

Serum α -macroglobulins in renal disease and preeclampsia

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SYNOPSIS The serum levels of α_2 -macroglobulin and pregnancy-associated globulin (another α -macroglobulin) have been measured by means of a radial immunodiffusion technique in (1) renal disease with and without proteinuria, (2) in age- and sex-matched controls, (3) in preeclampsia with and without proteinuria, and (4) in normal pregnant controls. There are significant increases in α_2 -macroglobulin and pregnancy-associated globulin in renal disease accompanied by proteinuria but normal levels are found in renal disease without proteinuria. Compared with normal pregnancy, α_2 -macroglobulin is significantly raised in preeclampsia with proteinuria but normal in preeclampsia without proteinuria. In contrast, serum pregnancy-associated globulin is significantly reduced in preeclampsia both with and without proteinuria when compared with normal pregnancy.

Maclaren, Kelleher, and Reid (1961) have described in pregnancy a serum α_2 -globulin, now known to be a macroglobulin (Maclaren and Alper, 1970), and have referred to it as 'pregnancy-associated globulin' (PAG). However, it has also been found in the serum of a small number (18%) of normal males and females (Maclaren, Reid, Konugres, and Allen, 1966). Since conventional serum α_2 -macroglobulin is known to be increased in the nephrotic syndrome (Schultze and Schwick, 1959; Steines and Mehl, 1966; Housley, 1968) and in preeclampsia accompanied by proteinuria (Horne, Howie, and Goudie, 1970a), it seemed likely that the serum levels of this other macroglobulin, PAG, might also increase in such diseases. Using a radial immunodiffusion technique we have determined the serum levels of α_2 -macroglobulin and PAG in 53 patients with various renal diseases and in age-sex-matched controls, and also in preeclampsia with or without proteinuria and in normal pregnant controls.

Subjects and Methods

Sera were obtained from 53 patients (37 males, 16 females, mean age 34, range 13-64) who had a variety of acute and chronic renal diseases, usually some form of glomerulonephritis (Table I), of whom 44 had proteinuria. Hospital inpatients with no known renal disease were chosen as age- and sex-matched controls.

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Renal Disease	No. of Cases
Acute and subacute glomerulonephritis	7
Chronic glomerulonephritis	6
Chronic proliferative glomerulonephritis	9
Chronic pyelonephritis	4
Focal glomerulonephritis	5
Membranous glomerulonephritis	6
Lupus nephritis	3
Miscellaneous	13
Total	53

Table I Clinical diagnosis in renal disease patients

Sera were also obtained from 37 preeclamptic patients (mean age 27.6 years, range 18-41, duration of pregnancy 37.1 weeks, range 30-41), 13 with proteinuria and 24 without proteinuria, and from 37 normal pregnant controls (mean age 27.5 years, range 18-41, duration of pregnancy 37.3 weeks, range 30-41) matched for age, parity, and duration of pregnancy. Most of the pregnant patients had been included in a previous study (Horne *et al.* 1970a).

Proteinuria was estimated in the renal disease patients by the Biuret method and in the preeclamptic patients by the Esbach technique.

All sera were stored at -20°C for up to two years before assay. Antiserum to PAG was prepared in rabbits by immunizing them with pregnant human serum, known to contain appreciable quantities of this protein, in Freund's complete adjuvant and absorbing the resultant antiserum with human serum which did not contain this pro-

tein (Maclaren *et al*, 1966). Antiserum to serum α_2 -macroglobulin was prepared by the method of Goudie, Horne, and Wilkinson (1966).

Assay of serum α_2 -macroglobulin and PAG was carried out using a radial immunodiffusion technique (Horne *et al*, 1970a). Each serum was tested in duplicate and where appropriate the control serum was included in the same assay plate to minimize the effects of interplate variation (Thompson, Horne, Steele, and Goudie, 1969). Serum α_2 -macroglobulin concentration was calculated from a calibration curve with a solution of a freeze-dried, reconstituted pooled human serum containing 3, 6, 12, and 18 g protein per 100 ml and standardized with reference to a serum (Behringwerke AG) containing a known amount of this protein. Pregnancy-associated globulin concentration was expressed as a percentage of a pregnant human serum known to contain appreciable quantities of this protein.

The statistical analysis of results was carried out using either Student's *t* test or the χ^2 test where appropriate.

Results

Table II shows a significant increase in serum PAG levels in renal disease with proteinuria (44 patients) compared with age- and sex-matched controls. Of the 44 patients with renal disease, only 18 have demonstrable PAG and of nine sera from renal

disease without proteinuria none contain PAG ($P < 0.10$). Of the 53 control sera, only eight contain PAG and this is significantly less than the number of PAG-positive sera in renal disease with proteinuria (18 out of 44) ($P < 0.05$). The mean urinary protein of the PAG-positive renal disease patients, 8.5 g/24 hours, is significantly more than that of the PAG negative, 4.0 g/24 hours ($P < 0.005$). A highly significant increase in α_2 -macroglobulin is observed in the sera of renal patients with proteinuria compared with sera from controls and renal patients without proteinuria (Table II). There is a highly significant correlation ($P < 0.001$) between α_2 -macroglobulin concentration and amount of proteinuria (Fig.) but no such correlation is noted between PAG and proteinuria. In addition there is no correlation between blood urea of creatinine clearance and the levels of α_2 -macroglobulin or pregnancy-associated globulin.

Table III shows significantly lower PAG levels in both preeclampsia groups compared with normal pregnancy, but there is no significant difference between preeclampsia with or without proteinuria. All preeclamptic and normal pregnant sera contain pregnancy-associated globulin. The difference in α_2 -macroglobulin between normal pregnancy and preeclampsia without proteinuria falls short of significance. In preeclampsia with proteinuria (mean level 4.4 g/100 ml), there is a significant increase in α_2 -macroglobulin compared with normal pregnancy and preeclampsia without proteinuria. No

	Renal Disease		Controls
	With Proteinuria (44 patients)	Without Proteinuria (9 patients)	
Serum PAG (% concentration)	8 \pm 17 ¹	0	3 \pm 12 ¹
Serum α_2 -macroglobulin (mg/100 ml)	557 \pm 302 ^{2, 3}	312 \pm 338 ³	328 \pm 116 ³

Table II Serum PAG and α_2 -macroglobulin levels (mean \pm SD) in 53 patients with renal disease and in age- and sex-matched controls

¹t test $P < 0.05$, ²t test $P < 0.0005$, ³t test $P < 0.0125$

	Preeclampsia		Normal Pregnancy
	With Proteinuria	Without Proteinuria	
Serum PAG (% concentration)	81 \pm 62 ¹	89 \pm 71 ²	127 \pm 73 ^{1, 2}
Serum α_2 -macroglobulin (mg/100 ml)	601 \pm 215 ^{3, 4}	423 \pm 215 ^{3, 5}	467 \pm 154 ^{4, 5}

Table III Serum PAG and α_2 -macroglobulin levels (mean \pm SD) in 37 preeclamptic patients and in matched normal pregnant controls

¹t test $P < 0.05$ ⁴t test $P < 0.01$
²t test $P < 0.05$ ⁵t test $P < 0.10$
³t test $P < 0.0025$

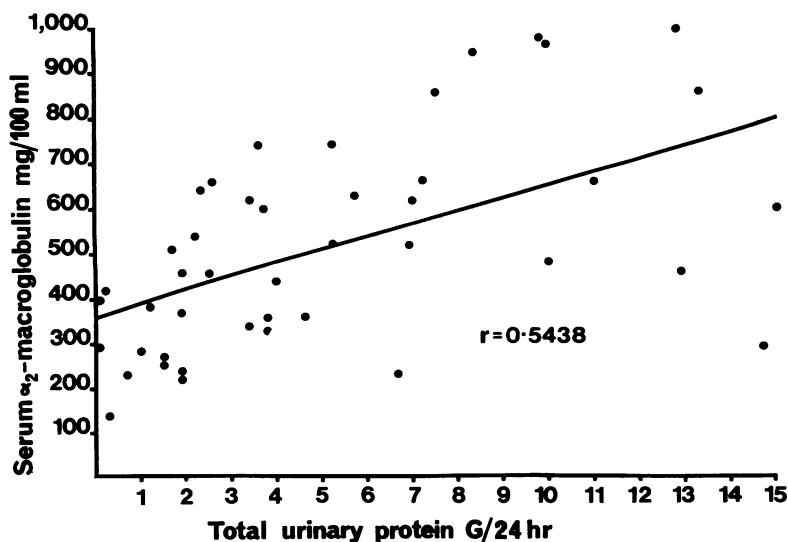


Fig. The relationship of serum α_2 -macroglobulin to total urinary protein.

significant correlation, however, is observed between the amount of proteinuria and PAG or α_2 -macroglobulin.

Discussion

The striking increase in serum α_2 -macroglobulin in the nephrotic syndrome is well known (Schultze and Schwick, 1959; Steines and Mehl, 1966; Housley, 1968). In our study those patients who had renal disease with associated proteinuria show an increase in serum α_2 -macroglobulin. In addition there is a highly significant correlation between α_2 -macroglobulin levels and the degree of proteinuria, a finding which does not appear to have been reported previously. Inspection of the Figure seems to indicate a proportional rather than a step-wise relationship between the amount of proteinuria and serum α_2 -macroglobulin and it would appear that raised α_2 -macroglobulin levels are not peculiar to the nephrotic syndrome. Although the reasons for the raised α_2 -macroglobulin levels are not clearly understood it would seem unlikely that marked increases in α_2 -macroglobulin could be attributed to non-specific factors such as diminution in plasma volume.

As anticipated there is an increase in serum PAG levels in patients with renal disease and associated proteinuria. Except for one case, however, the levels of PAG are low in comparison to those found in normal pregnancy. That PAG is demonstrable in the serum more often in renal disease than in other hospital patients lends support to the suggestion

that this macroglobulin may be present in all human sera but in such minute quantities that its presence is difficult to detect using conventional immunodiffusion techniques (Maclaren *et al*, 1966). Our failure to demonstrate a correlation between PAG and the amount of proteinuria may be due to the unsuitability of our radial immunodiffusion technique for the accurate measurement of very low levels of pregnancy-associated globulin.

In a previous study (Horne *et al*, 1970a) we demonstrated significantly lower α_2 -macroglobulin levels in 13 patients with preeclampsia unaccompanied by proteinuria compared with those in normal pregnancy but in the present study we have failed to confirm this finding in an extended group of 26 patients. The raised α_2 -macroglobulin levels in preeclampsia with proteinuria have been described in our previous study (Horne *et al*, 1970a). In preeclampsia we have found no correlation between α_2 -macroglobulin and the amount of proteinuria. The low serum PAG levels in preeclampsia unaccompanied by proteinuria are of interest and could be the result of decreased production of oestrogen and/or progesterone which are thought to occur in this disease (Klopper, 1968), particularly since it has been clearly shown that the levels of this protein increase following the administration of combined oestrogen/progesterone oral contraceptives (Maclaren *et al*, 1966). Unlike α_2 -macroglobulin serum PAG levels do not increase in preeclampsia with proteinuria.

Although both pregnancy and the administration of oestrogen/progesterone oral contraceptives result

in raised serum levels of α_2 -macroglobulin (Horne, Howie, Weir, and Goudie, 1970b) and PAG, it is clear that in renal disease and preeclampsia these serum α -macroglobulins behave in a dissimilar fashion.

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