised[15–17] the blood concentrations of α_1M undergo little change in many forms of inflammatory and neoplastic diseases.[11,21] In this paper we describe its blood concentrations and urinary excretion in diseases associated with acute or chronic tubular dysfunction and how its concentrations compare with other indicators of tubular activity.

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Patients and methods

Serum samples were obtained from volunteer blood donors giving one or two donations per year and representative populations of patients with diseases that may influence the excretion of α_1M in the urine. The sera were stored at −20°C.

Random urine collections were used for the majority of the studies. The concentration of the various analytes was adjusted for the urinary creatinine concentration. α_1M, RBP and β_2M concentrations were measured by single radial immunodiffusion (RID)[22] using antisera and standards provided by Behringwerke, Marburg/Lahn, Germany (α_1M and RBP) or purchased from Dako Immunoglobulins-a/s, Copenhagen, Denmark (β_2M). A urinary light chain assay was performed at the Department of Immunology, University of Birmingham and expressed in units/l (1 unit = approximately 1 g). No definitive standard is yet available; the working standard is a pool of monoclonal kappa and lambda light chains.

N-acetyl-β-D-glucosaminidase was measured by a fluorimetric method as described by Leback and Walker[23] and Price *et al*.[24] Two hundred units (1 unit = 1 nmol/h/mg creatinine) was taken as the upper limit of normal. Urinary creatinine was measured by Jaffé's method, adapted for use on a Pye Unicam Auto Chemistry Unit; serum creatinine concentrations taken from routine hospital analyses.

SDS polyacrylamide disc gel electrophoresis (SDS-PAGE) was carried out using 7% gel. Dialysed urine (20 μl) with a protein concentration adjusted to 10 mg/ml was applied to the gel with bromophenol blue marker and run for about one and a half hours at 5 mA per gel. The gels were stained with Coomassie blue.[25]

The experimental design involved (*a*) examination of random urines from healthy medical students and laboratory staff, aged 19–55 yr, referred to as normal controls; (*b*) the screening of the urinary α_1M excretion in patients in whom there was considered to be an increased probability of tubular proteinuria; these included bladder cancer, paraplegia and patients with an ileal conduit following total cystectomy; (*c*) the use of this screen in subjects who might have tubular proteinuria, workers in the chemical industry being screened for bladder cancer and patients with rheumatic diseases and (*d*) detailed studies of the evolution of acute tubular damage in burns and acute pancreatitis, and the chronic tubular damage in multiple myeloma. The latter were patients in the Medical Research Council IVth Myeloma Trial.

Only the abnormal urines as defined by an α_1M > 15 mg/g creatinine were investigated in detail in the screening survey (*b* and *c*). In (*d*) all urines were examined in detail whatever their α_1M concentration.

A total of 2000 measurements of urinary α_1M were made during this investigation.

STABILITY OF α_1M

Seven samples of urine with α_1M contents 3 mg–90 mg/l were divided into aliquots and the pH adjusted to 1–10 in steps of one pH unit. Each sample was split into two, one half stored at 4°C overnight, and the other half at 4°C for 11 days. There was hardly any change in the urine α_1M concentrations of the samples stored between pH 4·0 and 10·0, below pH 4·0 there was a loss of activity of approximately 50% as the pH fell to 2·0. This indicates the α_1M is stable in urine in the pathophysiological range of urine pH.

SERUM α_1M CONCENTRATIONS

The relation of serum α_1M concentrations to serum creatinine in a hospital population (excluding patients with myelomatosis) is illustrated in Fig. 1.

The serum values of α_1M in controls, pregnancy and various diseases, but excluding patients with a serum creatinine >200 μmol/l are shown in Table 1. The concentrations in burns and acute pancreatitis indicate that α_1M is not an acute phase reactant protein, as both these conditions are well known to provide a very powerful stimulus for the synthesis of acute phase proteins.

In multiple myeloma, in the absence of renal failure, the distribution of serum α_1M concentrations in patients with IgG myelomatosis was unimodal,

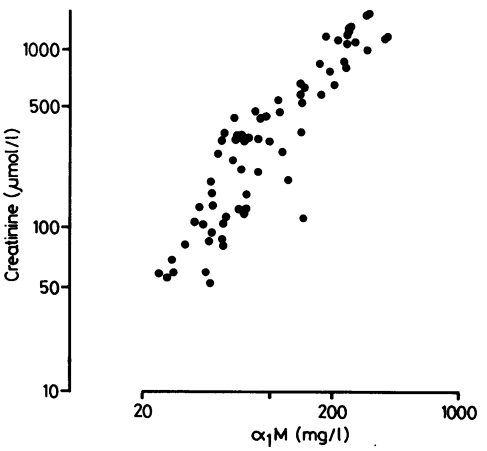

Fig. 1 *Relation between serum α_1-M creatinine concentrations in a hospital population (log scales)*

Table 1 *Serum α_1-microglobulin concentrations in controls and patients with serum creatinine <200 μmol/l*

Condition	*No of subjects*	*Mean ± SD (mg/l)*
Blood donors		
Male	20	32·0 ± 6·8
Female	20	27·9 ± 10·2
Pregnancy	40	27·3 ± 5·7
Liver disease	65	33·6 ± 10·2
Severe burns		
Day 1–2	16	37·6 ± 16·5
Day 10–12	16	54·3 ± 14·5*
Paraplegia	16	26·6 ± 11·2
Bladder cancer	13	45·4 ± 10·9*
Acute pancreatitis	16	33·1 ± 13·3

*The raised serum α_1M is associated with a reduced glomerular filtration rate.

whilst in patients with IgA myelomatosis the distribution was generally raised and showed a marked skewness with a few values >200 mg/l. These distributions are demonstrated in Table 2.

A similar skewness in the distribution was observed in rheumatoid arthritis but was not present in the other diseases listed in Table 1. Crossed immunoelectrophoresis, with anti-IgA in an intermediate gel showed the high concentrations α_1M in the serum of patients with IgA myelomatosis were associated with the serum α_1M being in two forms: a free form with an α_1M electrophoretic mobility and a form bound to IgA.

URINE ANALYSIS

The normal range of urinary α_1M excretion was 4·2 ± 5·6 mg/l (mean ± 2 SD) (range 0·5–17·2 mg/l) or 4·2 ± 6·0 mg/g (mean ± 2 SD) creatinine (range 0·2–15·0 mg/g creatinine) based on 102 normal subjects. Arbitrarily, we take 15 mg/g creatinine as a normal cut-off level.

When urinary α_1M was used to screen a population to assess the possibility of occult nephrotoxicity in 500 workers in the chemical industry, four cases of tubular proteinuria were detected and confirmed by their SDS-PAGE patterns (see Table 3).

The value of α_1M in detecting unsuspected tubular proteinuria in lower urinary tract disease is illustrated by the studies of bladder cancer and paraplegic patients. In general, considering all the diseases in the study, when the urinary α_1M is >15 mg/g creatinine and the ratio of α_1M : total urinary protein is >1:30, SDS-PAGE demonstrates the presence of tubular proteinuria or a mixed glomerular and tubular pattern.

The distribution of urinary NAG activities in patients with urinary α_1M >15 mg/g creatinine is illustrated in Fig. 2. In 128 patients, 79 were found to have a NAG >200 units. In contrast, only 37 out of 280 patients with α_1M <15 mg/g creatinine were found to have abnormal NAG activities. As urinary β_2M is degraded in acid urine (pH <5·5), the comparison between urinary β_2M and α_1M concentrations using a RID screen could only be made in about one third to half of the samples with the exception of the paraplegics nearly all of whom have alkaline urine. Using an arbitrary cut-off for β_2M of 2 mg/l as determined by the sensitivity of the RID, then the relation between a raised α_1M and β_2M in the chronic disorders is shown in Table 4. We have excluded patients with myelomatosis from the

Table 2 *Distribution of serum α_1M in patients with untreated myelomatosis (serum creatinine <200 μmol/l)*

	α_1M (mg/l)						
	<20	*21–40*	*41–60*	*61–80*	*81–100*	*>100*	*Total*
No of patients with IgA myeloma	1	7	12	14	8	42	83
No of patients with IgG myeloma	5	45	12	—	1	—	63

Table 3 *Percentage distribution of urinary α_1M concentrations*

		Percentage of distribution				
	Total	*<15 mg/g cr*	*15–30 mg/g cr*	*31–45 mg/g cr*	*46–60 mg/g cr*	*>60 mg/g cr*
Normal controls	102	100				
Chemical workers	500	97	3			
Paraplegia	105	72	12	3	4	10
Rheumatic diseases	84	64	16	8	3	10
Myelomatosis	99	40	23	11	12	13
Superficial bladder cancer	139	74	18	4	0	4
Advanced bladder cancer (T_2–T_4)	64	66	21	4	2	8
Ileal conduit	44	68	10	5	5	12

cr = creatinine

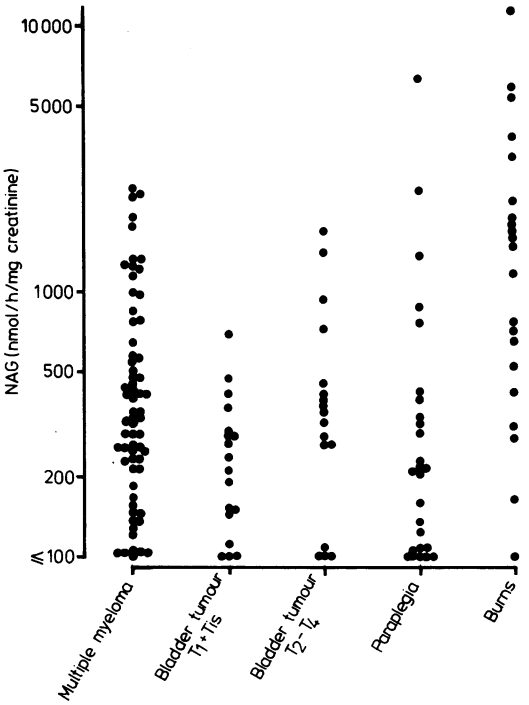

Fig. 2 *Distribution of urinary NAG for patients with urinary α_1M concentrations > 15 mg/l. T_1–T_4 = tumour stages; Tis = tumour in situ.*

analysis shown in Table 4 as in this disease the β_2M can appear in the urine due to overload from the high concentrations in the glomerular filtrate.[31] When urinary β_2M was detectable by RID then the correlation coefficient of α_1M to β_2M concentration was r = 0·60 (n = 68). The urinary light chain and α_1M concentration in multiple myeloma were less well correlated (r = 0·35, n = 80). Crossed immunoelectrophoresis of the urinary α_1M showed that in renal failure and in myelomatosis, including IgA myelomas, the α_1M ran as a single peak that was not bound to other proteins.

ACUTE CHANGES IN RENAL TUBULAR FUNCTION

Changes in urinary α_1M concentrations during a period of acute renal tubular damage and its recovery are illustrated by considering the urinary low molecular weight protein excretion patterns after burns. Figure 3 shows a patient with a 30% superficial burn not requiring antibiotics. A study of a further 24 burn injury patients with 100 samples indicated that β_2M and RBP concentrations are highly correlated (r = 0·89) whilst the correlation is less for α_1M to β_2M (r = 0·55) or α_1M to RBP (r = 0·48). Generally the urinary α_1M, RBP and β_2M concentrations follow the same pattern of increase and return to normal but the magnitude of change of β_2M and RBP is two to three times greater than that of α_1M. NAG runs a parallel course but it appears to lag behind the changes in the reabsorption of lower molecular weight proteins by the proximal tubular cells. Urinary α_1M concentrations in acute pancreatitis show a similar pattern to that in burns with an α_1M to β_2M correlation coefficient of r = 0·59 and α_1M to RBP correlation coefficient of 0·50.

Discussion

The distribution of serum α_1M in controls and patients with renal failure is similar to that recorded by other authors.[11 20 21] In normal subjects there is no diurnal variation of serum α_1M concentrations.[30]

IgA myelomatosis is a condition in which the well established property of α_1M to bind to IgA appears to influence strongly the blood concentrations of α_1M; this also seems to be an occasional effect in rheumatoid arthritis but not in liver disease where increases of the IgA concentrations are commonplace. This suggests the monomers and polymers of IgA do not bind α_1M to the same extent. However, it is clear that it is the free α_1M that is filtered by the glomerulus as the relation of urinary α_1M to light chain excretion is similar in IgA and IgG myelomas and electrophoretic analysis has demonstrated the α_1M excreted in the urine is an unbound protein.

Table 4 *Relation between urinary α_1M and β_2M*

Urinary concentration of α_1M and β_2M	*Chemical workers*	*Paraplegics*	*Rheumatic diseases*	*Bladder cancer*		*Ileal conduits*
				T_1 + *Tis*	T_2 – T_4	
Normal α_1M Normal β_2M	485	77	54	108	44	24
Raised α_1M Normal β_2M	11	14	15	16	11	4
Normal α_1M Raised β_2M	0	2	0	1	1	0
Raised α_1M Raised β_2M	4	12	15	14	8	8
Total	500	105	84	139	64	36

Normal = α_1M <15 mg/g creatinine; β_2M <2 mg/g creatinine by RID.
Raised = α_1 M ≥ 15 mg/g creatinine; β_2 M ≥ 2 mg/g creatinine by RID.

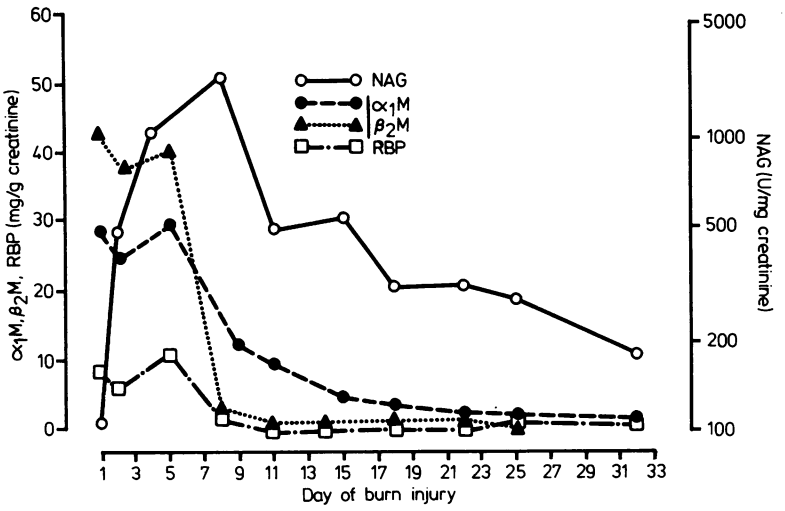

Fig. 3 *Pattern of evolution of urinary low molecular weight protein excretion and NAG activities after a mild burn injury*

Urinary excretion of α_1M in normal subjects has been estimated to be 9 mg/24 h[4] and 5·7 mg/24 h[19] using RID and 10 mg/l[15] and 1·3 mg/24 h using an electroimmunoassay.[16] These figures reflect the different reference standards and methods used in the assays. Our assay, using the Behringwerke standard, for normal urine and serum concentrations gives similar values to those reported by Takagi *et al.*[19] This study has shown that the measurement of urinary α_1M can provide information about the renal filtration and reabsorption of low molecular weight protein. The SDS gel patterns and correlations with other low molecular weight proteins (RBP and β_2M) confirm that in the absence of renal failure a raised urinary α_1M is an indicator of tubular proteinuria. When there is considerable reduction of the glomerular filtration rate the concentrations of α_1M are increased in the serum and an increase of urinary excretion under these circumstances could be partly due to overload of the tubule and partly due to a mixed glomerular and tubular lesion. In practical terms in a patient with a serum creatinine <200 μmol/l, a urinary concentration of α_1M of ≥ 15 mg/g creatinine is an indication of tubular proteinuria although an SDS gel is needed to confirm the diagnosis. In this respect, the α_1M test using RID is less sensitive than the measurement of urinary β_2M by RIA and cannot demonstrate falls of 1 or 2% of tubular reabsorption efficiency which can be discriminated by the RIA or ELISA measurement of β_2M. However, the α_1M test has the advantage of simplicity, low cost and α_1M is stable in the urine over a range of pH found in routine practice. On the other hand, β_2M is unstable at pH $<5·5$ and could give false-negative results if it is measured in acidic urine.[10 26] In this respect, α_1M shows similar advantages to those suggested for RBP as a practical method of screening of renal tubular function.[10] However, an RID assay for RBP is too insensitive to identify very small changes of tubular function but when tubular function is impaired by a factor of 10% or over, then concentrations of RBP and β_2M are highly correlated. The measurement of urinary α_1M appears to be free from interference from the effects of urinary infection and haematuria or from the liberation of tissue breakdown products in bladder cancer. Whether the more recently developed radioimmunoassays or enzyme linked immunosorbent assays[27] will have advantages for urine screening is uncertain. The assays have been modified for nephelometric techniques which could provide a rapid assay needed when there is a probability of occult acute tubular damage.[31]

The relation between urinary α_1M concentrations and light chain excretion is of particular interest as measurement of α_1M can provide an indication of the incidence and severity of tubular disorder that accompanies myelomatosis. This test has potential in helping to investigate the nephrotoxic effects of light chains that is evident in this series; as in other studies,[28] not all light chain excretion, even when a large amount, will produce renal damage. The nephrotoxic effects of myelomatosis—in general α_1M and light chain excretion are correlated, but these proteins can show marked discordance drawing attention to the non-nephrotoxic forms of light chain excretion[28] or tubular damage in the absence of increased light chain excretion. In monitoring of patients with myelomatosis urinary α_1M measurements have some advantages, especially as the assay

does not present the technical difficulties in measuring light chain excretion.

The interrelations of urinary α_1M, β_2M, and RBP are all partially explained by consideration of the time-point during an acute event when the urine was sampled. In long-standing tubular proteinuria α_1M excretion can be raised when there is a generalised abnormality of proximal tubular function affecting all proteins, or it can be part of a selective tubular proteinuria that seems to affect the relatively large low molecular weight protein such as α_1-acid glycoprotein (MW 44 000 daltons).[29]

The correlation of urinary α_1M and NAG excretion in acute tubular damage indicates that they can both reflect proximal tubular cell injury but the intensity of their disturbance as indicated by the failure to reabsorb a protein or the excessive discharge of NAG, a lysosomal enzyme, into the urine are not synchronous. In chronic tubular disorders the relation of the absorption of the low molecular weight proteins and the excretion of NAG are far more reliable. The very high NAG activities seen in acute lesions are rare. The precise interpretation of a high NAG is uncertain and depends on the clinical condition. For example, in paraplegia it would suggest an upper renal tract infection; in myelomatosis, an exacerbation of interstitial nephritis. High NAG activities are a well recognised feature of certain forms of drug-induced renal tubular damage, particularly that caused by aminoglycosides.[13] The present studies suggest that the urinary measurement of α_1M can be a useful method of screening populations in whom there is a risk of tubular proteinuria whatever the underlying cause.

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