ORIGINAL ARTICLE

Neuroendocrine alterations in lung cancer patients

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Abstract

BACKGROUND: A number of qualitative and quantitative changes in hormonal secretion pattern have been found in subjects suffering from neoplastic disease. The aim of this study was to evaluate the presence of alterations in neuro-endocrine system function and in the pattern of endocrine secretion in patients with lung cancer.

METHODS: Cortisol, melatonin, growth hormone (GH), insulin-like growth factor I (IGF-I), thyrotropin-releasing hormone (TRH), thyroid-stimulating-hormone (TSH), and free thyroxine (FT_4) serum levels were measured on blood samples collected every four hours for 24 hours from ten healthy old subjects aged 65–79 years (mean age±s.e. 67.28±3.11) and from ten subjects suffering from untreated non small cell lung cancer aged 65–78 years (mean age±s.e. 68.57±1.81). Areas under the curve and mean diurnal and nocturnal levels were compared and the presence of circadian rhythmicity was evaluated.

RESULTS: When hormone levels were expressed as area under the curve GH levels were higher ($p=0.004$) and IGF-I levels were lower ($p=0.006$) in patients with lung cancer than in normal subjects. The evaluation of melatonin/cortisol ratio in all subjects showed a significant difference between the control group and the group of cancer patients ($p<0.05$). When we compared mean diurnal levels (mean of 06.00–10.00–14.00h) GH levels were higher ($p<0.0001$) and IGF I levels were lower ($p<0.0001$) in cancer patients; when we compared mean nocturnal levels (mean of 18.00–22.00–02.00h) cortisol ($p=0.03$), TRH ($p=0.02$), and GH ($p=0.001$) levels were higher in cancer patients, while melatonin ($p=0.04$), TSH ($p=0.04$) and IGF I ($p<0.0001$) levels were higher in control subjects. A clear circadian rhythm was validated for time related changes of cortisol, melatonin, TRH, TSH and GH in control subjects and for time related changes of melatonin in cancer patients.

CONCLUSION: These data suggest that lung cancer patients show alterations of hormone secretion and neuroendocrine system function.

Introduction

Neurotransmitters, hormones and cytokines mediate interactions among the nervous, endocrine and immune system. The function of these systems shows patterns of circadian rhythmicity [1–7]. The hypothalamus-pituitary axis plays a central role in neuro-immune-endocrine system function releasing hormones and neuropeptides with direct modulatory action on the immune effectors or regulating the hormonal secretion of peripheral endocrine glands. Reciprocal influences among hypothalamus, pituitary, thyroid, adrenal, pineal gland and immune system have been evidenced [8–13]. In patients with neoplastic disease a number of alterations have been evidenced in neuro-endocrine and immune system function. Oncologic patients present alterations in cytokine levels and in proportions and nyctohemeral profiles of various lymphocyte subsets and endocrine alterations have been described in cancer patients by many studies [14–22] The purpose of this study was to evaluate the presence of alterations in neuro-endocrine system function and in the pattern of endocrine secretion in patients with lung cancer.

Subjects and methods

The study was approved by the local Scientific and Ethical Committee and the subjects (ten healthy old subjects aged 65–79 years, mean age±s.e. 67.28±3.11, mean body mass index±s.e. 24.40±1.65, and ten subjects suffering from untreated lung cancer aged 65–78 years, mean age±s.e. 68.57±1.81, mean body mass index±s.e.25.95±1.82) were studied in hospital between October and November, submitted to the same social routine, with identical mealtimes and sleep/wake cycle (lights on at 06.30h and lights out at 21.30h, 15:9 L:D). The extent of the tumor was evaluated by clinical examination, bronchoscopy, computed tomography (CT) of the brain, chest, upper abdomen and ultrasonography of the liver; tumor cell type was determined by biopsy (5 non small cell lung cancer I–II stage and 5 non small cell lung cancer III–IV stage). An indwelling catheter, kept patent with a slow infusion of 0.9% NaCl, was inserted in an antecubital vein and blood samples were drawn at 4-hourly intervals for 24 hours starting at 06.00h. During the overnight sampling period a dim blue light (<100 lux) was used. In each blood sample we measured cortisol, melatonin, thyrotropin-releasing hormone (TRH), thyroid-stimulating hormone (TSH), free thyroxine (FT_4), growth hormone (GH) and insulin like growth factor I (IGF I) on serum. To measure serum hormone concentrations blood samples were centrifuged immediately after collection and frozen at –20 °C for later determination. All samples were analyzed in duplicate in a single assay; the intra-assay and inter-assay coefficients of variation were below 10% and 9% respectively for cortisol, 13% and 16% for melatonin, 5% and 6% for TRH, 8% and 7% for TSH, 4% and 6% for FT_4, 5% and 3% for GH, 3% and 8% for IGF I. Standard curves were run with every assay and the experimental values were derived from the curves. We measured cortisol by polarized light immuno-fluorescence assay (Cortisol TDx/TDxFLx,Abbott Laboratories), melatonin by radioimmunoassay (Melatonin Radioimmunoassay Kit, Nichols Institute Diagnostics), TRH by radioimmunoassay ("Frederic Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene, Budapest, Hungary), TSH by immunoenzymatic assay (Enzymun-Test TSH, Boehringer Mannheim Immunodiagnostics), FT_4 by immunoenzymatic assay (Enzymun-Test FT_4, Boehringer Mannheim Immunodiagnostics), GH by immunoenzymometric assay (AIA-PACK HGH, Tosoh, Japan), IGF I by radioisotopic assay (IGF I 100T Kit, Nichols Institute Diagnostics).

Statistical analysis

The results were statistically evaluated by not inferential descriptive biometric analysis (Student's *t-test* and Mann-Whitney rank sum test, as indicated, on mean diurnal and nocturnal levels and on areas under the curve, AUC, calculated according to the trapezoidal method) and by inferential temporal descriptive biometric analysis to find and quantify the parameters (MESOR, amplitude and acrophase) of the circadian rhythm for each studied factor, using the methods named Single Cosinor and Population Mean Cosinor, which entail fitting sine curves to the data from individual subjects and from groups respectively, testing whether the variables of this curves showed consistent pattern of circadian variation. *MESOR* , acronym for Midline Estimating Statistic of Rhythm, defines the rhythm-determined average. *Amplitude* is the measure of one half the extent of rhythmic change in a cycle estimated by the function used to approximate the rhythm. *Acrophase*, measure of timing, is the phase angle of the crest time in the function appropriately approximating a rhythm, in relation to the specified reference timepoint [23,24]. Results were considered statistically significant if $p < 0.05$ in testing the null hypothesis.

Results

Figure 1 shows 24-hours profiles of GH, IGF-I, melatonin, cortisol, TRH, TSH, and FT_4 serum levels in healthy controls and in lung cancer patients. Table 1 shows integrated time-qualified 24-hours values expressed as AUC±s.e. and table 2 shows chronobiological data derived from best fitting sine curves (fitted period: 24 hours = 360°).

When we compared AUC, GH levels were higher ($p=0.004$) and IGF-I levels were lower ($p=0.006$) in lung cancer patients than in normal subjects; there was not a statistically significant difference in cortisol, melatonin, TRH, TSH and FT4 levels between the groups. The evaluation of melatonin/cortisol ratio in all subjects showed significant differences between the control group and the group of cancer patients ($p<0.05$).

When we compared mean diurnal levels (mean of 06.00–10.00–14.00h) GH levels were higher ($p<0.0001$) and IGF I levels were lower ($p<0.0001$) in cancer patients; when we compared mean nocturnal levels

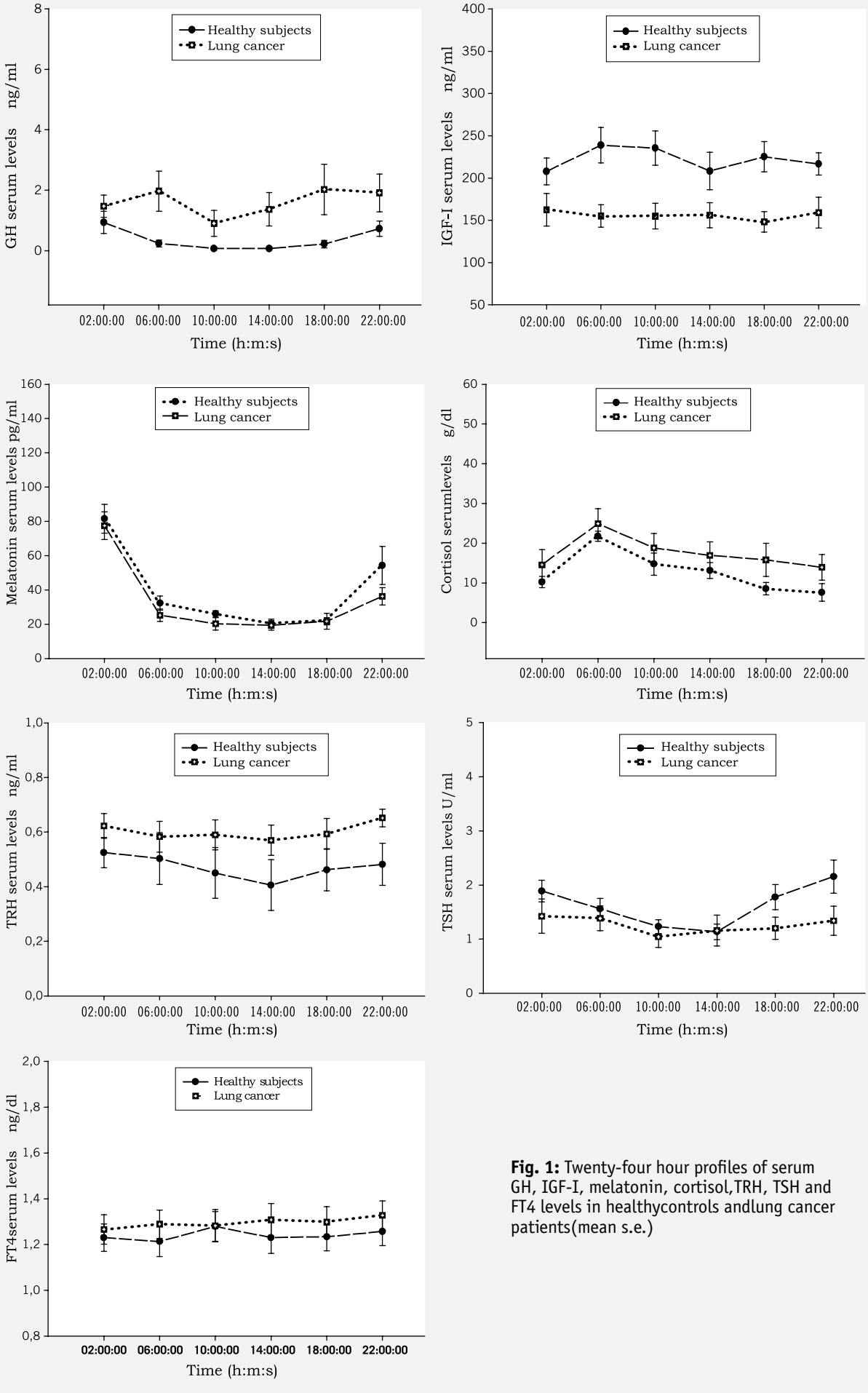

Fig. 1: Twenty-four hour profiles of serum GH, IGF-I, melatonin, cortisol,TRH, TSH and FT4 levels in healthycontrols andlung cancer patients(mean s.e.)

Table 1. Integrated time-qualified 24-hours values expressed as AUC±s.e.

	Healthy subjects	Lung cancer patients
Cortisol	278.68±27.92	383.23±75.73
Melatonin	624.42±36.28	525.48±74.03
TRH	9.25±1.57	11.80±1.15
TSH	28.62±3.12	27.20±7.18
FT_4	24.74±1.26	26.04±1.43
GH	6.23±1.50	32.91±9.73 *
IGF-I	4512.88±366.50	3084.51±267.34 *
Melatonin/cortisol ratio	2.37±0.18	1.62±0.24 *

Units: µg/dl for cortisol, pg/ml for melatonin, ng/ml for TRH, µU/ml for TSH, ng/dl for FT4, ng/ml for GH,
ng/ml for IGF-I; all parameters analyzed in all the subjects; $P<0.05$ *

Table 2. Chronobiological data derived from best fitting sine curves (fitted period: 24 hours = 360°)

		Healthy subjects		
Factor	***p***	**MESOR±s.e.**	**Amplitude±s.e.**	**Acrophase±s.e. (°)**
Cortisol	0.029	12.53±1.41	5.90±2.00	–121.9±19.4
Melatonin	0.011	38.81±5.60	26.29±7.92	–18.8±17.3
TRH	0.021	0.46±0.01	0.04±0.01	–41.9±17.4
TSH	0.018	1.39±0.05	0.46±0.07	–347.8±9.0
FT4	0.898	1.24±0.01	0.01±0.02	–238.7±121.0
GH	0.041	0.37±0.07	0.45±0.09	–5.3±12.1
IGF–I	0.585	222.00±5.88	9.44±8.32	–126.2±50.5
		Lung cancer patients		
Factor	**P**	**MESOR±s.e.**	**Amplitude±s.e.**	**Acrophase±s.e. (°)**
Cortisol	0.142	17.93±1.09	4.19±1.46	–134.9±22.9
Melatonin	0.039	33.19±7.48	23.17±10.58	–22.2±26.2
TRH	0.243	0.59±0.01	0.03±0.01	–353.4±26.4
TSH	0.327	1.34±0.07	0.17±0.10	–1.1±31.5
FT4	0.375	1.30±0.01	0.01±0.01	–280.9±34.4
GH	0.445	1.59±0.18	0.37±0.25	–332.9±39.1
IGF–I	0.425	155.93±1.90	4.07±2.68	–40.8±37.8

(mean of 18.00–22.00–02.00h) cortisol ($p=0.03$), TRH ($p=0.02$), and GH ($p=0.001$) levels were higher in cancer patients, while melatonin ($p=0.04$), TSH ($p=0.04$) and IGF I ($p<0.0001$) levels were higher in control subjects.

A clear circadian rhythm was validated for time related changes of cortisol, melatonin, TRH, TSH and GH in control subjects and for time related changes of melatonin in cancer patients.

Discussion

Many physiological, biological and immunological factors influence the appearance and the development of neoplastic disease. The nervous, endocrine and immune systems might act as an integrated unit to maintain body defense against this pathological process and reciprocal influences have been evidenced among hypothalamus, pituitary, thyroid, adrenal, pineal gland and immune system [25–29]. Cortisol has a well recognized influence on immune function, inducing significant immunosuppression, characterized by the reduced cellular and humoral response of monocytes and B and T lymphocytes [30]. Melatonin, hormone secreted by the pineal gland, is able to influence the secretion of many endocrine glands, modulates the function of the immune system and its production is under the control of the nervous system. It directly stimulates activated helper T lymphocytes and plays an immunomodulatory role by opioid peptides and by a thyroid-independent influence on the thymic function mediated by TRH and TSH. A possible involvement of melatonin in the control of carcinogenesis has been evidenced by studies on neoplastic cells in vitro and by clinical trials with melatonin as second line therapy for neoplastic diseases [31–41] An immunostimulatory role of growth hormone and insulin-like growth factor I has been highlighted by recent studies: GH and IGF-I have an important role in stimulating lymphocyte production and function and IGF-I assists the maturation of lymphocytes in bone marrow and their function in the periphery [42]. Neoplastic diseases are characterized by an altered pattern of hormonal secretion, with changes that may be qualitative (loss of circadian rhythmicity) and/or quantitative (increase or decrease) as variously described by many scientific studies. [43–48]

Hormone serum levels found in our healthy volunteers are comparable to values obtained by precedent studies [49–52]

The data obtained in the present study indicate that hormonal secretion is altered in patients suffering from lung cancer, with loss of circadian rhythmicity of GH, cortisol, TRH and TSH serum level changes. GH serum levels are significantly higher and IGF-1 serum levels are significantly lower in our cancer patients during a 24-hour period and diurnal hours; mean cortisol, GH and TRH nocturnal levels are higher and mean melatonin, TSH and IGF I nocturnal levels are lower in cancer patients as compared with those of control subjects. These alterations may play an important role in the natural history of neoplastic disease: IGF-1 is one of the most important growth factors for normal cell proliferation, several tumor cell lines have recently appeared to be also stimulated by this mitogen and an autocrine or paracrine GH/IGF-1 system have been evidenced in lymphoid tissues, capable of influencing lymphopoiesis and immune function [53–59]. Studies conducted on GH-deficient patients have demonstrated that different time treatment schedules of GH administration have different effects on IGF-1 serum levels and the closest similarity to normal hormone and metabolite patterns and relationships is reached by GH injection in the evening, so that the altered time structure of GH secretion evidenced in our cancer patients may impair the regular biosynthesis of IGF-1 in liver and in other

organs and tissues [60–65]. Besides the changed time structure and serum levels evidenced for hypothalamic (TRH), pituitary (TSH) and adrenal (cortisol) secretion may be produced by and may increase the altered interaction among nervous, endocrine and immune systems. Melatonin plays a role of immunomodulation mediated by TRH, TSH and opiatergic ways and stimulates activated helper T lymphocytes to produce opioid agonists and cytokines (IL-2 and IL-4); the pineal hormone directly inhibits normal or neoplastic cellular proliferation, has a powerful anti-oxidant action and influences the secretion of many endocrine glands, in particular hypothalamus-hypophysis-adrenal axis function. The immune system is partially regulated by glucocorticosteroids, that induce significant immunosuppression, with less secretion of immune response mediators and decreased availability of specific cell types such as natural killer cells. In vitro and in vivo studies have evidenced that endogenously produced TSH can regulate immune response and that lymphocyte production of TSH may fall under the positive regulatory influence of hypothalamic TRH. [66–69] The loss of normal rhythmic pattern of GH, cortisol, TRH and TSH secretion evidenced in our cancer patients may cause disregulation of hormone actions at cellular and systemic level, altering the equilibrium of factors shared by both the immune and neuroendocrine systems and used for intra- and inter-system communication. As evidenced by our data the melatonin/cortisol ratio is decreased in a significant way in cancer patients and may represent a useful index of altered function of regulatory mechanisms of neuro-endocrine secretion in the presence of neoplastic disease.

In conclusion, patients suffering from lung cancer exhibit altered patterns of hormonal secretion and neuroendocrine system function that may be important for the progression of neoplastic disease and that must be considered in devising and evaluating chronotherapeutic regimens of anticancer treatments.

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