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The Serum Pregnancy Associated α_2-Glycoprotein Level in Patients undergoing Chronic Hemodialysis*)

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ABSTRACT

The concentration of serum pregnancy associated α_2-glycoprotein (α_2-PAG) was determined in 157 healthy controls and 76 chronic hemodialysis (HD) patients. The results obtained were as follows.

1) Healthy females showed a significant increase as compared to healthy males ($p<0.001$), and the values increased with age.
2) HD male patients showed significantly higher values than healthy males ($p<0.001$).
3) A significant relationship was observed between serum α_2-PAG value and age, serum creatinine value, peripheral lymphocyte count, PPD and serum IgG value in HD male patients. However, a significant relationship was not seen between duration of hemodialysis and BUN.

INTRODUCTION

α_2-PAG, a pregnancy-associated protein, is a glycoprotein with a molecular weight of about 300,000 which was isolated and purified by Bohn[3]. It is present in minute volumes in healthy individuals, but its serum concentration increases markedly with pregnancy. There are reports that the concentration also increases in the presence of malignant tumors and inflammatory diseases such as rheumatoid arthritis. However, reports on the α_2-PAG behavior in renal diseases is limited to that of Horne et al.[7] who noted an increase in serum concentration in those with nephrotic syndrome. We have made studies on the α_2-PAG behavior in uremic patients undergoing chronic hemodialysis, and the results of our findings are reported.

MATERIALS AND METHODS

1) Materials

The subjects were 75 HD patients (44 males and 31 females). The ages of males ranged from 21 to 76 (mean 45.3±15.1) and females from 24 to 71 (mean 51.2±13.9). The duration of dialysis was 0.5 to 10.7 years (mean 3.9±2.6) for males and 1.1 to 9.5 years (mean 4.5±2.4) in females. The cause for chronic renal failure was primarily chronic glomerulonephritis.

A comparison group of 157 healthy controls (89 males and 68 females) whose ages in both sexes ranged from those in their second to seventh decades was selected. The specimens used for determination was serum for both study groups.

2) Methods

Serum α_2-PAG value was determined by use of Laurell's[9] immunoelectrophoresis method. That is, first a 1% agarose gel plate which contains 2% anti-human α_2-PAG rabbit serum is prepared. Wells of 2.5 mm in diameter are made 2 cm from the cathode and 5 μl of standard serum (made by Behringwercke Co.)

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and test serum are injected into each well. Electrophoresis is carried out for 6 hours at 10 V per cm at 4°C. The standard curve based on the length of the precipitation line was drawn and the α_2-PAG concentration in the test serum was determined. The sensitivity of determination by this method was 0.5 mg/dl.

RESULTS

1) Serum α_2-PAG values in healthy controls

Those in whom a precipitation line was not obtained on immunoelectrophoresis by Laurell's method were classified as negative and those in whom the line was present were classified as positive. Study was made of the difference in α_2-PAG values by sex in the healthy controls, and it was found that of the 89 males, 66 (74.2%) were negative and 23 (25.8%) were positive, which of the 68 females only 10 (14.7%) were negative and 58 (85.3%) were positive. The females had a significantly higher positive rate than males ($p<0.001$). The mean measured values were 0.27 ± 0.52 mg/dl for healthy males and 1.58 ± 1.21 mg/dl for females with the females demonstrating a significantly higher value than males ($p<0.001$) (Fig. 1).

Next, study was made of the difference in α_2-PAG values by age. In healthy males, there was no significant difference by age with the exception of those in their sixties who showed a slight increasing trend (Fig. 2). While in females, the value increased with age, and reached an almost set value at 30 and over (Fig. 3).

2) Serum α_2-PAG values in HD patients

As a difference in values was observed in the healthy controls, the HD patients were also grouped into males and females and comparison was made against the controls. Results showed that whereas only 23 out of 89 (25.8%) healthy males were positive, there were 32 out of 44 (72.7%) HD males who were positive, showing a significantly higher positive rate than healthy males($p<0.001$). Further, the healthy males had a mean measured α_2-PAG value of 0.27 ± 0.52 mg/dl, whereas the value for HD males was 0.97 ± 0.86 mg/dl indicating that the value for the latter was significantly higher than the former ($p<0.001$) (Fig. 4).

On the other hand, of the 68 healthy females, 58 (85.3%) were positive, while of the 31 HD females, all (100.0%) were positive. The mean

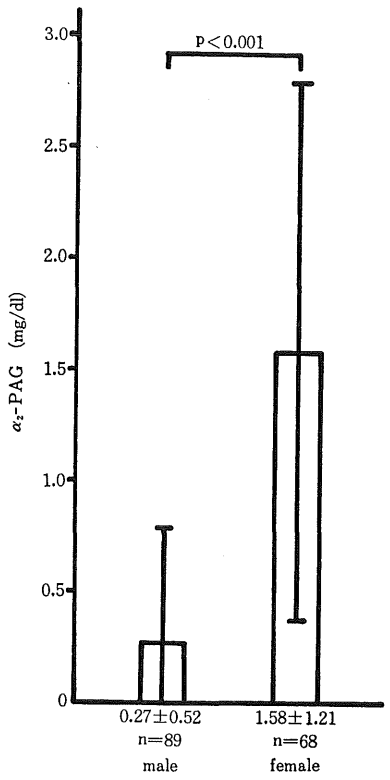

Fig. 1. Serum α_2-PAG values in healthy controls

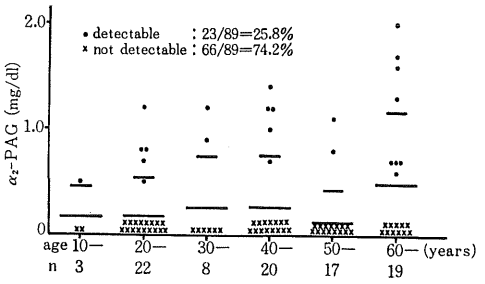

Fig. 2. Serum α_2-PAG values by age in healthy males

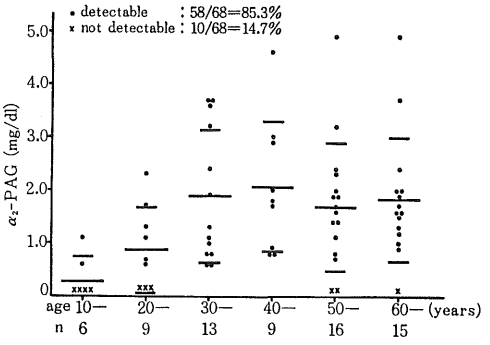

Fig. 3. Serum α_2-PAG values by age in healthy females

Table 1. Serum α_2-PAG values and other laboratory data in male patients undergoing chronic hemodialysis

No.	Case	Age	α_2-PAG (mg/dl)	HD Duration (years)	BUN (mg/dl)	Cr. (mg/dl)	Lymphocytes (/mm^3)	PPD	IgG (mg/dl)
1	T.D.	28	1.7	2.8	82.2	20.6	2450	—	925
2	N.M.	39	0.5	5.1	66.1	19.7	1496	n.d.	1575
3	T.K.	42	1.3	4.8	61.4	20.1	1242	n.d.	1675
4	M.O.	32	0	4.8	92.4	17.4	620	−	875
5	T.M.	76	0	2.0	44.4	6.3	874	−	2925
6	Y.O.	46	0.9	1.8	69.4	9.6	1342	−	975
7	S.K.	35	0.9	0.8	78.3	12.4	1400	+	1300
8	T.N.	38	2.2	1.0	54.0	10.7	1406	+	1350
9	T.K.	31	0.7	3.3	89.2	14.8	1056	+	1025
10	K.N.	55	3.8	2.1	68.1	10.0	1792	+	1250
11	H.S.	53	1.6	5.8	78.7	13.4	1717	+	1500
12	H.T.	45	1.1	1.8	84.0	13.0	988	+	2500
13	Y.N.	73	0.9	3.1	31.4	8.5	1332	+	2125
14	A.M.	58	2.7	2.4	80.1	19.5	1152	+	975
15	H.M.	62	0.7	5.0	63.1	15.5	870	+	2250
16	T.S.	21	0	0.6	106.1	15.7	1855	n.d.	925
17	T.K.	23	0	2.9	79.3	16.3	2848	−	2125
18	S.F.	38	0.9	8.4	57.0	14.8	1540	+	1125
19	H.K.	33	0.9	10.7	57.7	16.0	1715	−	2050
20	M.K.	42	0.5	7.5	63.0	13.5	2139	+	1800
21	S.M.	71	1.5	3.3	57.0	10.2	1012	+	2250
22	T.N.	50	0	7.5	51.2	10.7	720	+	1250
23	M.I.	36	0.7	6.0	40.2	12.1	966	n.d.	1575
24	Y.I.	26	1.1	0.5	79.5	15.3	1536	n.d.	2575
25	K.H.	31	1.1	4.1	73.4	12.9	1400	n.d.	775
26	Y.O.	64	2.7	6.1	38.6	6.5	1848	+	1625
27	Y.M.	62	1.4	0.1	55.0	12.4	900	−	1350
28	K.N.	34	1.8	4.1	77.9	12.7	2016	+	1925
29	T.K.	52	0	7.3	68.2	14.5	1271	−	2450
30	S.O.	23	0	0.5	60.7	11.0	840	n.d.	1175
31	T.T.	67	1.0	3.8	71.5	10.5	950	+	1575
32	S.H.	48	1.0	8.0	71.0	22.1	850	+	1575
33	A.I.	32	0	4.1	70.6	13.3	770	+	2250
34	Y.N.	37	0.9	1.8	66.6	15.4	1980	+	1775
35	T.T.	38	0	3.9	97.8	20.6	1742	+	1750
36	Y.Y.	62	0.9	7.8	80.2	13.9	2112	−	2050
37	T.O.	30	0	2.4	86.8	23.3	880	−	1125
38	M.I.	41	0	2.9	65.4	16.0	836	−	1300
39	M.O.	56	0	1.3	105.7	16.8	1953	−	1175
40	K.K.	47	2.2	3.4	78.4	11.7	770	+	650
41	Y.N.	32	1.3	1.8	56.6	13.6	1026	−	1400
42	S.A.	61	2.0	9.5	52.0	9.3	1515	+	2125
43	K.B.	69	1.1	2.0	51.3	8.8	1608	+	2650
44	M.N.	67	0.7	3.1	66.1	12.7	1200	+	2500

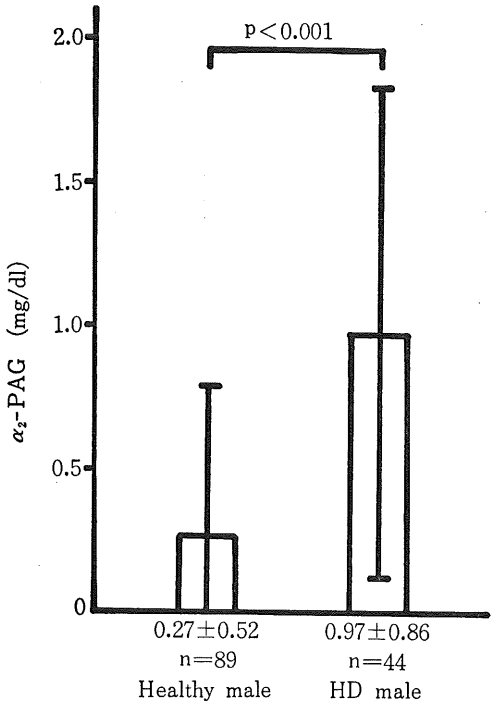

Fig. 4. Serum α_2-PAG values in healthy males and male patients undergoing chronic hemodialysis

measured α_2-PAG value of healthy females was 1.58±1.21 mg/dl, while that for HD females was 1.89±1.53 mg/dl, indicating the HD females tended to show higher values, but the difference was not significant.

3) Comparison of α_2-PAG values and various other test results in HD males

As elevation of α_2-PAG value was noted in HD males only, comparison of various test values was made at varying α_2-PAG levels to ascertain the clinical implications. The α_2-PAG value and the various clinical laboratory test values of the HD males are shown in Table 1.

(1) Age

The mean age of HD males was 45.3±15.1, thus they were separated into those under 45 and 45 and over, and comparison was made of their respective serum α_2-PAG values. There were 23 in the under 45 group whose mean α_2-PAG value was 0.72±0.65 mg/dl while the over 45 group consisted of 21 patients whose value was 1.25±0.97 mg/dl. Thus, the over 45 group showed a significantly higher value than the under 45 group ($p<0.05$) (Fig. 5).

(2) Duration of hemodialysis

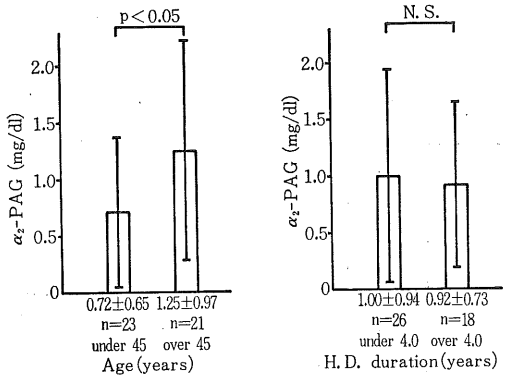

Fig. 5. Comparison of α_2-PAG values and age, hemodialysis duration in male patients undergoing hemodialysis

The mean duration of hemodialysis was 3.9±2.6 years. Thus, the patients were divided into those whose duration of dialysis was under 4 years and 4 years and over, and comparison was made with their respective serum α_2-PAG values. There were 26 whose duration of dialysis was under 4 years, whose mean α_2-PAG value was 1.00±0.94 mg/dl, while there were 18 in the over 4 years group whose value was 0.92±0.73 mg/dl, thus, no significant difference would be observed between the two (Fig. 5).

(3) BUN level

The mean BUN level was 68.8±16.4 mg/dl. Thus, the patients were divided into those whose BUN values were under 70.0 mg/dl and those 70.0 mg/dl and over, and comparison was made with their respective serum α_2-PAG values. There were 24 with values under 70.0 mg/dl, whose mean α_2-PAG value was 1.04±0.90 mg/dl, while there were 20 with values over 70.0 mg/dl, whose value was 0.89±0.80 mg/dl. Thus, no significant difference was observed between the two (Fig. 6).

(4) Serum creatinine level

The mean serum creatinine level was 13.9±4.0 mg/dl. Thus, the group was divided into those whose values were under 14.0 mg/dl and 14.0 mg/dl and over, and comparison was made of their respective α_2-PAG values. There were 25 with values under 14.0 mg/dl, whose mean α_2-PAG value was 1.21±0.88 mg/dl, and 19 with values over 14.0 mg/dl whose value was 0.65±0.71 mg/dl. Thus, the under 14.0 mg/dl group had a significantly higher α_2-PAG value

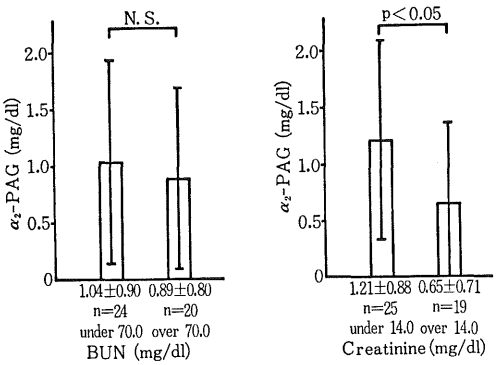

Fig. 6. Comparison of α_2-PAG values and BUN, creatinine levels in male patients undergoing hemodialysis

than the over 14.0 mg/dl group ($p<0.05$) (Fig. 6).

(5) Peripheral blood lymphocyte count

The peripheral blood lymphocyte count was divided into two groups, those under 1000/mm^3 and 1000/mm^3 and over, and comparison was made with the respective α_2-PAG values. There were 14 with values under 1000/mm^3 whose mean α_2-PAG value was 0.58 ± 0.69 mg/dl, while there were 30 with values over 1000/mm^3 whose value was 1.15 ± 0.89 mg/dl. Thus, the over 1000/mm^3 group had a significantly higher value than the under 1000/mm^3 group ($p<0.05$) (Fig. 7).

(6) PPD

PPD was performed on 37 out of the 44 patients. There were 13 with negative findings, whose mean α_2-PAG value was 0.55 ± 0.65 mg/dl, while 24 were positive with a value of 1.29 ± 0.94 mg/dl. As a result, the PPD positive patients had a significantly higher α_2-PAG value than the negative patients ($p<0.02$) (Fig. 7).

(7) Serum IgG level

The serum IgG value was divided into those whose value was under 1000 mg/dl and 1000 mg/dl and over, and comparison was made of the respective α_2-PAG values. There were 5 with values under 1000 mg/dl, whose mean α_2-PAG value was 1.72 ± 0.75 mg/dl, while 39 had values over 1000 mg/dl and a value of 0.87 ± 0.84 mg/dl. Thus, the under 1000 mg/dl group had a significantly higher serum α_2-PAG value than the over 1000 mg/dl group ($p<0.05$) (Fig. 7).

DISCUSSION

In 1955, Smithies[11] discovered a protein specific to serum of pregnant women and called it pregnancy zone protein (PZP). Subsequently, Bohn[3] in 1971 succeeded in isolating four other pregnancy associated proteins, namely, 1) human placental lactogen (HPL), 2) pregnancy specific β_1-glycoprotein (SP_1), 3) pregnancy associated β_1-glycoprotein (SP_2), and 4) pregnancy associated α_2-glycoprotein (SP_3). Of

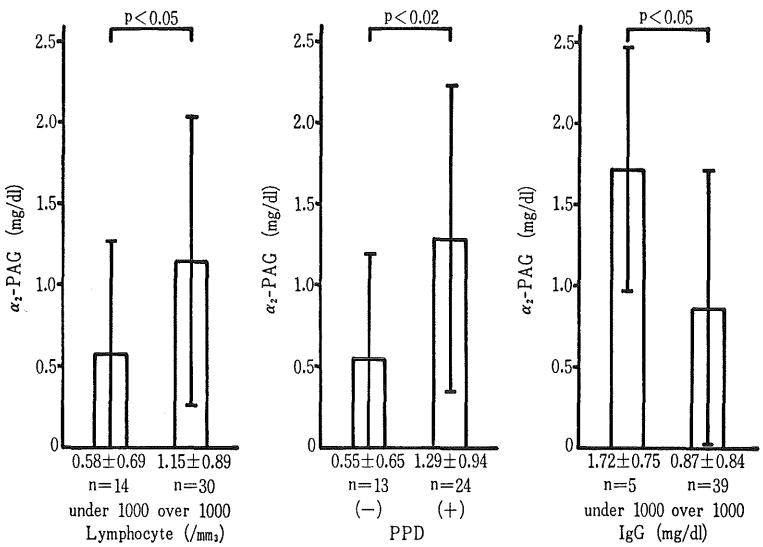

Fig. 7. Comparison of α_2-PAG values and lymphocytes count, PPD and IgG levels in male patients undergoing hemodialysis

these, HPL and SP_1 are pregnancy specific, but SP_2 and SP_3 are said to be present in minute amounts in healthy persons as well. It is also reported that the serum concentration of SP_3 is increased not only in pregnancy, but also in other disease states such as malignant tumors, inflammatory diseases and others[4]. Particularly, there are many reports[1,2,5,10,18,19] on its relationship with malignant tumors, with interest now being focussed upon it as a tumor marker. On the other hand, reports on its relationship with renal diseases have been limited to that of Horne et al.[7] in which it was noted that the serum concentration was increased in those with high degree of proteinuria.

Therefore, the authors undertook a study to review the serum α_2-PAG behavior in those undergoing chronic hemodialysis due to uremia, the terminal stage of renal diseases, and a group of healthy controls. Although a significant increase in the values of HD females as compared to healthy females was not observed, there was a remarkable increase in HD males as compared to their healthy controls. In an attempt to determine the mechanism which causes the increase, study was made of the relationship between the α_2-PAG value and age, duration of dialysis, BUN and serum creatinine levels, lymphocyte count, PPD and serum IgG levels.

Our findings show that there was a significant increase in the value with age in healthy females, and although a significant increase could not be observed in healthy males, an increasing trend was seen in those 60 and over when compared with those of younger age. Yamagishi et al.[19] and Folkersen et al.[6] also have reported the value increases with age. The mechanism responsible for the increase in value with age is yet unknown, but it is presumed it is related with decrease in immunological function due to aging.

Next, review was made of the relationship with duration of dialysis, but no significant association was found.

The relationship with BUN and serum creatinine levels was studied. Those with high BUN levels showed a lesser degree of α_2-PAG increase than those with low levels, but the effect was not significant. On the other hand, in those whose serum creatinine value was high, the degree of α_2-PAG increase was significantly small. This may be due to an inhibitory effect of serum creatinine on the α_2-PAG producing cells.

The relationship between α_2-PAG and peripheral blood lymphocyte count, PPD and serum IgG value showed that the α_2-PAG value increase was small in those whose lymphocyte count was low and PPD was negative, while the increase was large in those with low IgG values. These findings are considered to be related to the site of α_2-PAG production and the effect of α_2-PAG on the immunity. That is, the site of α_2-PAG production is considered to be in the lymphocytes, and it is said that α_2-PAG has immunosuppressive effect. Stimson et al.[14] report that when the leukocytes of peripheral blood of pregnant women, women on oral contraceptives and healthy males are cultured, α_2-PAG can be found in the culture media. Further, it has been demonstrated by indirect immunofluorescent antibody method that α_2-PAG is present on the surface of peripheral lymphocytes of pregnant women[13]. Thomson et al.[16] have removed α_2-PAG from the lymphocytes of healthy persons with trypsin, and after culturted in the cells for 20 hours, have demonstrated by direct immunofluorescent antibody method that α_2-PAG has reappeared on the surface, which suggests the possibility that α_2-PAG is produced by the lymphocytes. On the other hand, Von Schoultz[17], Than et al.[15], Stimson[12] and Kasukawa et al.[8] have reported that as their findings indicate α_2-PAG reduces PHA activity of peripheral lymphocytes in vitro and inhibits MLC and E-rosette formation, it has immunosuppressive action. However, there are yet many unknown points such as the site of α_2-PAG production and its role. The results of the author's study on HD patients have shown that the serum α_2-PAG value in HD males is higher than that of healthy males and that there are associations with a number of factors, but there are yet many points which require clarification, thus it is considered further study must be carried out in the future.

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