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Serum Glycoprotein Hormone Alpha Sub-unit Values and Survival in Metastatic Melanoma Patients

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Abstract—*Serum α subunit of the glycoprotein hormones was measured in 370 samples from 55 patients with metastatic malignant melanoma before, during and after chemo-immunotherapy. Ten of 20 premenopausal, one of 11 postmenopausal and one of 24 male patients had levels above the upper limit of normal before treatment. There was a statistically significant reduction in survival for the 10 premenopausal patients with elevated pretreatment α-levels (median 19 weeks) compared to the 10 premenopausal patients with normal pretreatment α levels (median 83 weeks). Also pretreatment α-levels were significantly lower in the premenopausal patients who responded to chemo-immunotherapy (median 0.5 ng/ml) than those who failed to respond (median 4.9 ng/ml). Maximum α-levels during clinical course, α-levels at regression or progression of disease and α-levels at the end of therapy did not influence patient survival. The presence of pretreatment elevated α-subunit levels could be taken as indirect evidence of a more malignant, unresponsive type of melanoma in a proportion of premenopausal patients.*

INTRODUCTION

THE FOUR glycoprotein hormones, follicle stimulating hormone (FSH), luteinising hormone (LH), thyroid stimulating hormone (TSH) and human chorionic gonadotrophin (HCG) are composed of two dissimilar polypeptide chains—the α and β subunits. The α chains of all four hormones are structurally similar and are immunologically indistinguishable. Elevated α subunit values have been observed in the blood of patients with malignant neoplasms of differing histological types [1–3]. The α subunit is released by malignant cells cultured *in vitro* [4–6] and excessive production has been claimed to be a property of malignant, but not benign, functioning islet cell tumours [5].

Little information is available concerning the clinical significance of α subunit. The production of this subunit was more frequent in prognostically unfavourable (node positive) patients with breast cancer [6, 7] and lack of response to chemotherapy has been associated with subunit release in trophoblastic tumours [8].

This study reports the α subunit levels in the blood of patients with metastatic malignant melanoma (using the α TSH assay) before and during chemo-immunotherapy. This is the first report of an association between the α subunit level, chemotherapy response and survival in patients with malignant disease.

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MATERIALS AND METHODS

Patients

Three-hundred and seventy serum samples from 55 patients with metastatic malignant melanoma were assayed. All patients had histologically proven melanoma and no patient had received previous chemotherapy or immunotherapy. The median ages and ranges

were as follows: 24 male patients, median age 46 yr (range 25–64), 20 premenopausal patients, median age 37 yr (range 21–48), 11 postmenopausal patients, median age 62 yr (range 54–74). Blood was taken immediately before the first injection of treatment pulses. Serum was stored in aliquots at −20°C and all samples from individual patients were measured in the same assay.

The chemo-immunotherapy regime has been described in detail previously [9], briefly DTIC was given i.v. daily for 5 consecutive days and on the first day a single i.v. vincristine injection was also given. Courses were repeated every 28 days. BCG was administered by multiple puncture gun between courses, 10 days from the start of the preceding chemotherapy pulse. Response to therapy was taken as a 50% or greater reduction of tumour size in the product of two diameters taken perpendicular to each other. The response had to last at least 2 months without disease progression elsewhere. Chemotherapy was discontinued on progression of disease.

Controls

Forty-nine premenopausal women, 53 postmenopausal women and 27 men served as controls. These were healthy laboratory personnel and blood donors. The median age and ranges of the controls were: men, median age 40 yr (range 21–65); premenopausal women, median age 31 yr (range 17–52); postmenopausal women, median age 55 yr (range 46–65).

α TSH assay

This was measured by the method of Kourides *et al.* [10], using double antibody radioimmunoassay. The α TSH for iodination and standards and its antibody were a gift from N.I.A.M.D.D., U.S.A. Cross reaction studies on a weight for weight basis at 50% binding in the assay were: TSH 17% (MRC 68/38); LH 19% (MRC 68/40) and FSH 1.7% (MRC 68/104). The sensitivity of the assay was 0.5 ng/ml and standards were made up with an appropriate volume of serum obtained from a T_3 suppressed male control. The interassay coefficient of variation was 12%. The upper limit of normal defined as the 95% confidence limit for α subunit was 2.3 ng/ml in men and premenopausal women and 9.5 ng/ml for postmenopausal women.

RESULTS

Serum α subunit levels, before any chemo-immunotherapy in the 24 male patients, 20 premenopausal patients and 11 postmenopausal patients, were compared with controls and are displayed in Fig. 1. The median pretreatment value for male patients was 0.5 ng/ml (range <0.5–10), for premenopausal patients 2.3 ng/ml (range <0.5–10) and for postmenopausal female patients 3.5 ng/ml (range 0.5–13). The corresponding values for the control groups were in men <0.5 ng/ml (range <0.5–2.4) premenopausal women 0.7 ng/ml (range <0.5–4.2) and postmenopausal women 4.4 ng/ml (range 1.3–10). There was a significant difference between pretreatment α levels in premenopausal patients compared with the premenopausal control group, $P=0.005$ (Mann–Whitney two-tailed U-test).

There were 10 premenopausal patients with levels above the defined upper limit of normal, median 6.8 ng/ml (range 2.5–10), but only one male patient and one postmenopausal female patient had levels above the upper limit of normal, Fig. 1. During therapy one further premenopausal, one postmenopausal and four male patients developed α-levels above the upper limit of normal. Median values after the last course of therapy were 0.5 ng/ml (range <0.5–3.6) in male patients, 0.55 ng/ml (range <0.5–7.5) in premenopausal patients and 3.5 ng/ml (range <0.5–7.8) in postmenopausal patients.

There was no difference in survival between the three groups: male, premenopausal and postmenopausal, $P=0.47$ (log rank, Chi-square test). Keeping the three groups separate, we compared the survival of those patients with normal pretreatment α-levels against those with elevated pretreatment α-levels. There was a significant reduction in survival for those 10 premenopausal women with elevated α-levels, median 19 weeks, compared to the 10 premenopausal women with normal pretreatment α-levels, median survival 83 weeks, $P=0.015$ (Fig. 2). In the postmenopausal patients the median survival of the group with normal pretreatment α-levels was 27 weeks. The one postmenopausal patient with a raised pretreatment α-level survived 20 weeks. In men, the median survival of the patients with normal pretreatment α-levels was 37 weeks but the one male patient with a raised pretreatment α-level survived only 8 weeks. The pretreatment α-value was also significantly different, $P=0.02$ (Mann–

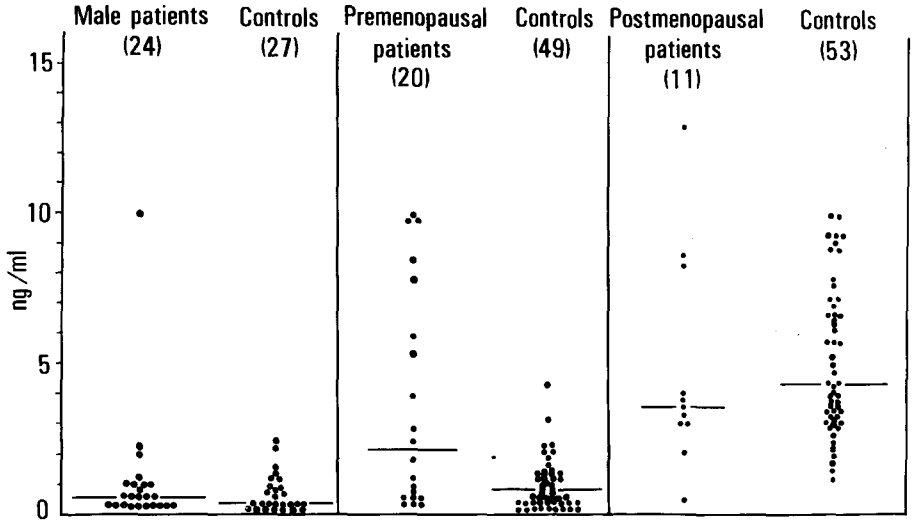

Fig. 1. Melanoma; α subunit pre-treatment with median levels.

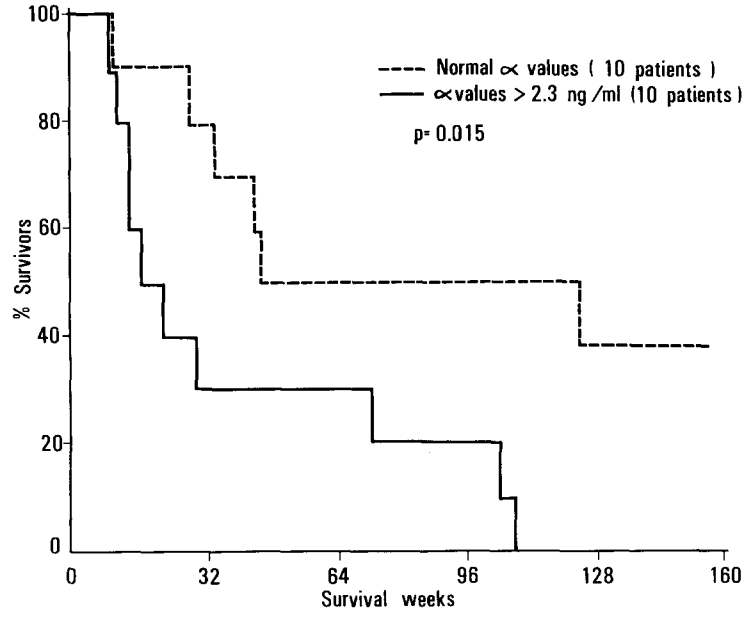

Fig. 2. Survival of premenopausal patients with normal and elevated subunit values.

Whitney two tailed U-test), between those premenopausal patients who responded, median 0.5 ng/ml (range <0.5–5.4) and those premenopausal patients who failed to respond to chemoimmunotherapy, median 4.9 ng/ml (range 0.5–10). The maximum α-level attained during the patients clinical course, the levels at documented regression or progression of the disease and the levels at the end of therapy did not statistically significantly ($P>0.05$) influence the patients survival.

DISCUSSION

This is the first demonstration of an assoc-

iation between serum α subunit levels and survival in patients with metastatic melanoma. There was a statistically significant reduction in survival of premenopausal patients with elevated pretreatment α subunit levels compared to those premenopausal patients with normal α subunit levels. However, there was no significant difference in the survival curves of the premenopausal patient group as a whole compared with the postmenopausal and male groups ($P=0.57$). This finding agrees with a recent report which also found no survival difference between men and women with advanced melanoma [11].

Tumour markers are know to vary with extent of disease and pregnancy-associated globulin has been shown to rise with increasing disease stage in melanoma [12]. The differences in α subunit values observed in the present study were not explicable by variation in stage as all patients had metastatic melanoma and there was no clinical difference in metastatic pattern within the patient groups. The elevations in α subunit levels found in our patients were only relatively small and variable levels are found during the menstrual cycle. Increases up to 5 ng/ml have been found on the day of ovulation in premenopausal women [13]. However, it is unlikely that coincidental ovulatory increases could explain the difference in prognosis of all 20 patients in the premenopausal group. Patient intake was staggered for this study over 18 months.

Therefore, an association between prognosis and α subunit levels within a group of premenopausal melanoma patients exists which is not readily explicable by other factors capable of influencing either the α subunit level or survival. The statistically significant difference was only observed for pretreatment values and other subunit values taken during the clinical course did not influence outcome. The reduction in survival of the elevated subunit group could be taken as indirect evidence of genetic derepression of a more malignant, unresponsive type of melanoma, as has been suggested for insulinoma [5]. Another consideration is the immuno-suppressive effect exerted by the HCG whole molecule [14]. This could reduce an effective host defence mechanism which is occasionally clinically evident in malignant melanoma [15]. However, there is no evidence that α subunit alone exerts an immuno-suppressive effect and the small increases in α subunit found in our patients make this even more unlikely. This work would be strengthened if similar findings were observed in a larger study of premenopausal patients with melanoma. Several blood samples should be taken during the menstrual cycle in view of the transient increases in α subunit during ovulation, a procedure which is now under consideration.

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REFERENCES

1. M. Dosogne-Guérin, A. Stolarczyk and A. Borkowski, Prospective study of the alpha and beta subunits of human chorionic gonadotrophin in the blood of patients with various benign and malignant conditions. *Europ. J. Cancer.* **14,** 525 (1978).
2. E. M. Rutanen, The circulating alpha subunit of human chorionic gonadotrophin in gynaecologic tumours. *Int. J. Cancer*, **22,** 413 (1978).
3. P. Franchimont, U. Gaspard, A. Reuter and G. Heyner, Polymorphism of proteins and polypeptide hormones. *Clin. Endocr.* **1,** 315 (1972).
4. J. M. Lieblich, B. D. Weintraub, S. W. Rosen, N. K. Ghosh and R. P. Cox, Secretion of HCG alpha sububit and HCG by HeLa strains. *Nature (Lond.)* **265,** 746 (1977).
5. C. R. Kahn, S. W. Rosen, B. D. Weintraub, S. S. Fajans and P. Gorden, Ectopic production of chorionic gonadotrophin and its subunits by islet cell tumours. *New Engl. J. Med.* **297,** 565 (1977).
6. A. Howell, S. C. H. Smith and D. H. Cove, Glycoprotein hormone alpha subunit in human breast tumours. In *Biological and Immunological Aspects of Tumour Markers.* (Abstracts of Free Communications) p. 34. Wembley, England (1977).

7. R. A. WALKER, Significance of alpha subunit of HCG demonstrated in breast carcinoma by the immunoperoxidase technique. *J. clin. Path.* **31,** 245 (1978).
8. J. L. VAITUKAITIS and E. R. EBERSOLE, Evidence for altered synthesis of human chorionic gonadotrophin in gestational trophoblastic tumours. *J. clin. Endocr.* **42,** 1048 (1976).
9. N. THATCHER, M. K. PALMER, N. GASSIUNAS and D. CROWTHER, Lymphocyte functions and response to chemo-immunotherapy in patients with metastatic melanoma. *Brit. J. Cancer.* **36,** 751 (1977).
10. I. A. KOURIDES, B. D. WEINTRAUB, M. A. LEVKO and F. MALOOF, Alpha and beta subunits of human thyrotrophin; purification and development of specific radioimmunoassays. *Endocrinology* **94,** 1411 (1974).
11. H. M. SHAW, G. W. MILTON, G. FARAGO and W. H. MCCARTHY, Endocrine influences on survival from malignant melanoma. *Cancer (Philad.)* **42,** 669 (1978).
12. H. W. BAUER, K. E. M. DEUTSCHMANN, H. H. PETER and H. BOHN, Pregnancy associated alpha$_2$ glycoprotein in malignant melanoma. *Europ. J. Cancer.* **15,** 123 (1979).
13. I. A. MACFARLANE, Unpublished observations.
14. S. F. CONTRACTOR and HELEN DAVIES, Effect of human chorionic somatomammotrophin and human chorionic gonadotrophin on phytohaemagglutinin—induced lymphocyte transformation. *Nature New Biol.* **243,** 284 (1973).
15. J. C. EVERSON, Spontaneous regression of cancer. *Ann. N. Y. Acad. Sci.* **114,** 721 (1964).