The Serum Pregnancy Associated $\alpha_2$-Glycoprotein Level in Patients with IgA Nephropathy

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The 1st Department of Pathology, Hiroshima University School of Medicine (Received February 9, 1984)

Key words: Pregnancy associated $\alpha_2$-glycoprotein, IgA nephropathy

ABSTRACT

Serum pregnancy associated $\alpha_2$-glycoprotein levels in 51 IgA nephropathy patients (28 males and 23 females) and 46 healthy subjects (31 males and 15 females) were determined by enzyme-immunoassay. The results showed the serum pregnancy associated $\alpha_2$-glycoprotein levels to be significantly increased in the IgA nephropathy patients over the healthy subjects in both sexes (males: $p<0.01$, females: $p<0.001$). Further, in the male patients, the level was significantly increased in those with proteinuria, also there was a significant negative correlation between the concentration and PSP 15 minute value and GFR. On the other hand, in the female patient group there was a significant positive correlation between serum total cholesterol level.

On the basis of these findings, we presume that the determination of serum pregnancy associated $\alpha_2$-glycoprotein level may be useful in assuming the prognosis of IgA nephropathy.

INTRODUCTION

Pregnancy associated $\alpha_2$-glycoprotein (referred to here after as $\alpha_2$-PAG) is a glycoprotein with a molecular weight of approximately 360,000 which increases in serum during pregnancy, and has been reported to increase also in patients with malignant tumors, Behçet's disease, rheumatoid arthritis etc. However, reports on the serum $\alpha_2$-PAG behaviour in chronic glomerulonephritis are still rare. Thus, we undertook review of the serum $\alpha_2$-PAG behaviour in IgA nephropathy which is said to have the highest frequency among chronic glomerulonephritis patients.

MATERIALS AND METHODS

The subjects were 51 IgA nephropathy patients (28 males and 23 females), excluding those currently pregnant or who had complication of malignant tumors. The ages were males 26.2±12.6 and females 22.5±10.1. As controls, a group of 46 healthy subjects (31 males and 15 females) whose ages were males 21.3±2.6 and females 20.1±1.7 was selected.

Determination of serum $\alpha_2$-PAG level was made by enzyme-immunoassay (Fig. 1), and standard curve was shown (Fig. 2). The lower limit of determination was 0.1 µg/ml.

RESULTS

As there is a sex difference in $\alpha_2$-PAG level, all subsequent review was made by sex.

The serum $\alpha_2$-PAG level in male IgA nephropathy patients was 0.93±1.20 µg/ml, while that for healthy subjects was 0.23±0.57 µg/ml, thus indicating a significant increase ($p<0.01$). The same for female patients was 14.48±12.92 µg/ml, while that for healthy females was 1.99±1.93 µg/ml, indicating a significant increase ($p<$
Mix together 0.01ml of test sample or standard α2-PAG solution and 0.5ml of PBS.

Add immunobead and incubate at room temperature for 2 hr.

Wash bead with PBS-Tween 20.

Add 0.5ml of HRPO labelled anti-human α2-PAG antibody solution.

Incubate at 4°C over night.

Wash bead with PBS-Tween 20.

Add 0.5ml of substrate solution.

Add 2ml of 1N HCL.

Centrifuge.

Measure absorbance of supernatant at 492nm.

**Fig. 1.** Method for determination of serum α2-PAG level by enzyme-immunoassay

**Fig. 2.** Standard curve for determination of serum α2-PAG level

**Fig. 3.** Serum α2-PAG levels in healthy subjects and IgA nephropathy patients

Next, comparison of serum α2-PAG levels was made between the proteinuria positive and negative groups in male IgA nephropathy patients. Results showed that there was a significant increase in the positive group as compared with the negative group (p<0.05). However, no significant difference could be demonstrated between the positive and negative groups in the female patients (Fig. 4).
Further, comparison of serum $\alpha_2$-PAG levels between hypertensive and normotensive individuals among IgA nephropathy patients, showed the hypertensives tended to show higher levels in both sexes, but the values were not significant (Fig. 5).

On the other hand, study of the relationship with renal function showed that there was a significant negative correlation between PSP 15 minute value and GFR in male patients (PSP: $p<0.02$, GFR: $p<0.05$) (Fig. 6). However, no such significant correlation was observed in female patients.

Although a significant correlation between serum $\alpha_2$-PAG and total cholesterol levels could not be demonstrated in male patients, there was a significant positive correlation in female patients ($p<0.05$) (Fig. 7).

Further, no significant correlation between serum $\alpha_2$-PAG and BUN, serum creatinine, serum uric acid and immunoglobulin levels could be found in both male and female patients.
DISCUSSION

Serum $\alpha_2$-PAG is a glycoprotein with a molecular weight of approximately 360,000, glucose content of 12.2% and belongs to the $\alpha_2$-globulin zone by electrophoresis with isoelectric points ranging from 4.5 to 4.7, and was first reported by Smithies.<ref>. He made electrophoretic studies of serum of pregnant women using starch gel, and discovered a specific band which was not observed in non-pregnant women or males, and thus named this protein "pregnancy zone protein" (PZP). Subsequently, many researchers have reported on pregnancy proteins under a variety of names.<ref>. In 1971, Bohn<ref> carried out studies on serum of pregnant women and succeeded in isolating four different types of pregnancy associated proteins. He named them 1) human placental lactogen (HPL), 2) pregnancy specific $\alpha_1$-glycoprotein (SP₁), 3) pregnancy associated $\alpha_1$-glycoprotein (SP₂) and 4) pregnancy associated $\alpha_2$-glycoprotein (SP₃). HPL and SP₁ appear only during pregnancy and are considered pregnancy specific, but SP₂ and SP₃ are present in minute volumes in the serum of non-pregnant women and in males. Moreover, as they show marked increases with pregnancy, they were called "pregnancy associated proteins". Subsequent study revealed that SP₃, that is pregnancy associated $\alpha_2$-glycoprotein
Pregnancy Associated α₂-Glycoprotein

(α₂-PAG), was the protein, PZP, discovered by Smithies\(^9\).

Quantitation of α₂-PAG can be done by 1) single immunodiffusion\(^1\), 2) Laurell’s immunoelectrophoresis method\(^2\), 3) radioimmunoassay\(^3\), 4) enzyme-immunoassay\(^4\), 5) solid-phase fluoroimmunoassay\(^5\), etc., and basic and clinical studies on it are being performed.

The clinical implications of α₂-PAG have not been established, but many researchers have reported on its behaviour in a variety of diseases. MacLaren et al.\(^6\) have reported that this protein increases with the use of oral contraceptives. It is also reported to be increased in serum of patients with Behcet’s disease\(^7\) and rheumatoid arthritis\(^8\). There are also reports that it is increased in patients with malignancies such as breast cancer\(^9,10\), ovarian cancer\(^11\), choriocarcinoma\(^12\), gastric cancer\(^13\), urinary bladder cancer\(^14\) and malignant melanoma\(^15\). α₂-PAG has become one of the many tumor markers, and much attention is now being focussed upon it.

On the other hand, reports on its behaviour in renal diseases are rare. In 1972, Horne et al.\(^1\) determined the α₂-PAG concentration in 53 patients with renal diseases, and reported that the level was significantly higher in patients with proteinuria than in those without it. In 1983, we reported that the level was higher than healthy subjects in patients undergoing chronic hemodialysis\(^2\) and those with nephrotic syndrome\(^3\), and also noted that there was a correlation with the clinical activity in the latter. However, no reviews have been made of the relationship between the serum α₂-PAG level and histological type of glomerulonephritis. Thus, we made a study of the α₂-PAG behaviour in IgA nephropathy patients. The results showed the level in IgA nephropathy patients was increased as compared with healthy subjects. Also in male patients the level was significantly higher in those with proteinuria than in those without it, and further there was a negative correlation between renal function. Care must be exercised in the interpretation of these results, but as there are reports that α₂-PAG has some sort of immunosuppressive substance\(^4,5\), we presume α₂-PAG is involved in some form in the development of IgA nephropathy which is induced by abnormalities in the immune system. Further, it is considered possible that the determination of serum α₂-PAG may be useful in assuming the prognosis of IgA nephropathy. However, there are yet many unknown aspects of α₂-PAG such as in what organ it is produced and its role. Thus, it is considered further study is required.

ACKNOWLEDGEMENT

We would like to express our appreciations to Research Laboratory of Diagnostic Reagents in Mochida Pharmaceutic Co. Ltd. for its cooperation in serum α₂-PAG determinations and also to Dr. H. Bohn who so generously provided us with standard α₂-PAG serum.

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