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SIGNIFICANCE OF PREGNANCY-ASSOCIATED α_2-GLYCOPROTEIN (α_2-PAG) IN PATIENTS WITH BREAST, GASTRIC AND COLORECTAL CANCER[1]

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SUMMARY Pregnancy-associated α_2-glycoprotein (α_2-PAG) levels were measured in human sera by a modification of Laurell's electroimmunoassay using rabbit anti-α_2-PAG serum. Sera were obtained from healthy controls (32 males and 46 females), patients with benign breast diseases (55 cases), and patients with breast (82 cases), gastric (89 cases), or colorectal (22 cases) cancers.

In healthy controls, the mean α_2-PAG value for females was higher than that for males ($p<0.05$), so α_2-PAG values for males and females were considered separately in this study.

Serum α_2-PAG levels in patients with benign breast tumors were almost the same as those of controls.

In patients with primary breast and gastric cancer, α_2-PAG levels were higher than those of controls ($p<0.005$) and tended to increase with progress of the disease. Raised α_2-PAG levels decreased in these patients after curative surgery.

These results show that serum α_2-PAG is useful as a marker in both the initial diagnosis and follow-up of breast and gastric cancer.

The reliability of α_2-PAG as a tumor-associated marker was reinforced by comparison of the positive rates of the three parameters α_2-PAG, carcinoembryonic antigen (CEA), and immunosuppressive acidic protein (IAP) in patients with breast and gastric cancer.

INTRODUCTION

The existence of a pregnancy-associated serum protein was first detected by Smithies using starch gel electrophoresis in 1959 (Smithies, 1959).

In 1971, Bohn detected four different pregnancy-associated proteins by the Ouchterloney gel diffusion technique with antisera which had been prepared by immunizing rabbits with

1 A summary of this work was reported at the 18th Annual Meeting of the Japan Society for Cancer Therapy in Tokyo, in September, 1980.

protein fractions from human placentas (Bohn, 1971): These were human placental lactogen (HPL), pregnancy-specific β_1-glycoprotein (SP_1), pregnancy-associated β_1-glycoprotein (SP_2), and pregnancy-associated α_2-glycoprotein (SP_3, α_2-PAG).

Among these proteins, α_2-PAG is a high molecular weight glycoprotein (M.W.=300,000–500,000), with a sedimentation coefficient of 12S and isoelectric point of pH 4.6–4.7 (Stimson et al., 1972; Schoultz et al., 1973a). It has been reported to have immunosuppressive properties in vitro (Schoultz et al., 1973; Than et al., 1975a; Stimson, 1976), but its biological role in vivo has not yet been clarified.

In pregnant women, the serum level of α_2-PAG begins to rise markedly from the 16th week pregnancy, reaches a maximum in the third trimester, and decreases to the normal level after delivery (Stimson, 1974; Schoultz, 1974).

An increased serum level of α_2-PAG has been found in patients with malignancy (Stimson, 1975a, b; Than et al., 1975b; Wood et al., 1978a; Cove et al., 1979).

We studied the clinical significance of α_2-PAG as a tumor-associated protein by comparison of the serum α_2-PAG levels in healthy controls, patients with benign breast tumors, and patients with breast, gastric and colorectal cancers. To assess the relative merits of tumor-associated markers, we also compared the levels of α_2-PAG with those of carcinoembryonic antigen (CEA) and immunosuppressive acidic protein (IAP) in cancer patients.

MATERIALS

Sera of patients with benign breast tumors (55 cases), breast cancers (82 cases), gastric cancers (89 cases) and colorectal cancers (22 cases) were obtained at the Hospital of the Research Institute for Microbial Diseases of Osaka University. Histological diagnoses were made in all cases. The stages of cancer were determined according to the TNM classification.

Sera obtained from 32 healthy males (25–50 years old) and 46 healthy females (20–50 years old) were used as controls. The standard serum used was pooled serum from three pregnant women in the third trimester. All samples were stored at −20 C before assay.

METHOD

Quantitative estimations of α_2-PAG were performed by the modification of Laurell's electroimmunoassay (Laurell, 1966) using rabbit antiserum to α_2-PAG (Behring Institute).

Agarose was diluted (to 1%) in Veronal buffer (pH 8.6, r=0.05) and anti-α_2-PAG serum was used at a concentration of 350 μl per 25 ml of agarose containing 0.1% NaN_3. Gel plates of 2 mm thickness were made on glass plates, and wells of 2.5 mm diameter were cut in the gel. Samples of 5 μl were introduced into wells under 8 V/cm, and electroimmunoassay was performed at a constant voltage of 30 V/cm for 3 h. A standard curve was obtained by running standard serum (a pooled pregnant serum) with test samples at appropriate dilutions. The precipitated antigen-antibody complex was stained with Coomassie Brilliant Blue R-250 and the distance from the center of the well to the tip of the rocket-shaped precipitate was measured.

The concentration of α_2-PAG was calculated from a standard curve obtained by testing 2.5, 5, 10 and 20 U of α_2-PAG prepared by dilutions of the standard serum (pooled pregnant serum) that was assumed to contain 100 U α_2-PAG/μl.

The upper limit of the normal range for α_2-PAG was defined as the mean value of controls plus 2 times the deviation ($x+2\sigma$) in each sex group. With this definition, there were only 2 false-positive cases among 32 males (6.25%) and 2 among 46 females (4.34%).

Serum CEA was determined in duplicate by the sandwich method using a Dinabot RIA kit, originally devised by Nishi and Hirai (Hirai, 1977). The upper limit of the normal range for CEA was 2.5 ng/ml.

The serum immunosuppressive acidic protein (IAP) level was measured by single radial immunodiffusion (Mancini et al., 1965) using agarose gel plates involving rabbit anti-IAP serum which were supplied by Kayaku Antibiotics Research Co. The upper limit of normal for IAP was 500 μg/ml.

The Student's t-test was used for statistical analyses.

RESULTS

1. *Healthy controls (Fig. 1)*

α_2-PAG was found in sera of 11 of 32 male controls (34.4%) and 26 of 46 female controls (56.6%). The mean serum level of α_2-PAG of female controls (2.6 ± 3.00 U/μl) was statistically higher ($p<0.05$) than that of male controls (0.6 ± 1.01 U/μl).

2. *Benign breast tumors (Fig. 1)*

The mean value of serum α_2-PAG in patients with benign breast tumors was 2.94 ± 2.47 U/μl, and there was no difference between the values in these patients and female controls.

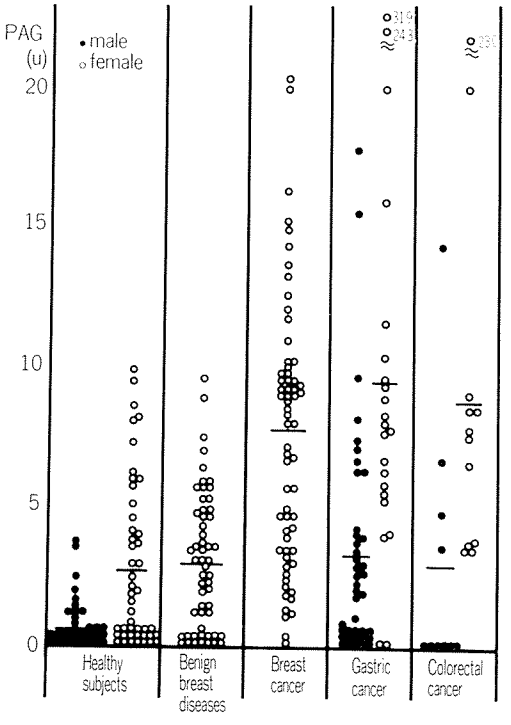

FIGURE 1. Serum levels of α_2-PAG in several subjects. α_2-PAG levels of males (●) and females (○) are shown. Values shown in this figure do not include those for recurrent cases. Estimations of serum α_2-PAG levels were performed preoperatively in all cancer cases. Bars indicate means.

3. *Primary cancer patients (Fig. 1)*

The mean value for patients with primary breast cancer was 7.86 ± 5.14 U/μl, which was statistically higher ($p<0.005$) than that of female controls.

Primary gastric cancer patients showed statistically higher ($p<0.005$) mean values of α_2-PAG than those of controls when the values for males and females were compared separately.

Female patients with colorectal cancer showed statistically higher ($p<0.05$) mean values of α_2-PAG than female controls, but male patients did not show significantly different values from male controls.

α_2-PAG could not be detected in sera from 6 of 10 patients with colorectal cancers.

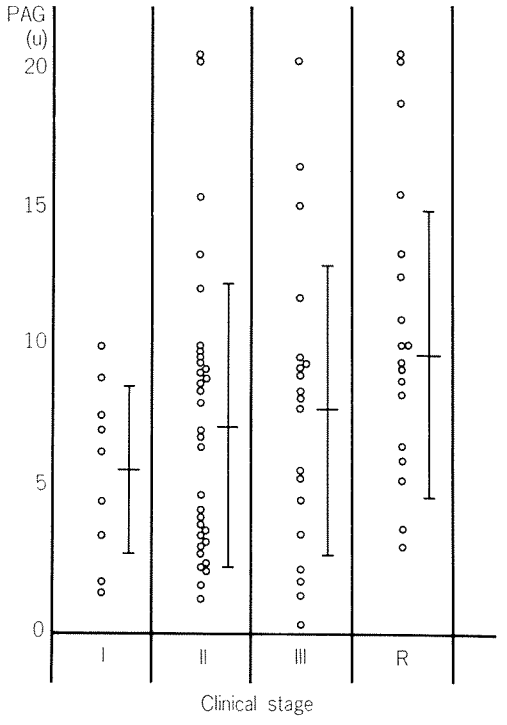

FIGURE 2. Serum levels of α_2-PAG in patients with breast cancer of different stages judged by the TNM classification. R: Recurrence

4. *α_2-PAG levels in relation to clinical stages of cancer (Fig. 2, 3)*

In patients with breast cancer, α_2-PAG levels tended to rise with increase with the clinical stage. Recurrent cases showed statistically higher ($p<0.05$) values of α_2-PAG than those of cases at stage I (Fig. 2). This tendency was not so clear in patients with gastric cancer as in those with breast cancer (Fig. 3). α_2-PAG could not be detected in 5 of 14 male patients with gastric cancer of stage IV (Fig. 3).

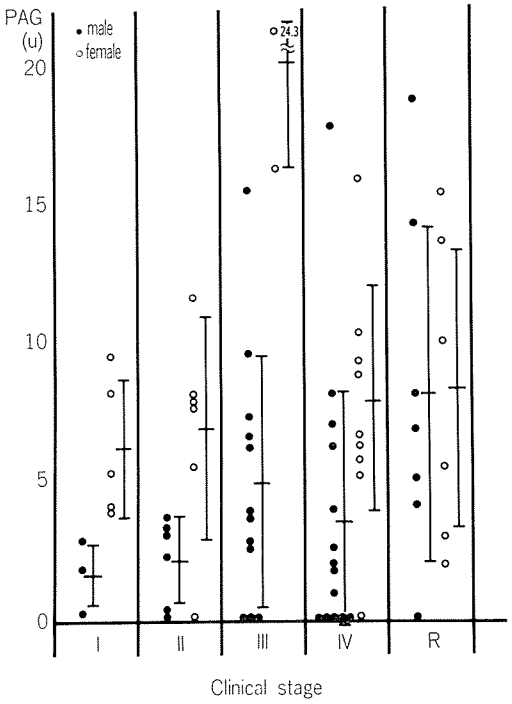

FIGURE 3. Serum levels of α_2-PAG in patients with gastric cancer, at different stages judged by the TNM classification. α_2-PAG levels for males (●) and females (○) are shown. R: Recurrence

5. *Changes in serum α_2-PAG levels of cancer patients after curative surgery (Fig. 4, 5)*

In most cases of both breast and gastric cancer, α_2-PAG levels decreased within a month or two after removal of the tumor.

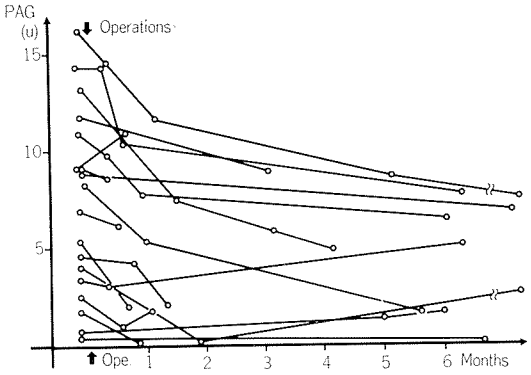

FIGURE 4. Alteration in serum α_2-PAG levels of breast cancer patients after curative surgery. Operations were carried out at the time indicated by the arrow.

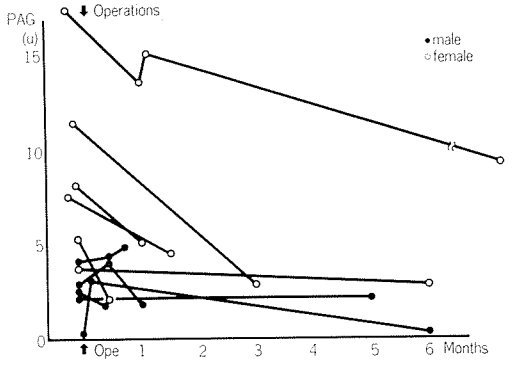

FIGURE 5. Alteration in serum α_2-PAG levels of gastric cancer patients after curative surgery. α_2-PAG levels for males (●) and females (○) are shown. Operations were carried out at the time indicated by the arrow.

6. *α_2-PAG levels in relation to histology (Fig. 6)*

In patients with gastric cancer, no difference in α_2-PAG levels was found between the group with poorly differentiated adenocarcinoma and that with tubular adenocarcinoma.

Among breast cancer patients, α_2-PAG levels were high in those with the scirrhous type and low in those with the medullary tubular type. But the deviations of values in each group were very wide and there was no statistical difference between values in different groups.

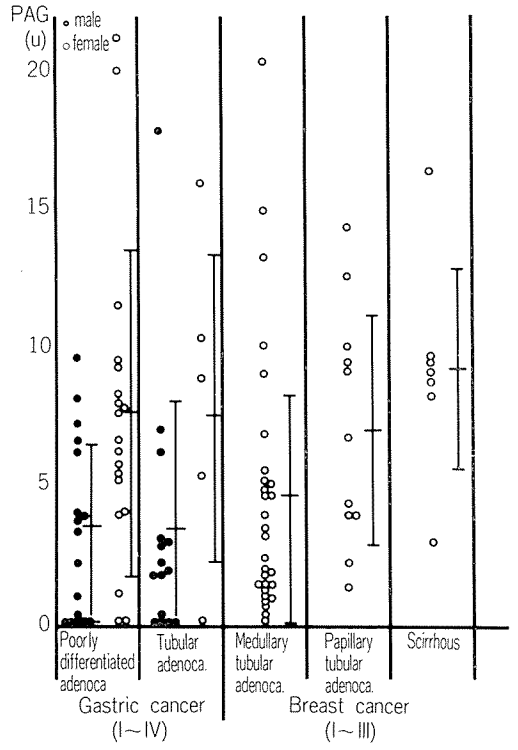

FIGURE 6. Serum α_2-PAG levels of patients with gastric and breast cancer of different histological types. α_2-PAG levels for males (●) and females (○) are shown. Values for recurrent cases are not involved.

7. *Comparison of the values of α_2-PAG, CEA, and IAP in cancer patients (Table 1, 2, 3)*

Table 1 shows that the positivity of α_2-PAG was significantly higher than those of the other two parameters at all stages in breast cancer.

In patients with gastric cancer, α_2-PAG positivity was higher than the other parameters at an early stage, but almost the same as those of the other parameters at an advanced stage (Table 2).

In colorectal cancer patients, CEA showed the highest positive ratio at all stages (Table 3).

DISCUSSION

The serum level of α_2-PAG rises remarkably during pregnancy and decreases to a comparatively insignificant value within 6 weeks after delivery (Schoultz, 1974), but it has been reported that very small amounts of α_2-PAG can be detected even in non-pregnant women and healthy males. Than et al. reported that α_2-PAG was found in the sera of 8 of 30 healthy non-pregnant women and 1 of 10 male controls (Than et al., 1975b). In our study, α_2-PAG was found in 26 of 46 non-pregnant female controls and 11 of 32 male controls. The mean value of serum α_2-PAG of female controls (2.6 ± 3.00 U/μl) was statistically higher ($p<0.05$) than that of male controls (0.6 ± 1.01 U/μl).

Raised serum levels of α_2-PAG have been found in females taking oral contraceptives containing estrogen components (Horne et al., 1973), but the concentration of estrogen and that of α_2-PAG in the serum were not correlated (Schoultz et al., 1973c; Bohn et al., 1973). At present, the reason why α_2-PAG levels of female controls are significantly higher than those of males is unknown.

It is possible that the serum α_2-PAG changes during the menstrual cycle in woman. So now we are measuring α_2-PAG levels serially in sera of healthy adult females with a regular menstrual cycle. Results suggest that the extent of change is not too wide to influence the initial diagnosis of cancer patients.

Owing to the sexual difference, we considered the α_2-PAG levels for males and females separately.

Primary breast cancer patients showed statistically higher ($p<0.005$) values of α_2-PAG than controls (Fig. 1) and raised α_2-PAG levels were lowered by removal of tumor (Fig. 4), but they were markedly elevated in recurrent cases (Fig. 2).

Anderson and Stimson reported similar results to ours on breast cancer patients (Stimson, 1975; Anderson et al., 1976). Patients with benign breast tumors showed almost the same α_2-PAG levels as female controls.

These results clearly showed that α_2-PAG is a reliable marker for not only initial diagno-

TABLES 1–3. *Results are expressed as total numbers of patients having higher values than the upper limit of normal/total number of patients studied.*

TABLE 1. *Comparison of values of α_2-PAG, CEA, and IAP in breast cancer patients*

Clinical stage	PAG	CEA	IAP
I	2/9 (22.2%)	2/19 (10.0%)	1/9 (11.1%)
II	14/32 (43.8%)	3/29 (10.3%)	4/32 (12.5%)
III	8/19 (42.1%)	6/20 (30.0%)	3/19 (15.8%)
R	12/18 (66.7%)	6/15 (40.0%)	5/16 (31.3%)
Total	36/78 (46.2%)	17/83 (20.5%)	13/76 (17.1%)

TABLE 2. *Comparison of values of α_2-PAG, CEA, and IAP in gastric cancer patients*

Clinical stage	PAG	CEA	IAP
I	3/9 (33.3%)	0/9 (0%)	0/7 (0%)
II	4/11 (36.4%)	1/5 (20.0%)	2/10 (20.0%)
III	10/14 (71.4%)	0/7 (0%)	7/12 (58.3%)
IV	10/23 (43.5%)	12/20 (60.0%)	9/19 (47.4%)
R	9/13 (66.2%)	6/9 (66.7%)	6/11 (54.5%)
Total	36/70 (51.4%)	19/51 (37.3%)	24/59 (40.7%)

TABLE 3. *Comparison of values of α_2-PAG, CEA, and IAP in colorectal cancer patients*

Clinical stage	PAG	CEA	IAP
I	0/3 (0%)	0/6 (0%)	0/2 (0%)
II	1/3 (33.3%)	1/3 (0%)	0/2 (0%)
III	5/12 (41.7%)	5/11 (45.5%)	5/12 (41.7%)
IV	1/3 (33.3%)	2/3 (66.7%)	3/3 (100%)
V・R	7/10 (70.0%)	12/14 (85.7%)	5/9 (55.6%)
Total	14/31 (45.2%)	20/37 (54.1%)	15/29 (51.7%)

sis of breast cancer but also follow-up of cancer patients undergoing surgery. The reliability of α_2-PAG as a cancer-associated marker was reinforced by comparison of α_2-PAG levels with those of CEA and IAP in breast cancer patients (Table 1).

Than reported that none of the 6 patients with gastric cancer they examined had high α_2-PAG values (Than et al., 1975b). On the contrary, we found that the mean α_2-PAG levels of primary gastric cancer patients were statistically higher ($p<0.005$) than those of controls (Fig. 1) and that the raised α_2-PAG levels decreased after curative surgery (Fig. 5). It should be emphasized that rise in the α_2-PAG level occurred at earlier stages than rises in CEA and IAP levels (Table 2). It is, therefore, concluded that α_2-PAG may also be a reliable marker of gastric cancer of all clinical stages as well as of breast cancer.

In case of colorectal cancer, elevated α_2-PAG levels were also detected, indicating that α_2-PAG may be useful for initial diagnosis of colorectal cancers. But CEA and IAP were more reliable than α_2-PAG as indicators of these tumors and CEA was the best marker in

detecting recurrency (Table 3). Wood et al. also reported that serum α_2-PAG levels were increased in advanced cases of colorectal cancers, but that this protein was less reliable than CEA for detecting recurrent tumors (Wood et al., 1978b).

The above findings indicate that α_2-PAG was not a specific marker for one kind of cancer, but a common marker of several kinds of cancers.

It is concluded that the accuracy of diagnosis of cancers may be improved by adding measurements of the new cancer-associated marker, α_2-PAG, to the conventional diagnostic procedures.

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