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Pregnancy Associated α_2-Glycoprotein in Malignant Melanoma

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Abstract—*Pregnancy associated α_2-glycoprotein is a high molecular weight, carbohydrate containing plasmaprotein with unknown biological function. Serum levels of α_2-PAG were measured by electro-immunoassay in 97 melanoma patients and 97 healthy controls. A clear-cut stage dependent increase of the mean α_2-PAG levels during tumour progression was noticed, being particularly pronounced in females. The significance of α_2-PAG measurements in melanoma patients for monitoring the disease is discussed.*

INTRODUCTION

PREGNANCY associated α_2-glycoprotein (α_2-PAG) was first demonstrated in sera of pregnant women [1]. A later work showed that it is readily detectable in trace amounts in most sera of normal males and females [2]. Elevated serum levels were found during oestrogen medication and trophoblastic disease [3].

The interest in α_2-PAG rose when an association with malignant tumours was demonstrated [4] and confirmed by several groups [2, 5]. There is no association with any particular malignant tumour but rather a direct relationship between the serum concentration of α_2-PAG and the degree of tumour dissemination. Stimson [6] was the first to observe this relationship in breast carcinoma. He reported that α_2-PAG serum levels fell after excision of the primary tumour and rose again with the appearance of metastases. This finding has been confirmed [7] and extended to bronchial carcinoma [8, 9].

In the present study, serum concentrations of α_2-PAG are determined in patients with malignant melanoma and the results were related to the clinical stage of the disease.

MATERIALS AND METHODS

Two hundred and six serum samples were obtained from 97 adult melanoma patients aged from 22 to 80 yr. Control sera collected from 97 healthy blood donors. Pregnant women and females receiving oestrogen medication were excluded from the analysis. All melanoma patients were regularly examined in the policlinic of the Medizinische Hochschule Hannover. Patients were grouped according to the clinical stage of their disease.

Stage I defined patients with primary malignant melanoma of Clark's grades III–V [10] without regional lymph node involvement. Patients in clinical stage II had histologically proven lymph node involvement but presented no clinical, radiological and scintigraphical signs of distant metastasis. Stage III included all patients with a single distant metastasis or disseminated disease.

In stages I and II the therapy consisted of radical surgery either alone or followed by adjuvant therapy with BCG, DTIC or DTIC plus BCG. Stage III patients received DTIC, CCNU or palliative radiation therapy.

Serum samples were coded, stored at −70°C until use and tested as unknowns. α_2-PAG concentrations were determined using the electroimmunoassay according to Laurell [11]. To 15 ml agarose‡ solution (1%) in barbital buffer pH 8, 6, a rabbit anti-α_2-PAG antiserum* was admixed in a final concentration of 1% and diffusion gels were poured on 10 × 10 cm glass plates. Wells of 2.5 mm diameter were punched into the gel and filled with either 5 µl of α_2-PAG standard* solution or with the same volume of undiluted serum. Electrophoresis was carried out at 2 V/cm for 16 hr. Statistical analysis was performed by

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means of the non-parametric statistical test (Wilcoxon).

RESULTS

The high degree of reproducibility of the electro-immunoassay was confirmed by testing repeatedly a coded reference serum. Moreover, serum samples from the same patient taken at different times showed little variation of the α_2-PAG level as long as the clinical stage remained unchanged. Since α_2-PAG values are commonly sex-dependent, results had to be presented for males and females separately. As can be seen from Figs. 1 and 2 the mean normal α_2-PAG serum level amounted to 1.56 ± 1.30 mg/100 ml in females and to 0.58 ± 0.41 mg/100 ml in males. In melanoma patients a clear-cut stage dependent increase of the mean α_2-PAG levels was noticed, being particularly pronounced in females (Figs. 1 and 2). This tendency was also observed in several individual cases in which α_2-PAG serum concentrations were determined in stage II and after progression to stage III (Fig. 3). In male melanoma patients the stage-dependent increase of α_2-PAG was less obvious; only in stage III the values differed significantly from the control group.

Females

1. Controls: stage I $P = <0.01$.
2. Controls: stage II $2P = <0.001$.
3. Controls: stage III $2P = <0.001$.
4. Stage I: stage II N.S.
5. Stage I: stage III $2P = <0.001$.
6. Stage II: stage III $P = <0.03$.

Males

Controls: stage I N.S.
Controls: stage II N.S.
Controls: stage III $P = <0.05$.

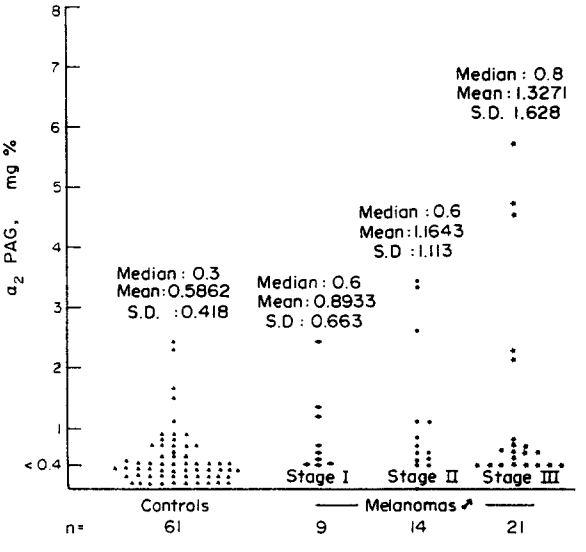

Fig. 2. Serum levels of α_2 PAG according to the clinical stage of male melanoma patients.

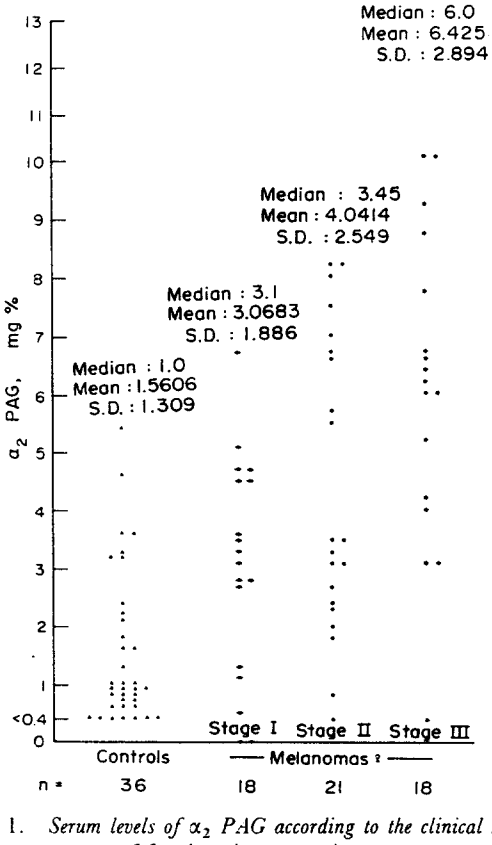

Fig. 1. Serum levels of α_2 PAG according to the clinical stage of female melanoma patients.

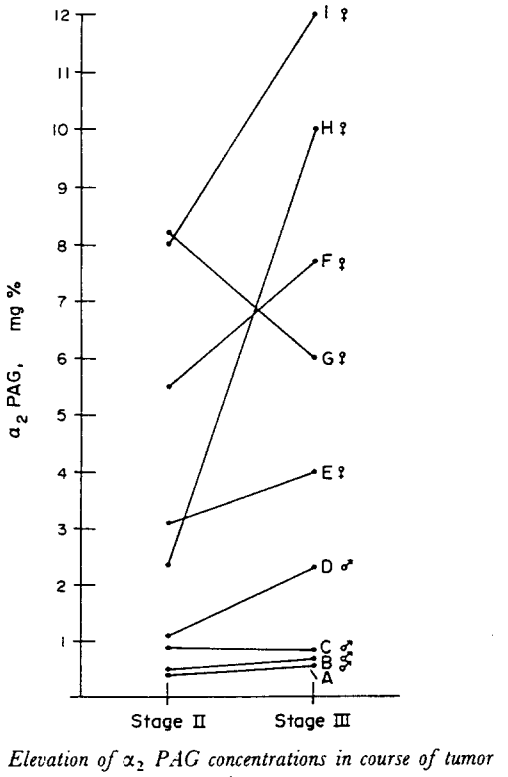

Fig. 3. Elevation of α_2 PAG concentrations in course of tumor progression.

DISCUSSION

α_2-PAG is a high mol. wt (360,000) carbohydrate (12%) containing protein. It is composed of two subunits having a mol. wt of 180,000 which are held together by disulfide bounds. The isoelectric point was found by isoelectric focussing to be 4.7 [12].

The origin, fate and biological role of α_2-PAG are unknown. It has been suggested that it may function as an immunosuppressive factor [13].

These findings and its relationship to the cancer-host and fetus-mother complexes, indicate that it may be one component of the complicated series of reactions involved in suppressions of the immunological rejection of elements of non-self.

On practical grounds the significant elevation of serum α_2-PAG in malignant melanoma and other tumours suggests that determination of this glycoprotein may become a useful screening test for pre-clinical malignancy, provided that oestrogen treatment and pregnancy can be excluded. Moreover, since type of therapy, infections or other co-existing diseases have so far not been found to substantially influence α_2-PAG serum concentrations, the stage dependent rise of this protein in malignant melanoma suggests a quantitative relationship with the tumour load. For the clinical routine a simple, inexpensive laboratory test monitoring the natural course of malignant melanoma as well as its response to therapy would be extremely useful. Among the biochemical methods so far, the detection of melanogens, notably 5-S-cysteinyl-DOPA, in the urine [14] seems to be a sensitive assay in primary melanoma but may not accurately reflect the presence of amelanotic tumours. The measurement of circulating tyrosinase in the serum on the other hand, may be of practical value in detecting occult metastasis [15]. Both methods have, however, the disadvantage of being laborious, expensive and technically difficult. The recently reported increase of the IgG 4 subclass in metastatic melanoma [16] provides similar advantages as α_2-PAG with respect to low costs and simple methodology, but both tests share a histogenic unspecificity. It will be of interest to examine in the future the value of these different tests in a comparative study.

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