VITAMIN A LEVELS IN LUNG CANCER PATIENTS A CASE-CONTROL STUDY

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SYNOPSIS

A case-control study was made for vitamin A levels in lung cancer patients. Although there was a highly significant association with cigarette smoking, no significant difference in the levels of serum vitamin A was observed between the cases and controls. The vitamin A levels were found to vary over a wide range in both groups having values from around 20 μg per dl to 120 μg per dl.

INTRODUCTION

Besides forming part of the light-sensitive retinal proteins, vitamin A (retinol) compounds are known to play a major role in maintaining the healthy states of epithelia throughout the body. Deficiency states are associated with metaplasia and hyperkeratosis of the epithelia. Thus, when various workers suggested the possible inhibitory effects of vitamin A on epithelial carcinogenesis, it was not at all unexpected. Such effects have in fact been demonstrated in laboratory animals (1,2).

But while vitamin A has been shown to prevent neoplastic change in intact animals and in cultured cells, does it have any such activity in humans? Some epidemiological studies based on dietary histories seem to suggest a possible relationship between lowered levels of the vitamin and the onset of lung cancer (3,4,5).

MacLennan et al (5), in a study of lung cancer in Chinese females in Singapore, made the incidental finding of a significant increase in risk for women with low consumption of selected green leafy vegetables. The index of consumption used was an arbitary one, and there was no information on other sources of vitamin A. Nevertheless, the suggestion of a possible negative relationship between vitamin A and lung cancer is worth exploring.

A case-control study was designed to look at serum vitamin A levels. This approach will provide some clues within a short period, although it is generally recognised that taking patients alive at any one point in time may lead to identification of factors associated with the course of illness rather than the risk of developing the disease. Such a drawback can be minimised by restricting cases to newly diagnosed patients.

The objective of the study was to compare serum vitamin A levels between pairs of newly-diagnosed lung cancer cases and matched controls. Smoking histories were also obtained in both groups, to allow for stratified analysis according to presence and absence of smoking.

MATERIALS AND METHODS

All the cases and controls were taken from one hospital (Tan Tock Seng) in Singapore. Cases selected had to be newly diagnosed, with subsequent histological confirmation. The controls were matched for age (± 5 years), sex and ethnic group. These were patients who had diseases other than lung cancer and those conditions which were likely to have an effect on vitamin A levels (e.g. gastrointestinal and liver conditions).

All the subjects were interviewed by one of the authors, covering mainly the smoking histories. They were also weighed.

Chemicals and reagents

Trifluoroacetic acid, β-carotene and retinol acetate were purchased from Sigma Chemical Co. The chloroform, acetic anhydride, 95% ethanol and petroleum ether (b.p. 30°-40°C) were obtained from E. Merck, W. Germany. The TFA (trifluoroacetic acid) reagent was prepared by mixing TFA:chloroform in 1:2 (v/v).

Serum Vitamin A determination

The procedure used in the estimation of serum vitamin A levels was according to Neeld and Pearson (6). 2.0 ml plasma was mixed with 2.0 ml of alcohol. To this mixture was added 3.0 ml petroleum ether. The tube was mixed well and centrifuged at low speed. Aliquots of 2.0 ml of the supernatant petroleum ether layer were removed and pipetted into the cuvette and the O.D. read immediately at 450 nm. The petroleum ether in the cuvette was then evaporated off to dryness by incubating the cuvette at 40°C in a water-bath. The residue was redissolved in 0.1 ml chloroform and 0.1 ml of acetic anhydride was added. Then 1.0 ml of TFA (trifluoroacetic acid) reagent was added to the mixture. The tube was well mixed over the vortex mixer and the O.D. was read at 620 nm.

A stock solution of 1 mg/ml of β-carotene standard dissolved in chloroform was used. The O.D. was read initially a 450 nm and the chloroform solvent was evaporated off by passing a constant flow of oxygen-free nitrogen gas into the solvent incubated at 40°C in a water-bath. The dry residue was taken up again in 0.1 ml chloroform and 0.1 ml of acetic anhydride was added followed by 1.0 ml of the TFA reagent. The O.D. was read at 620 nm. The carotene correction factor was calculated using concentrations ranging from 4.0, 8.0 and 10.0 μg/ml and the factor was calculated as equal to

$$\frac{\text{O.D. value at 620 nm}}{\text{O.D. value at 450 nm}^*}$$

Similarly stock solution of 1 mg/10 ml of vitamin A acetate standard in chloroform was also prepared. For the calculation of the vitamin A factor, a series of stock solutions containing 5, 10, 15, 20, 25 and 30 μg/ml of vitamin A were prepared. 0.1 ml aliquots of each stock solution was used to carry out the O.D. determinations after reaction with TFA reagent and read at 620 nm. The vitamin A factor was calculated from the formula μg vitamin A/tube ÷ O.D. value at 620 nm.

A Coleman Junior Spectrophotometer Model 6A was used for determining the O.D.

RESULTS

Subjects studied

48 pairs of cases and controls were studied. On subsequent checking, only 33 cases were histologically confirmed. As such, analysis of data will be confined to the 33 pairs. For each group, there were 29 (88%) males and 4 (12%) females. The age range was from 44 to 76 years, with the mean at 62.6 years.

Body weights

The mean body weights for cases and controls were 51.2 kg and 51.7 kg respectively (paired t-value = 0.21 not significant). The distributions are shown in Table 1.

Smoking history

From Table 2, the association between smoking and lung cancer is highly significant ($p < 0.01$). The odds ratio based on discordant pairs is equal to 8 (Mantel-Haenszel estimate).

Among the cases, mean duration of smoking was about 43.1 years and mean dosage of cigarettes was 23.6 sticks per day.

Table 1

Body-weights of cases and controls

Body-weight (kg)	Case	Control
30 —	3	4
40 —	13	11
50 —	11	7
60 —	3	10
70 +	3	1
Total	**33**	**33**
Mean weight	**51.2**	**51.7**
Paired t-value	**0.21 (n.s.)**	

Table 2

Smoking histories of cases and controls

Control \ Case	Smoking	Not Smoking	Total
Smoking	11	2	13
Not smoking	16	4	20
Total	**27**	**6**	**33**

McNemar's Test = 3.06, $p < 0.01$

Serum Vitamin A

The distributions of serum vitamin A levels for cases and controls seem fairly consistent between the 2 groups (Table 3). The mean levels are 52.26 μg per dl for cases and 61.82 for controls. The differences are found to be statistically not significant (paired t-value = 1.55, $p>0.1$).

When the vitamin A readings were regrouped under 'smoking' and 'non-smoking' categories, there was again no significant difference between cases and controls in both categories, although in both situations the mean levels appeared lower among the cases (Table 4).

Table 3

Serum vitamin A levels of cases and controls

Serum Vitamin A (μg per dl)	Case	Control
0 —	—	1
20 —	10	9
40 —	14	8
60 —	5	6
80 —	2	6
100 +	2	3
Total	**33**	**33**
Mean level	**52.26**	**61.82**
Paired t-value	**1.55 (n.s.)**	

Table 4

Mean serum vitamin A levels between cases and controls, by smoking history

Subject	No. of subject	Mean vitamin A level (μg per dl)	Unpaired t-value
Smokers:			
Cases	27	55.2	0.70
Controls	13	60.9	(n.s.)
Non-smokers:			
Cases	6	39.3	1.86
Controls	20	62.4	(n.s.)

DISCUSSION

In this matched case-control study, although there is a highly significant association with cigarette smoking, there is no significant difference in the levels of serum vitamin A. The vitamin A readings span over a wide range in both groups, from around 20 μg per dl to 120 μg per dl.

Studies on normal subjects have shown that, except in states of gross deficiency, large doses of vitamin A intake result only in minor changes of serum levels. There is a very wide range for the limits of normality. According to the review by Sauberlich et al (7), the mean level for normal adults is approximately 45-65 μg per dl, with higher levels in the older age-groups. The U.S. National Academy of Sciences — National Research Council (8) recommends a serum level of 20 μg per dl as the lower cut-off point for adequacy.

The majority of subjects in this study do not have unusually low serum levels of vitamin A. As such, they are not considered deficient in this vitamin. But the situation that one has to consider may not be a state of hypovitaminosis, with very low serum levels. Instead it may be just a state of lower than optimal levels of vitamin A in subjects who have apparently adequate nutrition. Even then, this study has not been able to demonstrate that cases had significantly lower serum levels than controls.

Bjelke (3) and Hirayama (4) both found a negative association between the consumption index for vitamin A and lung cancer, at all levels of cigarette smoking. But these cohort studies were based on self-administered questionnaires on dietary histories, and the indices of consumption were arbitrarily fixed. In the case of Hirayama, he merely dichotomised between daily consumers of vegetables and non-daily consumers. Such histories are difficult to validate in terms of consistency and actual influence on serum vitamin A levels. Furthermore, the relative risks of 1.5 and 2.0 are rather weak, especially when the indices used are quite crude.

Case-control studies of serum vitamin A levels have also shown significantly lower values in cases than in controls. Basu et al (9) reported this on a small case-control study and they found significantly lower levels in lung cancer cases, especially those with squamous cell and cat-cell types. This was also found in stomach and oesophageal cancers. Ibrahim et al (10) also made similar findings in a study of oral cancer cases. Thus, a wide variety of epithelial cancers seem to be associated with lowered levels of vitamin A.

A prospective cohort study on the effects of low serum cholesterol has been reported recently by Kark et al (11). The negative association of cholesterol with the development of cancers in general was thought to be secondary to the effects of lowered vitamin A. The evidence, though inconclusive, seems to point to the likely role of vitamin A in inhibiting carcinogenesis.

What needs to be done now is for a few more well-designed studies to be conducted elsewhere to test this hypothesis. Recently, a suggestion has been made that, perhaps, one should also consider carotene as a likely factor in view of its close relationship to vitamin A and the better correlation between dietary intake and serum levels.

The large variations in serum vitamin A between adequately nourished subjects will certainly make it difficult to detect any real difference between cases and controls. This preliminary study has not been able to show any significant difference between the two groups, among smokers as well as non-smokers. Whether it is the carotene or the retinol, the factor certainly requires further scrutiny.

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