Serum α_2 -macroglobulin levels in diabetes

K JAMES¹, J MERRIMAN¹, RS GRAY², LJP DUNCAN², AND R HERD³

From the ¹Department of Surgery, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG, ²Diabetic and Dietetic Department, Royal Infirmary, Lauriston Place, Edinburgh EH3 9YW, and ³Medical Computing and Statistics Unit, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG, UK

SUMMARY Serum α_2 -macroglobulin levels have been determined in diabetic patients by quantitative radial immunodiffusion and compared with those observed in age- and sex-matched controls. In addition, the results in diabetics have been analysed with respect to such variables as the age and sex of the patient, the duration of disease, treatment, control, and the occurrence of retinopathy or nephropathy.

The α_2 -macroglobulin levels in diabetic patients were found to be significantly higher than in age- and sex-matched controls, thus confirming previous observations. However, these differences were most apparent in the more extreme age groups. Multiple regression analysis also revealed that the only variables contributing significantly to the regression apart from age and sex were control and retinopathy.

Although there have been a number of reports indicating that serum α_2 -macroglobulin levels may be elevated in diabetes, few of these studies have taken into consideration the known variation in the level of this protein with age and sex,¹ or the possible influence of any therapy employed, or the duration of the disease.

In view of these limitations we decided to undertake further studies of serum α_2 -macroglobulin levels in diabetic patients. Furthermore, such studies seemed desirable in the light of recent suggestions that the incidence of peripheral vascular complications in diabetic patients might be linked to high serum α_2 -macroglobulin levels,² and the report that this protein is intimately associated with human vascular endothelium.³

Material and methods

PATIENTS AND CONTROLS

The diabetic patients studied were regularly attending the Diabetic and Dietetic Department of the Royal Infirmary, Edinburgh. Further details on the number of patients treated with diet, oral hypoglycaemic agents, and insulin, their ages, and the duration of diabetes in relation to treatment are recorded in Table 1.

Table 1 Patients studied according to sex, treatment, age, and duration of diabetes

Treatment	Sex	No. of patients	Age (yr)	Duration (yr)
Diet	M	36	54.6 $\pm$ 12.7	3.8 $\pm$ 4.0
	F	17		
OHA	M	44	59.8 $\pm$ 13.5	6.0 $\pm$ 5.3
	F	37		
Insulin	M	46	41.3 $\pm$ 17.2	10.8 $\pm$ 9.1
	F	46		

Diabetic control was assessed on the basis of the mean plasma glucose concentration observed at the time of study and the previous two outpatient attendances at the clinic. Mean plasma glucose levels of <9, 9-14, >14 mmol/litre were regarded as indicating good, moderate, and poor control respectively.

The ocular fundi of each patient were examined under clinic conditions, and retinopathy status was recorded as DR-negative (no retinopathy or mild background retinopathy) or DR-positive (moderate to extensive background retinopathy with or without new vessel formation).

A urine sample was obtained from all patients at every clinic attendance and tested for protein using Multistix (The Ames Co, Slough, UK). Those without proteinuria were considered to have no nephropathy (ie, grade 0), while those with intermittent or

persistent proteinuria were considered to have grades 1 and 2 nephropathy respectively.

The normal human serum samples (ages 18-70) were freshly collected by the Blood Transfusion Service, The Royal Infirmary, Edinburgh. The older controls were obtained from geriatrics with no apparent clinical abnormalities. All serum samples were stored at -20°C before assay.

ESTIMATION OF α_2 -MACROGLOBULIN

Serum α_2 -macroglobulin levels (expressed as mg per dl) were measured by a standard Mancini gel diffusion method.⁴ A range of α_2 -macroglobulin standards were included on every plate, and all samples were assayed in duplicate. The standard serum had been calibrated against a human α_2 -macroglobulin reference standard obtained from Meloy Laboratories Inc, Springfield, Virginia, USA. The antiserum to human α_2 -macroglobulin was produced in rabbits as previously described.⁵

STATISTICAL ANALYSES

The results obtained were submitted to a number of statistical analyses. Initially, the levels of α_2 -macroglobulin in diabetics were compared with those observed in age- and sex-matched normal controls using both Student's *t* test and Welch's test, which allows for differences in variance. As preliminary analysis indicated that the significance levels obtained by both tests were similar, the data were subsequently analysed by conventional procedures. The values obtained in diabetics were also subjected to both

individual and multiple regression analysis of $\alpha_2\text{M}$ values on sex, age, treatment, control, duration, retinopathy, and nephropathy.

Results

An overall analysis of the results indicated that serum α_2 -macroglobulin levels were significantly higher ($P < 0.001$) in diabetics than in normals, thus confirming the results of previous observations.^{1,6} However, in the present study, additional points emerged. Thus while α_2 -macroglobulin levels in diabetics also varied with age, the pattern of variation was somewhat different from that noted in normals (Fig. 1; Table 2). These differences were most apparent in the younger and older patients, the α_2 -macroglobulin levels in the 41-50 age group being relatively similar. It should perhaps be noted that while the data in Table 2 suggest that these differences are more apparent in female diabetics we believe that this is simply a reflection of small sample sizes.

The results obtained in diabetic patients were subjected to further analyses. Initially, the α_2 -macroglobulin levels were regressed on each of the variables individually in turn. From these preliminary analyses each variable appeared significantly to influence the α_2 -macroglobulin level in diabetics (Table 3). Thus, as in normal controls, the levels of this protein were significantly higher in both females and males and there was a significant quadratic relationship with age. Furthermore, significant differences were noted with respect to such variables as

The values observed in male and female diabetics have been pooled and compared with those in pooled normal controls. Note that in both groups the α_2 -macroglobulin levels exhibit a quadratic variation with age, but the pattern in diabetics is somewhat different from that in normals. Thus the levels of this protein in diabetics appear to be most significantly elevated above control values in the younger and older age groups, while those in the 41-50 range are not significantly different from normal.

Table 2 Levels of α_2 -macroglobulin in diabetic patients and sex- and age-matched controls

Age	Normal		Diabetics		Significance level
	No.	α_2M Conc.*	No.	α_2M Conc.	
<i>A Males</i>					
18-20	21	452 $\pm$ 82.7	4	483 $\pm$ 220.9	Not significant
21-30	29	338 $\pm$ 88.9	16	496 $\pm$ 156.3	P < 0.001
31-40	20	300 $\pm$ 53.6	20	343 $\pm$ 100.1	Not significant
41-50	20	264 $\pm$ 46.5	26	309 $\pm$ 77.1	P < 0.05
51-60	19	271 $\pm$ 59.6	16	375 $\pm$ 120.7	P < 0.01
61-70	20	287 $\pm$ 55.4	30	411 $\pm$ 153.1	P < 0.01
71 +	14	399 $\pm$ 115.9	14	481 $\pm$ 160.9	Not significant
<i>B Females</i>					
18-20	30	463 $\pm$ 80.0	5	672 $\pm$ 167.5	P < 0.001
21-30	43	395 $\pm$ 87.8	9	466 $\pm$ 182.3	Not significant
31-40	23	355 $\pm$ 70.4	13	430 $\pm$ 173.1	Not significant
41-50	20	362 $\pm$ 87.6	14	358 $\pm$ 105.8	Not significant
51-60	21	366 $\pm$ 75.0	18	399 $\pm$ 85.0	Not significant
61-70	18	355 $\pm$ 92.4	29	469 $\pm$ 127.7	P < 0.01
71 +	13	317 $\pm$ 68.9	12	537 $\pm$ 197.9	P < 0.002

*Arithmetic mean value $\pm$ standard deviation (mg per dl).

the duration of the disease, the form of treatment, the degree of control, and the incidence of retinopathy. However, it should be noted that where multiple comparisons are being made (Tables 2 and 3) small P values (eg, 0.05) are not necessarily significant.

When more detailed analyses of these results were undertaken, namely, multiple regression analysis, it became apparent that the only variables contributing significantly to the regression, apart from age (P < 0.001) and sex (P < 0.05), were control (P < 0.025) and retinopathy (P < 0.001). The actual regression equation was as follows:

$$Y = 935.8 + 42.8 (\text{if female}) - 2.310 \times \text{Age} + 0.2387 \times (\text{Age})^2$$

$$- \begin{cases} 54.38 \text{ if moderate control} + 91.85 \text{ (if} \\ 85.53 \text{ if good control} & \text{retinopathy + ve)} \end{cases}$$

where Y is the α_2M level.

Discussion

The present results confirm those of previous workers, indicating that α_2 -macroglobulin levels are elevated in diabetic patients.^{6,7} They also re-emphasise that, as in normal individuals, the level of α_2 -macroglobulin in diabetics is significantly influenced by the sex and age of the patient⁷ though the pattern of variation with age may be different from that in normal controls.

However, of most interest in relationship to the present study are the results of multiple regression analysis, which indicate the variables most significantly influencing the regression in diabetics. Apart from sex and age, these appear to be the control of blood sugar level and the presence of retinal complications. All the other variables studied,

Table 3 Influence of various factors on α_2 -macroglobulin levels in diabetic patients—a summary of individual regression analysis on each variable

Variable investigated		No. of samples	α_2M^* concentration	Significance level
Age	Linear	226	395 $\pm$ 145.5	P < 0.001
	Quadratic			
Sex	Male	126	454 $\pm$ 156.1	P < 0.005
	Female	100		
Duration	Positive	53	358 $\pm$ 106.1	P < 0.001
	Increase			
Treatment	Diet	81	409 $\pm$ 161.9	P < 0.001
	OHA	92	468 $\pm$ 153.6	
	Insulin	21	388 $\pm$ 164.9	
Control	Good	107	433 $\pm$ 156.7	P < 0.005
	Moderate	98	519 $\pm$ 135.4	
	Poor	183	401 $\pm$ 141.6	
Retinopathy	Zero	43	506 $\pm$ 170.1	P < 0.001
	Positive	217	417 $\pm$ 150.2	
Nephropathy	Grade 0	7	481 $\pm$ 157.9	P < 0.05
	1	2	660 $\pm$ 294.9	

*Arithmetic mean value $\pm$ standard deviation (mg per dl).

including duration of disease, treatment, and nephropathy, were not independent of the above and so became redundant. In short they failed to contribute significantly to the regression.

These observations indicate that there is a strong association between poor control and high α_2 -macroglobulin levels. Likewise, there is a similar association between high levels of this protein and the incidence of diabetic retinopathy, an observation noted by other workers using less sophisticated statistical analyses.⁸ However, in contrast to the present observations, others have noted that the elevation of α_2 -macroglobulin levels in diabetics is directly related to the duration of the disease⁷ and is not significantly different in patients with vascular complications apart from nephropathy.^{7,9}

The present results indicate that the determination of α_2 -macroglobulin levels in diabetic patients may provide useful additional information with respect to both the ease with which control of blood sugar levels can be achieved and the propensity to develop retinal complications. It is appreciated, however, that more detailed studies on larger groups of patients will be necessary to establish unequivocally the true relevance of these observations and the biochemical events leading to these perturbations. Nevertheless the present observations, together with the known biochemical^{10,11} and possible immunological properties of α_2 -macroglobulin,¹² emphasise that this is an area worthy of further investigation.

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Requests for reprints to: Dr Keith James, Department of Surgery, University Medical School, Teviot Place, Edinburgh EH8 9AG, UK.