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ORIGINAL ARTICLE

Serum retinoic acid, retinol and retinyl palmitate levels in patients with lung cancer

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Objectives: Epidemiological studies have shown an inverse relationship between dietary vitamin A intake and the risk of developing lung cancer. The aim of this study was to investigate the vitamin A status in patients with lung cancer, by determining the serum levels of retinoic acid, retinol and retinyl palmitate.

Methods: In total, 36 patients with lung cancer and 27 controls were assessed. Of the patients 14 had squamous cell carcinoma, 3 adenocarcinoma, 15 non-small cell lung cancer and 4 small cell lung cancer. Serum retinoic acid, retinol and retinyl palmitate levels were determined with HPLC and UV detection, after liquid extraction.

Results: Serum retinol levels did not differ between patients (733.5 ± 326.4 ng/mL) and controls (734.5 ± 337.1 ng/mL). The retinyl palmitate concentration tended to be lower in patients (14.3 ± 9.7 ng/mL) than in controls (16.7 ± 13.7 ng/mL) The serum retinoic acid levels were significantly lower in patients (1.9 ± 0.6 ng/mL) than in controls (2.5 ± 1.1 ng/mL, $P < 0.05$). A positive correlation was observed between the retinol and retinoic acid levels and retinyl palmitate and retinoic acid levels.

Conclusions: The lower levels of retinoic acid in patients with lung cancer suggest there may be a deficiency or impairment in retinol metabolism in these patients. Further studies with larger numbers of patients are needed to evaluate the possible relationship between serum retinoid levels and lung cancer.

Key words: chemoprevention, lung cancer, retinoic acid, retinoid, vitamin A.

INTRODUCTION

Vitamin A is an essential micronutrient and is involved in a large number of physiological functions including vision, reproduction, cell growth, differentiation, apoptosis and immune modulation. The term vitamin A refers to the retinoids, a family of fat soluble compounds that have vitamin A activity. Carotenoids are pigments that can be converted to vitamin A. Retinol is the predominant form in the circulation whereas retinyl palmitate is considered to be the storage form of vitamin A. Retinoic acid is the active form of vitamin A. The actions of the retinoids are mediated by the retinoid receptors, which are members of the steroid hormone receptor family. Retinoic acid binds to retinoid receptors and promotes expression of specific genes.[1,2]

Numerous investigations have shown that there is a strong relationship between vitamin A and cancer development.[2–5] This connection was made soon after the discovery of the vitamin and its chemical structure. Wolbach and Howe in their pioneering work in 1925 found that vitamin A deprivation of cattle led to increased incidence of lung and upper aerodigestive tract cancers.[6] Vitamin A deficiency in experimental

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animals has been associated with higher incidence of cancer and increased susceptibility to carcinogens. Several *in vitro* and animal studies have shown that the retinoids can prevent or delay tumour development.[7]

Epidemiological studies have shown an inverse relationship between dietary vitamin A intake and risk of development of various forms of cancer including lung cancer.[2–5] Epidemiological data connecting lung cancer with vitamin A have been reported as early as 1975.[8] The majority of epidemiological data support the finding that increased vitamin A intake, especially in the form of carotenoids, is associated with decreased risk of lung cancer.[9] There is some controversy in reports about retinol intake and retinol levels and the risk of cancer. Increased preformed retinol intake, has been associated with increased risk for lung cancer in a study in southwest England.[10] Decreased levels of retinol have been associated with higher risk of lung cancer in a study with Asian population.[11]

Certain retinoids may act as inhibitors of carcinogenesis in certain tissues and carcinogenesis models but retinoids may also act as cancer enhancers in different conditions.[2] Although epidemiological studies supported the relationship between vitamin A intake and reduced cancer risk, large intervention studies with vitamin A supplementation to high risk groups have failed to produce any benefit. In some studies, an increased rate of cancer and mortality was associated with vitamin A supplementation in certain groups of people such as heavy smokers.[12–14]

Previous studies have shown that retinol levels are normal in lung cancer patients whereas there is little data concerning retinyl palmitate and retinoic acid levels in such patients.[15–17] Because serum retinol levels are under homeostatic control, possible deficiencies are not acutely reflected in retinol levels. The simultaneous determination of serum retinoic acid, retinol and retinyl palmitate offers a better estimation of vitamin A status.[18] The aim of the present study was to investigate possible deficiencies or impairment of vitamin A metabolism in patients with lung cancer, by assessing the vitamin A status with the determination of the serum levels of retinoic acid, retinol and retinyl palmitate.

METHODS

Patients and controls

The study was conducted in the Pneumonology Department of the University Hospital of Thessaly, in cooperation with the Technological Education Institute of Larissa, Greece. The local ethics committee approved the study and written informed consent was obtained from each participant.

Serum samples were obtained from 36 patients (35 men and 1 woman, age 66.2 ± 12.3 years, mean ± SD) who were admitted to the pneumonology clinic and were diagnosed to have lung cancer. Only patients with a primary diagnosis of untreated lung cancer were included in the study. Patients with an abnormal CXR underwent CT. If the CT scan did not exclude malignancy, patients underwent endoscopic examination of the tracheobronchial tree with a flexible brochofibrescope. Bronchial brushings and biopsies were examined. Transthoracic needle aspiration of the lung was performed on some patients, under CT guidance, and the aspirate was examined by a cytopathologist. Accurate staging was completed using other imaging techniques including ultrasound and abdominal CT scan, bone scan and CT/MRI of the brain. Staging was based on the tumour nodes metastasis (TNM) system as it was revised in 1997 in order to address the heterogeneity of end results within groups and the lack of specificity in stage classification and was accepted by the AJCC (American Joint Committee on Cancer) and the UICC (International Union Against Cancer).

The histological classification of the lung cancer patients was as follows: 14 squamous cell carcinoma, 3 adenocarcinoma, 15 were characterized as non-small cell lung cancer and 4 as small cell lung cancer. All patients were in good clinical condition (performance status 0–1). Patients with histological types of lung cancer other than small cell cancer were classified as following: one in stage IA, three in stage IB, three in stage IIA and five in stage IIB. Furthermore, five patients were classified as in stage IIIA, eight in stage IIIB and finally seven patients were considered to have stage IV disease. Two of the four patients with small cell lung cancer were considered to suffer from limited disease whereas the other two patients were considered to suffer from extensive disease.

All the patients completed a questionnaire about their demographic data, lifestyle, dietary and smoking habits. The mean cigarette consumption in the cancer patients was 58.7 pack-years (range 0–120) and there were only three non-smokers. The mean alcohol consumption per day in the cancer patients was 3.6 units (range 0–10). One unit is defined as the equivalent of 7.9 g of alcohol.

Serum samples were obtained from 27 people (21 men, 6 women, age 44.5 ± 16.7 years) who were admitted to the orthopaedic clinic for various reasons related to benign diseases and served as a control group.

There were no differences in the dietary habits of patients and controls. The diet of both groups was based on a traditional Mediterranean diet with influences from western dietary habits. They consumed meat two to three times a week and fish usually once a week. The main source of dietary lipids was olive oil included in cooked food and in salads with vegetables and legumes. They consumed fruit on a daily basis. None was receiving dietary supplements and they were not on any special diet in the months before entering the study.

HPLC determination of retinol and retinyl palmitate

Serum retinol and retinyl palmitate levels were determined by HPLC, as described previously.[18] The method was evaluated for use with human serum and

the results obtained were well within the reported values.

In brief, 0.3 mL of serum sample was mixed with 0.9 mL of an extraction solvent mixture consisting of 2-propanol and dichloromethane (2:1, v/v) in a 5-mL centrifuge tube. The content of the tube was vortexed for 30 s and centrifuged at 3000 *g* for 5 min. Following centrifugation, the supernatant solution was transferred into another tube, evaporated to near dryness under a gentle nitrogen stream, carefully dissolved in 100 µL of a solvent mixture of tetrahydrofuran and methanol (15:85, v/v), made up to 300 µL with methanol, and an aliquot of 100 µL was injected into the liquid chromatography system.

Liquid chromatography was carried out on a Hewlett–Packard system (HP1100, Agilent Technologies, Palo Alto, CA, USA) consisting of a G1311A quaternary pump, a G1322A vacuum degasser, a Model 7725, injection valve (Rheodyne, Cotati, CA, USA) equipped with 100 µL loop and a G1314A variable wavelength detector.

Liquid chromatography analysis was performed at ambient temperature on a reverse phase column (MZ, Mainz, Germany), 250 × 4.0 mm, packed with Hypersil MOS, C_8, 5 µm.

The solvents used for the mobile phase were methanol containing 0.1 M sodium acetate and 0.01 M acetic acid (solvent A), water containing 0.1 M sodium acetate and 0.01 M acetic acid (solvent B), and tetrahydrofuran (solvent C). At the beginning of each run, the composition of the mobile phase was set at 87% solvent A, 12.9% solvent B and 0.1% solvent C, and the elution was maintained isocratic for 9 min. A short linear gradient from the initial mobile phase composition to 94% solvent A, 1% solvent B and 5% solvent C, was applied between 9 and 9.5 min, followed by isocratic elution for an additional 10 min. The flow rate of the mobile phase was set at 1 mL/min. The detection was made by UV absorbance at 350 nm for retinoic acid and at 325 nm for retinol and retinyl palmitate.

Blood collection

Blood was obtained by venepuncture using vacuum tubes. The blood collection tubes were covered with aluminum foil for protection from light and stored at 4°C. Within 1 h after venepuncture, serum was obtained by centrifugation (10 min at 3000 *g* and 4°C). Subsequently, serum was collected in cryovials covered with aluminum foil and stored in the dark at –70°C, until analysed.

Statistical analysis

Statistical analysis was performed using the SPSS statistical package (SPSS, Woking, Surrey, UK). Data were expressed as means ± SD. A *P*-value less than 0.05 was considered as the limit of significance and all *P*-values were two-sided. The Student's *t*-test was used for comparison of the means between patients and controls after testing for normality with Lilliefors test. Simple regression was used for the calculation of correlation coefficients.

RESULTS

Serum retinol levels did not differ significantly between patients (733.5 ± 326.4 ng/mL) and controls (734.5 ± 337.1 ng/mL) (Fig. 1). The retinyl palmitate concentration in subjects tended to be lower in patients (14.3 ± 9.7 ng/mL) than in controls (16.7 ± 13.7 ng/mL) (Fig. 2). Serum retinoic acid levels were significantly lower in patients (1.9 ± 0.6 ng/mL) than in controls (2.5 ± 1.1 ng/mL) ($P < 0.05$, Student's *t*-test) (Fig. 3). Comparison between small cell cancer patients and the rest of the patients or between small cell cancer patients and controls showed no difference. A positive correlation was observed between the retinol and retinoic acid levels in patients ($r = 0.46$, $P < 0.01$) and controls ($r = 0.45$, $P < 0.05$). Positive correlation was also observed between retinyl palmitate and retinoic acid levels in patients ($r = 0.41$, $P < 0.05$) and in control subjects ($r = 0.48$, $P < 0.05$). Retinol and retinyl palmitate levels were positively correlated in patients ($r = 0.41$, $P < 0.05$) but not in controls ($r = 0.462$, $P = 0.057$). No correlation was observed between retinoid levels and pack-years in patients. Moreover, no correlation was observed between retinoid levels and age in patients and controls.

DISCUSSION

Serum levels of retinoic acid, retinol and retinyl palmitate were determined in patients with lung cancer.

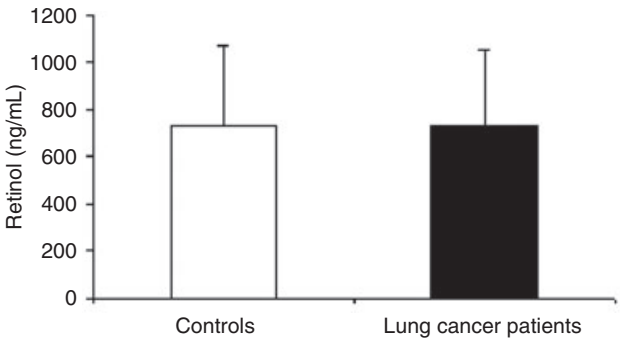

Figure 1 Serum retinol levels (ng/mL) in controls and patients with lung cancer.

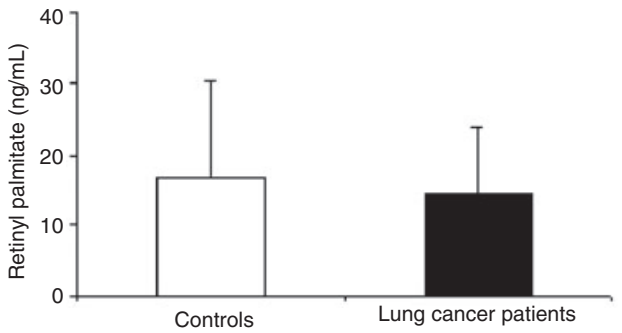

Figure 2 Serum retinyl palmitate levels (ng/mL) in controls and patients with lung cancer.

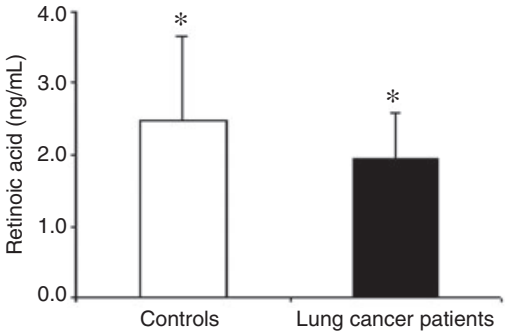

Figure 3 Serum retinoic acid levels (ng/mL) in controls and patients with lung cancer (*$P < 0.05$, comparison between patients and controls, Student's *t*-test, after Lilliefors test).

The serum retinoic acid levels were observed to be lower in patients than in controls, whereas retinol and retinyl palmitate levels were not different. The retinoid levels did not correlate with age.

Several epidemiological studies have suggested a possible relationship between vitamin A intake, especially in the form of carotenoids, and the risk of lung cancer.[2–5,7] Retinoids have been found to suppress carcinogenesis in several *in vitro*[19] and animal models.[7] Some clinical trials have shown beneficial effects of vitamin A treatment. For example, when large doses of vitamin A were given to patients with stage I non-small cell lung cancer after curative surgery, a statistically significant difference in favour of this treatment was observed with regard to the development of new primary tumours.[20]

Large-scale intervention studies have failed to establish any beneficial effect of vitamin A supplementation in reducing the development of cancer or in improving the survival of cancer patients.[12–14] The CARET intervention study tested the daily administration of a combination of 30 mg beta-carotene and 25 000 IU of retinyl palmitate against placebo. The participants were 18 314 men and women aged 50–69 years, all smokers and at a high risk of developing cancer. The study was stopped 21 months early, as there were 28% more lung cancers and 17% more deaths in the group receiving the vitamin A supplementation compared with the placebo group.[13,17] In the EUROSCAN study, 2-year supplementation with 300 000 IU of retinyl palmitate administered orally, had no effect on the survival, event-free survival or second primary tumours for patients with lung cancer or head and neck cancer.[14] In a prospective study of diet and cancer, conducted in China, serum retinol levels showed a threshold effect on lung cancer risk.[11]

A significant positive association between risk of lung cancer and preformed vitamin A was reported in a study with 1000 cases and 1500 controls in England.[10] In the same study, moderate but highly statistically significant negative associations between carotenoid-rich foods and lung cancer risk were also reported.

Although there is experimental evidence about a relationship between vitamin A status and cancer induction, the epidemiological findings do not always support these findings and not all compounds with vitamin A activity are equally potent in inhibiting cancer induction at different organ sites.[21] Nagata *et al.* found that the rate of progression of the cancer *in situ* or invasive cervical cancer was 4.5-fold higher in women with lower serum retinol levels than in those with higher serum retinol levels.[22] Kohlhaufl *et al.* in a pilot study found a 56% remission rate of metaplasia and dysplasia of respiratory epithelia after a 3-month treatment with retinyl palmitate in the form of an aerosol.[23]

In a placebo controlled study on patients with dysplasia and/or metaplasia, Lee *et al.* concluded that isotretinoin, a vitamin A analogue, had no effect on squamous metaplasia, a potential intermediate endpoint of bronchial carcinogenesis.[24]

The data of the present study show that there is no difference in the serum retinol levels between patients with lung cancer and controls. This finding is in accordance with previous reports.[15,16] Serum retinol levels are under homeostatic control, most probably mediated by the concentration of retinol binding protein.[25] Retinyl palmitate is the storage form of retinol. Retinol bound to retinol binding protein is released to the bloodstream from liver stores of retinyl esters.[26] Redlich *et al.* measured the α-tocopherol, retinol, beta carotene and total carotenoids levels in serum and in lung biopsies in patients with lung diseases. Lung tissue levels of retinol and α-tocopherol were found to be lower in lung cancer patients, suggesting that lung tissue levels of retinol may not be adequate in some patients. The serum and lung tissue levels of retinol did not correlate.[16] Normal serum levels of retinol A therefore do not necessarily reflect normal lung tissue levels.[25,26] No correlation was observed between age and retinoid levels. This observation is in accordance with previous studies including one based on a Greek population where retinol levels were not found to change significantly with age.[27,28]

Very few reports exist about retinyl palmitate levels in lung cancer patients for comparison. Lower geometric mean serum concentrations of retinyl palmitate in lung cancer patients have been reported by Goodman *et al.*[17] According to the data in the present study the retinyl palmitate levels tended to be lower in lung cancer patients than in control subjects, but this difference did not reach statistical significance. This could indicate that the vitamin A reserve in these patients is low.

There are no data in the literature concerning retinoic acid levels in patients with lung cancer. In the present study, the retinoic acid levels in patients were lower than in controls. The levels of retinoic acid, however, correlated with the corresponding levels of retinol and retinyl palmitate in patients and controls.

Retinoic acid is the active form of vitamin A. It acts through specific receptors, the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs), and induces or inhibits gene expression.[2] Retinol itself does not bind to the retinoid receptors. It has to be converted to retinoic acid in order to exert most of the biological action of vitamin A. Retinoic acid in the circulation is derived mainly from the conversion of a

small percentage of the dietary vitamin A in the intestine. It is not clear if other tissues contribute to the circulating levels of retinoic acid. In the human circulation both the all-*trans*- and 13-*cis*-retinoic acid isomers are normally present.[25] There is an established connection between the expression of specific retinoid receptors and lung cancer.[2] Decreased expression of all RAR and RXR receptor subtypes is a frequent event in tumours from non-small cell lung cancer patients.[29]

The lower levels of retinoic acid indicate that there may be a deficiency or impairment of the retinol metabolism in patients with lung cancer. It is not known if this phenomenon contributes to the pathogenesis or evolvement of lung cancer. The subcellular levels of retinoids including retinoic acid isomers could provide more information on this issue. Further studies with a larger number of patients as well as studies of the molecular mechanism of action of retinoids are needed to explain the possible relationship between serum retinoid levels and lung cancer.

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