Brief communication - Experimental

Elevated anti-cholesterol antibody levels in the sera of non-small cell lung cancer patients

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Abstract

There have been no published data yet on the serum level of antibodies against cholesterol (anti-cholesterol antibodies) in oncological patients. We decided to examine these levels in the sera of non-small cell lung cancer (NSCLC) patients. Measurements were performed by ELISA technique in the sera of 44 NSCLC patients and the results were compared to the anti-cholesterol antibody levels of 34 non-tumorous control subjects. Serum anti-cholesterol antibody levels were found to be significantly higher in NSCLC patients than in non-tumorous controls (40.35 arbitrary units/ml (AU/ml) versus 26.00 AU/ml, P=0.0003). The elevated anti-cholesterol antibody values were observable at different percentile values as well (25 percentile: 27.01 AU/ml in NSCLC patients, versus 17.33 AU/ml in controls; 75 percentile: 60.90 AU/ml in NSCLC patients versus 32.90 AU/ml in controls). These results suggest that anti-cholesterol antibodies might be applicable for the serodiagnosis of NSCLC. We emphasize the need for the collection of more data on anti-cholesterol antibody levels in NSCLC patients and in patients with different other malignant tumours in order to investigate the possible benefit anti-cholesterol antibodies might offer in clinical work.

Keywords: Anti-cholesterol antibodies; Non-small cell lung cancer; Serum tumour markers

1. Introduction

After in-vitro observations it was shown in animal experiments that both free- and bound serum cholesterol, which otherwise show very weak immunogenicity, in certain situations can be extremely immunogenic and can induce the production of anti-cholesterol antibodies (ACHAs) [1–3]. Anti-cholesterol antibodies found in the sera of the normal human population were hypothesized to originate when cholesterol meets with natural occurring bacterial endotoxins [4]. Serum ACHA levels, different from the ones of the normal population, were found in atherosclerotic diseases. No explanation was given for the significantly elevated serum ACHA levels in coronarosclerotic- and decreased serum ACHA levels in stroke- and peripheral arteriosclerotic patients [5]. Serum values of serum levels of anti-cholesterol antibodies in HIV and hepatitis C virus (HCV) patients [6,7] were explained by the hypothesis that when viruses take on parts of the host cells’ membrane during budding, cholesterol might be effectively exposed to the immune system [8]. Systemic lupus erythematosus and Trypanosoma cruzii protozoonosis (Chagas disease) were found to be associated with elevated serum ACHA levels too [9,10].

There have been no published data on the serum level of ACHAs in oncological patients up to now. In our paper we compare the serum level of anti-cholesterol antibodies of lung cancer patients to the serum level of ACHAs of control individuals without tumours.

2. Patients and method

We examined 44 non-small cell lung cancer (NSCLC) patients (21 men and 23 women), treated in our Thoracic Surgical Department between January and May 1999. The patients had Stage I–IV (mostly stage I–III) NSCLC. All sera were taken preoperatively. The control group consisted of 34 other volunteers, matched to the NSCLC patients in age and sex, smoking habits etc. No control or patient was accepted with a concurrent or previous history of malignancy. Informed consent was obtained from all subjects and the study was approved by the Ethics Committee of Bajcsy-Zsilinszky Hospital. The most important data of the patients and controls are listed in Table 1.

When selecting individuals for the control group, conditions that were previously reported in the literature as associated with elevated serum levels of ACHAs (coronarosclerosis, HIV-, HCV infection, Chagas disease, or SLE) [5–7,9,10] were not less frequent in the past or present medical history of controls, than in that of the studied NSCLC patients. Similarly, during selection we ensured that conditions, known to be associated with decreased ACHA serum levels, such as cerebrovascular, or peripheral arterial atherosclerosis [5], were not more frequent in the control group than in the study group.
The level of cholesterol-specific antibodies in the sera of patients and controls was measured by a solid phase enzyme immunoassay (ELISA). Polystyrene plates (Greiner, Frickenhausen, Germany) were coated with 5 μg/well cholesterol dissolved in 100 μl absolute ethanol, and incubated at +4 °C for 24 h. After being washed with phosphate buffered saline (PBS) and blocked with 0.1% casein (Reanal, Budapest, Hungary) in PBS, the wells were incubated with 100 μl of serum samples diluted to 1:800 in PBS, containing 0.1% casein. Binding of anti-cholesterol antibodies was detected by anti-human horseradish peroxidase conjugated gamma-chain specific rabbit antibodies (DAKO, Glostrup, Denmark); and with o-phenylene-diamine (Sigma, St Louis, MO) and H₂O₂ as substrate. The optical density was measured at 492 nm (reference at 620) and the mean value of duplicates was calculated. Serial dilution of a control serum was used as the standard in all experiments. Data obtained as optical density values were expressed in arbitrary units per millilitre (AU/ml) related to the standard curve. The coefficient of variation of the method was 18.7% (95% CI: 14.5–23.0%).

3. Results

We found that the level of ACHAs in the sera of NSCLC patients was significantly higher than in the sera of the control patients (median: 40.35 AU/ml versus 26.00 AU/ml). The elevated ACHA values were observable at different percentile values as well (25 percentile: 27.01 AU/ml in NSCLC patients, versus 17.33 AU/ml in controls; 75 percentile: 60.90 AU/ml in NSCLC patients versus 32.90 AU/ml in controls). We used the Mann–Whitney significance analysis test, P-value was 0.0003 (Fig. 1). We defined elevated ACHA levels, when they were higher than the median + 2 S.D. of the healthy controls’ ACHA titer (54.9 AU/ml). Elevated ACHA levels were measured in 13/31 (29.5%) of the patients and 2/32 (5.8%) of those in the control group (Fisher’s exact test P = 0.0095). In addition, we found that ACHA levels were lowest in Stage II and highest in Stage IV NSCLC, though there were only 3 patients in this latter group. There were non-significant differences in ACHA levels between the histological subtypes. Though serum cholesterol levels were only available in 14 NSCLC patients, we found a negative correlation between the serum level of ACHAs and cholesterol (Spearman rank correlation test, r = −0.406).

4. Discussion

We found serum levels of ACHAs significantly elevated in non-small cell lung cancer patients. We are aware that, for stronger results, more patients and controls should have been included. The progress of lung cancer patients should have been followed to see if increasing serum ACHA levels are indicators of recurrence. Other types of malignancies (e.g. colorectal) could also have been examined and the study groups should have been more homogenised. We think, however, that the significance of the elevation of ACHA levels we found in lung cancer patients made it worthwhile to publish a preliminary report. We are planning a new study with the abovementioned criteria (together with ACHA-immunostaining of the pathologic specimens), in order to investigate if the level of ACHAs as an easily measurable marker should be considered for future use in the serodiagnosis of non-small cell lung cancer, or other tumours.

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References