Original Article

EFFECT OF SERUM ANTIOXIDANTS (VITAMIN E, C AND A) IN LUNG CANCER PATIENTS

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ABSTRACT

Objective: Intake of vitamin E, C and A have been reported to reduce lung cancer risk because of their roles as regulators of cell differentiation (vitamin A), antioxidants (vitamins C and E), and modulators of DNA synthesis, methylation and repair. Some case-control studies have found inverse associations between intakes of these vitamins and lung cancer risk. However, most of the prospective studies evaluating these nutrients have not found clear inverse associations. Because many of these prospective studies have included less than 100 lung cancer cases, they lacked statistical power to detect modest inverse associations. Therefore, in order to address the national issue the present study attempted a little beat to explore the association between some antioxidant vitamins with lung cancer.

Methods: In this study we investigated cases (lung cancer) and control group (Smoker); serum Vitamin A, Vitamin C and Vitamin E level estimation were focused. Detailed information on these facts was collected by questionnaire and blood sample analysis, which were then complied and analyzed with a statistical software package.

Results: In this study we found that Serum vitamin C levels were significantly higher in case (0.8 ± 0.2 μg /dl) than in control (0.30 ± 0.1μg/dl). But Serum vitamin A and serum vitamin E level showed less difference between cases (75.89 ± 24.1μg/dl) and (657.25 ± 322.7μg/dl) and controls (74.87 ± 26.6μg/dl) and (641.55 ± 413.5μg/dl) respectively.

Conclusion: It can be suggested that vitamin E, C, and A has no effect on lung cancer patients.

Keywords: Lung cancer, Smoker, Vitamin (A, C, E).

INTRODUCTION

Lung cancer is an uncontrolled growth of abnormal cells that start off in one or both lungs; usually in the cells that line the air passages. If left untreated, this growth can spread beyond the lung in a process called metastasis into nearby tissue and eventually, into other parts of the body. Most cancers that start in lung, known as primary lung cancers, are carcinomas that derive from epithelial cells. Lung cancer can be broadly classified into two main types based on the cancer's appearance under a microscope: non-small cell lung cancer and small cell lung cancer. Non-small cell lung cancer (NSCLC) accounts for 80% of lung cancers, while small cell lung cancer accounts for the remaining 20%. The incidence of lung carcinoma has increased intensely during the 20th century. Hundred year's back it was not so severe disease to be reported but currently it is the commonest cause of death in developed as well as in developing countries [1]. On the basis of GLOBOCAN 2008 statistics lung cancer is the leading cancer in males, comprising 17% of the total new cancer cases and 23% of the total cancer deaths globally [2].

In Bangladesh it is also the most common cancer of males. A recent study conducted with 701 lung cancer patients for one year showed that 85% of them were male patient (608 male: 93 female- 6.53:1) [3]. It reveals the fatalities of lung cancer in Bangladesh. Main causes of the lung cancer are free radical or oxidant and genetic factors. Free radicals are highly reactive molecules that are produced in the body naturally as a byproduct of metabolism (oxidation), and by exposure to toxins in the environment such as tobacco smoke and ultraviolet light. Free radicals contain an unpaired electron. Simply put, they are in a constant search to bind with another electron to stabilize them -- a process that can cause damage to DNA and other parts of human cells. This damage may play a role in the development of cancer and other diseases, and accelerate the aging process. Actually free radical or oxidants oxidize DNA to change its sequence to form wrong sequence DNA. This DNA creates abnormal cell or malignant cell in lung. Thus result lungs becomes to cancer. Although tobacco smoking is claimed to be the most common cause of lung cancer (IARC, 2002) [4], respiratory diseases due to asbestos

inhalation are also responsible to increase the risk of developing lung cancer [5]. Cigarette smoke contains over 60 known carcinogens [6] or oxidizing agents including radioisotopes from the radon decay sequence, nitrosamine and benzopyrene. Additionally, nicotine appears to depress the immune response to malignant growths in exposed tissue [7]. Carcinogens are a class of substances that are directly responsible for damaging DNA and promoting or aiding cancer. Tobacco, asbestos, arsenic, radiation such as gamma, x-rays and the sun, and compounds in car exhaust fumes are all examples of carcinogens. When our bodies are exposed to carcinogens, free radicals are produced that try to steal electrons from other molecules in the body. These free radicals damage cells and affect their ability to function and divide normally.

If we suppress this oxidizing agent or carcinogen, must treat with antioxidant. Antioxidants are substances that may protect cells against the affects of free radical. Antioxidant substances include Beta-carotene, Lutein, Lycopene, Selenium, Vitamin A, Vitamin C, Vitamin E etc. Several studies have looked at the relationship between smoking and serum plasma level of various antioxidant nutrient statuses. Low plasma level of antioxidants has been reported in lung cancer patients [8] [9], have 12 times more risk of lung cancer having low level of vitamin E in serum than healthy person [10]. However, an investigation on the relation between lung cancer incidence fruits and vegetable consumption appeared to confer no protection from lung cancer [11]. While a study appeared to show that high dose of beta carotene supplementation increased the risk of lung cancer among current smokers [12]. Another study showed that lung cancer is not dependent on beta carotene and retinol supplementation rather more case and gender specific [13]. This study attempts to investigate whether any association of antioxidant vitamins A, C, and E with lung cancer or not.

MATERIALS AND METHODS

Population Study

The study included 120 people comprising 60 lung cancer patients and 60 smokers as cohort controls during June to October 2013.

Randomly selected lung cancer patients were admitted in to Dhaka medical college hospital, Mitford hospital, Cancer institute and some clinics in Dhaka city. The smoker controls were preferably from the community. The age limit was between 40 to 60 years and patients had with the strong clinical features of lung cancer and was in medication and vitamin supplementation.

Every cancer patient and smoker control was selected randomly on basis of the following inclusion criteria.

(ᵢ) Strong history of smoking

(ᵢᵢ) Long term more consumption of cigarette smoking

(ᵢᵢᵢ) No history of other drugs which can cause the drugs addiction

(ᵢv) Cancer patient have strong and long term smoking history

Resident physician of the respective hospital, on the basis of history, clinical and laboratory findings advised the cancer patients to take treatment either taking admission or outdoor treatment into the hospital. After briefing the purpose of the study, written consent was taken from the patients or from their guardian and then included in the study. After having the consent, participant was asked to report on a prefixed date for interview and blood sampling.

A questionnaire was developed to obtain the relevant information's. It included: general information, socioeconomic information, anthropometric data, dietary history, smoking habit, clinical signs and symptoms, vitamins and drug taking information.

After collection of the socio-demographic anthropometric and dietary data; 5.0ml of blood was drawn from the subject arm and aliquot of 2.0 ml of this blood sample was placed in a heparinized tube to collect the plasma sample for vitamin C estimation. The remaining 3.0 ml blood was centrifuge- test tube which was washed with acid and de-ionized water. Centrifuge tube was immediately wrapped in aluminum foil to protect vitamin A against degradation by light. The blood sample was allowed to stand at room temperature for approximately 2hrs. After centrifugation serum samples were separated frozen and stored at – 20 ^{0}C until further analysis.

Estimation of serum vitamin A (retinol) and vitamin E (α-tochopherol)

Serum vitamin A and vitamin E were measured simultaneously by a modification of the method of Bieri *et al*, using HPLC, equipped with reversed – phase column of Waters of Bondapak C 18 125 A°10μM (3.9 ×300mm) HPLC column. A guard column (5.0 cm x 0.5 cm) packed with octadecyl silica preceded the primary column. For extraction purpose absolute alcohol (BDH, UK.) and extra pure hexane (Merck, Germany) were used and the eluting solvent was extra pure methanol (Merck, Germany) water at ratio of 97.5: 2.5, where the injection volume during operation was 20 μl.

Trans-retinol (Sigma Chemical Company, Saint Louis, Missouri, USA) and dl-alpha- tocopherol (Sigma Chemical Company, Saint Louis, Missouri, USA) were used as the external standard for vitamins A and vitamins E respectively. Retinol acetate (Sigma, USA) and tocopherol acetate (Sigma, USA) were used as the internal standard for vitamins A and vitamins E respectively. Retinol standard (stock) Retinol (10.0 mg) was dissolved in 100 ml absolute alcohol and was stored at -20 ^{0}C. Retinol Acetate standard (stock) Retinol Acetate (10 mg) was dissolved in absolute alcohol (100ml) and stored at -20 ^{0}C. Alpha- tocopherol (stock).Alpha- tocopherol (10mg) was dissolved in absolute alcohol to make a final volume of 100ml and stored at -20 ^{0}C. Alpha- tocopherol Acetate (stock) Alpha- tocopherol acetate (10mg) was dissolved in 100ml of absolute alcohol and stored at -20 ^{0}C.

To measure vitamin A and vitamin E simultaneously, retinol stock standard was diluted to following concentration; 25, 50, 75, and 100 μg /dl and alpha- tocopherol was diluted to the following concentrations; 250, 500, 750 and 1000μg / dl. A constant amount of retinol acetate 50μg /dl and alpha- tocopherol acetate 5.0mg/dl were also added as internal standard to each of the concentration of retinol and alpha- tocopherol solution. For internal standard, Stock retinol acetate, 0.5ml and 50.0ml of tocopherol acetated were

diluted to 100ml with absolute alcohol to obtain a concentration of 50μg /dl retinol acetate and 5.0 mg/dl for tocopherol acetate. To determine the retention time of retinol, alpha- tocopherol, retinol acetate and tocopherol acetate, each was applied into HPLC instrument separately at a concentration of 100 μg /dl. The retention times were calculated from the chromatograms.

For analysis of serum retinol and α-tocopherol, serum sample (100μl) was mixed with 100μl of the internal standard solution to a concentration of 50 and 100 μg/dl for retinol acetate and 5.0 mg/dl for alpha-tocopherol acetate. The contents were mixed with a vortex mixer for 15 sec. For extraction of lipids, 500 μl of hexane was added and mixed for 45 sec in a vortex mixer. The tubes were centrifuged at 10000 rpm.for10 min and the top of the hexane layer was separated. The hexane was evaporated under a stream of nitrogen gas. The extracted vitamins A and E then re-dissolved in 100μl absolute alcohol. The solution was then injected in to the HPLC instruments. A solvents flow of 1.0 ml/ min was used. Elution was monitored at 291 nm. The pick height ratios for retinol and alpha-tocopherol were calculated and the amounts were read from the standard curve.

Day to day variation for vitamin A and E assay were determined using a polled serum sample for a period of 10 days. The inter assay variation for vitamin A 2.3% and for vitamin E was 3.3%.

Estimation of plasma ascorbic acid

Plasma ascorbic acid was measured by dinitriphenyl hydralazine method. In this method, an aliquot of 0.2 ml serum was taken in a 5.0ml test tube, 0.8ml 5.0% TCA was added and mixed immediately using a vortex mixer. The mixer was then centrifuged at 3000 rpm for 10 min. An aliquot of 0.6 ml supernatant was taken and 0.25 ml of DTC solution was added. The tube was covered with paraflim and then incubated at 60 ^{0}C for 1 hr in a water bath. Immediately after the incubation, the test tube was chilled in an ice cool water bath. 1 ml 65% sulphuric acid was then added drop wise and mixed well solution then. Incubated at room temperature 25 ^{0}C for 30 min. working standard solution, 0.2ml was taken and treated as serum sample. The blank was prepared by taking 0.6 ml 5.0% TCA and 0.25 ml DTC solution and treated in the same procedure as serum.

Statistically Analysis

SPSS software package (version 20.0, IBM SPSS Inc. Chicago, USA) was used to analyze the data. Descriptive statistics were calculated for all variables. Values were expressed as mean ± SD. Comparison of groups in Lung cancer patients and Control smoker were performed by independent sample p-test.

RESULTS AND DISCUSSIONS

Vitamin C and vitamin E are powerful antioxidants found in the lung where they protect against oxidative damage [14]. There is growing body epidemiologic evidence suggesting that antioxidants such as Beta-carotene, Vitamin C and alpha-tocopherol may modify the incidence the respiratory tract cancer both in smokers and nonsmokers. Decrease concentration of Vitamin C in plasma and Vitamin E in epithelial lining fluid has been observed in smokers, suggesting that cigarette smoking may induce the oxidative stress on respiratory system and that dietary antioxidants may diminish the deleterious effects of cigarette smoking. However, the therapeutic use of alpha-tocopherol and beta-carotene to ameliorate the adverse effects to cigarette smoke has been questioned recently. There are several studies that have analyzed the association between dietary intake of these antioxidant vitamins and respiratory function [15]. A large number of studies have found positive associations between pulmonary function and intake of vitamin C or foods high in vitamin C content (i.e., fresh fruit and vegetable) [16–20]. Fewer studies have analyzed the relation of dietary vitamin E intake with pulmonary function and have reported contradictory findings [17] [18].

In our Study, we found vitamin E in serum is 657.25±322.7 μg/dl and 641.55±413.5 μg/dl for lung cancer and control smoker respectively (Table-1). Slightly difference is found from these values. From these values serum Vitamin E level of Lung cancer patients is slightly higher than control smoker. This results in not significant.

Vitamin C in serum is 0.85±0.2 µg/dl and 0.30±0.1 µg/dl of lung cancer and control smoker respectively in our findings (Table-1). Vitamin C level in lung cancer patients is significantly higher than the control smoker. Vitamin A in serum level is 75.89±24.1 µg/dl and 74.87±26.6 µg/dl of lung cancer and control smoker respectively (Table-1). This difference is not significant. In our finding serum Vitamin E, C, A level is higher in lung cancer patients than control smokers.

Table 1: Serum vitamin E, C and A level of lung cancer patients and control smokers

Vitamin	Lung cancer	Control smoker	P value
Vitamin E	657.25±322.7	641.55±413.5	0.82
Vitamin C	0.85±0.2	0.30±0.1	0.00
Vitamin A	75.89±24.1	74.87±26.6	0.94

Level of significance: $p < 0.05$, All values unit= µg/dl, Data's are expressed as mean ± SD.

In true sense Vitamin E, C, A level should be lower in Lung cancer patients than control smokers. Because Lung cancer patients need more anti-oxidant than control smokers for diminish their carcinogen or free radical that produce cancer cell. But not occur that. It may be no effect of Vitamin E, C and A on lung cancer patients and control smokers.

The finding of this study was found consistent as reported by others. Tawee Tanvetyanon reported that high-dose of Beta-Carotene supplementation appears to increase the risk of lung cancer among current smokers [21]. A study found no reduction in the incidence of lung cancer among male smokers after five to eight years of dietary supplementation with alpha-tocopherol or beta carotene. In fact, this trial raises the possibility that these supplements may actually have harmful as well as beneficial effects [22]. Slatore CG reported that Supplemental multivitamins, vitamin C and vitamin E were not associated with a decreased risk of lung cancer. Supplemental vitamin E was associated with a small increased risk [23].

CONCLUSION

Based on the present experimental results, it can be suggested that vitamin E, C, and A has no effect on lung cancer patients. Higher level of Vitamin E, C and A in lung cancer patient may indicates high endogenous anti-oxidant secretes from the body due to inhibition of oxidation of cell.

ACKNOWLEDGEMENT

CONFLICT OF INTERESTS

Declared None**.**

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