Research Article

SERUM CONCENTRATIONS OF VITAMINS A, C AND D IN IRAQI PATIENTS WITH LUNG CANCER AND SMOKERS

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ABSTRACT

Aim: This study was designed to investigate the serum levels of vitamins A, C and D in Iraqi patients with lung cancer as well as in cigarette smokers and to compare them with those of healthy subjects. **Method:** Blood samples were collected from 48 lung cancer patients with age ranging between 45-75 years, and from 38 male smokers with age ranging between 20-25 years, as well as from 35 health volunteer subjects with age ranging between 30-65 years. Vitamins (A, C and D) levels were measured using isocratic mobile phase HPLC technique with UV-Visible detection. **Results:** The results of the current work indicated that the mean values of vitamin A and vitamin C were significantly lower in serum samples of both smokers ($p<0.001$) and lung cancer patients ($p<0.001$) than in controls. Whereas the concentrations of vitamin D were significantly higher in the serum samples of both smokers ($p<0.001$) and lung cancer patients ($p<0.05$) than in those of healthy control subjects. **Conclusions:** According to the present work, it is possible to conclude that adequate intake of vitamins A and C may have a protective role in occurrence of lung cancer. Also, it can conclude that exposure to cigarette smoke lead to the depletion of most of the antioxidants in human serum and thus impair oxidant defense system.

Keywords: Lung cancer, Smoker, Vitamin A, Vitamin C, Vitamin D, HPLC.

INTRODUCTION

Cancer is a large group of diseases characterized by uncontrolled growth and spread of abnormal cells, and in most cases it can result in death. Among all known cancer types, lung cancer is found to be the most common cause of cancer-related death worldwide[1-2]. According to WHO report, 1.8 million new cases (12.9% of all new cancer cases) and 1.6 million deaths (19.4% of all cancer deaths) occurred in 2012[3]. It has been reported that lung cancer is the leading cause of cancer mortality in both men and women in the European Union, Canada and United States[3-4].

Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Antioxidants are chemicals that interact with and neutralize free radicals, thus preventing them from causing damage. Antioxidants are also known as free radical scavengers. In fact, it was found that free radical damage may lead to cancer. Antioxidants terminate the chain reactions that damage cells by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves[5-6].

Vitamins are essential nutrients found in foods that are required in small amounts to maintain the health of the human body. They perform specific and vital functions in a variety of body systems, and are crucial for maintaining optimal health such as vitamins E, C and A that functions as antioxidants. It has been found that the most commune nutrient-derived antioxidants are ascorbic acid (vitamin C), Tocopherols and Tocotrienols (vitamin E), vitamin A and other low molecular weight compounds such as glutathione and lipoic acid[7-8].

MATERIALS AND METHODS

This study was conducted in Ibn Al-Bitar research Centre/ Ministry of Industry, Baghdad, Iraq between October, 2014 and June, 2015. The study includes 48 patients with lung cancer, 32 males and 16 females, with age ranging from 45-75 years; the mean age 63.4 years. The control group (Non-Smokers group) comprised of 35 volunteers, 23 males and 12 females, with age ranging from 30-65 years; the mean age 41.4 years. The study also includes 38 male smokers with age ranging from 20-25 years; the mean age 22.37 years. Patient samples were collected from the Oncology Teaching Hospital at Medical City/ Baghdad, control samples were collected from the teaching staff of Chemistry Department / College of Sciences at AL-Mustansiriya University, while smoker samples were collected from the undergraduate students of the Department of Chemistry/ College of Sciences/ AL-Mustansiriya University.

Serum sample preparation

Samples were prepared, in order to be ready for injection to the HPLC system, by adding 50 μl of (15%) 5-Sulfosalicylic acid dihydrate to 400 μl of serum, the solution mixed well and then centrifuge at 5000 rpm for 10 minutes. The supernatant was carefully removed and diluted 10-folds with distilled water, and then filtrated by passing through a Millipore 0.22 μm Millex-GS sterilizing filter unit.

Samples analysis

All serum pre-treatment samples and standard working solutions were chromatographically analyzed using SCL-10 AVP HPLC system, Shimadzu (Kyoto, Japan), with an analytical C_8, 4μm (100 × 4.5 mm) reverse phase column, under PC control. Different sets of mobile phasesand conditions were used. First mobile phase was methanol-acetonitrile-tetrahydrofuran (75-20-5%), flow rate 1.2 ml/min and UV-VIS detection at wavelength 290nm to measured vitamin A, second mobile phase was methanol–distilled water (90-10), flow rate 1.0 ml/min and UV-VIS detection at wavelength 290 nm to measure vitamin Cand third mobile phase using methanol-distilled water (75-25%), flow rate 1 ml/min and UV-VIS detection at wavelength 275 nm to measured vitamin D.

Data analysis

The statistical analysis was carried out using the Statistical Package for Social Sciences computer program version 20.0 for windows (IBM SPSS Statistic software, IBM Corporation, New York, United States). Statistical analysis was performed using one way analysis of variance (ANOVA test) for assessment of mean differences between three groups (patients, smokers and control groups). The statistical tests were considered to be significant at the $p<0.05$ with 95% Confidence Interval. The upper and lower limits were recorded for each element in this study, and the values were expressed as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

The mean values ± SD of vitamins (A, C and D) for the three study groups were calculated from HPLC chromatograms using the standard calibration curve of each vitamin separately. The collective results are presented in Table 1, where the data obtained were assigned for p values <0.05 with 95% Confidence Interval.

Table 1: Serum levels of vitamins (A, C and D) in patients with lung cancer, smokers and non-smokers (control group)

Vitamin	Control (Non-smokers)			Smokers			Patients			*P* value Non-Smokers & patients	*P* value Non-smokers & Smokers	*P* value Smokers & Patients
	Mean (mg/L)	SD (mg/L)	Upper & lower limit (mg/L)	Mean (mg/L)	SD (mg/L)	Upper & lower limit (mg/L)	Mean (mg/L)	SD (mg/L)	Upper & lower limit (mg/L)			
Vitamin A	58.5426	6.5119	62.1488 54.9364	48.6242	6.8689	51.2370 46.0114	35.7044	9.4979	40.7655 30.6433	High Sig. <0.001	High Sig. <0.001	High Sig. <0.001
Vitamin C	2.7631	0.3973	2.9142 2.6120	1.0321	0.31951	1.1536 0.9105	1.8123	0.1229	1.8583 1.7664	High Sig. <0.001	High Sig. <0.001	High Sig. <0.001
Vitamin D	6.2896	1.5867	6.9444 5.6340	62.9128	13.8542	68.1829 57.6427	10.9923	1.1602	11.4256 10.5587	Sig. 0.038	High Sig. <0.001	High Sig. <0.001

The serum mean value ± SD of vitamin A for control (non-smokers), smokers and lung cancer patients were found to be (58.5426 ± 6.5119 mg/L), (48.6242 ± 6.8689 mg/L) and (35.7044 ± 9.4979 mg/L) respectively, as shown in Table 3.1 and represented in Figure 1. These results indicated the presence of a highly significant decrease in vitamin A concentration in lung cancer patients compared with control (non-smokers) group ($p<0.001$), as well as a highly significantly lower of vitamin A concentration in smokers compared with control group ($p<0.001$). In addition, the results indicated that the serum level of vitamin A was highly significantly lower in patients with lung cancer as compared with the smokers group ($p<0.001$).

The results obtained in Figure 1 show that the vitamin A level of healthy subjects is hardly affected relative to those in lung cancer patients and smokers giving a positive indication of the presence of a significant impact of this vitamin on lung function.
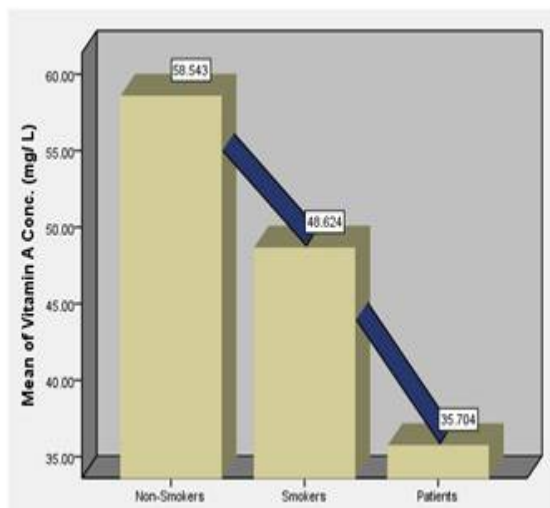

Fig. 1: Comparison between serum vitamin A concentration (mg/ L) in lung cancer patients and healthy non-smokers subjects (control group) as well as smokers group

These results are in agreement with the data of other previous studies such as(Basu*et al.*, 1976; Atukorala S., *et al.*, 1979; Çalışkan-Can *et al.*, 2008)[9-11], where they reported that the concentration of vitamin A in the serum of lung cancer patients was lower than in the healthy control group. While, they disagree with another previous study (Cohen M. H. *et al.*, 1977)[12], who reported that the concentration of vitamin A in the serum of lung cancer patients was higher than those in the healthy control subjects.Regarding with the smoking habit, it has shown that our findings disagree with those of the data published in previous study (Yeung D. L., 1976)[13], who found that cigarette smoking slightly increased the levels of vitamin A. The concentration of serum vitamin A in control (non-smokers group) was 32 ± 1.2 µg/ 100 ml, while it was 37 ± 2.3 µg/ 100 ml in smokers. Furthermore, it has been reported from (Chow C. K *et al.*, 1986; Faruque M. O. *et al.,* 1995; Davis *et al.,* 1983; Bolton-Smith *et al.,* 1991)[14-17] that the serum level of vitamin A is not significantly different between smokers and non-smokers subject groups.

Several studies documented that smoking habit may increase oxidative stress and impair oxidant defense system[18]. The cigarettes contain a lot of carcinogens and oxidizing substances, which produces a lot of free radicals. The accumulation of these dangerously reactive radicals is very harmful to the body cells and may increase the risk of lung cancer. So, it is most important for the human body to get rid of these toxic free radicals quickly. It is well known that vitamin A acts, in the body, as a free radical scavenger and antioxidant agent (a protective chemical that may reduce the risk of certain cancers). Therefore, in the present work, this is may be the reason of depletion of the vitamin A level in smokers group and lung cancer patients compared with the non-smokers sample group.

The results obtained for vitamin C showed clear differences in the mean values ±SD of the control group compared with the patients and smokers groups. The concentrations of vitamin C in the serum of the control group, lung cancer patients and smokers group were (2.7631 ± 0.3973 mg/L), (1.8123 ± 0.12119 mg/L) and (1.0321±0.3195 mg/L)respectively, as shown in Table 1 and Figure 2. These results indicated the presence of highly significant decrease in vitamin C concentration of lung cancer patients compared with the healthy control group ($p<0.001$). Similarly, the results obtained showed a highly significant decrease in the vitamin level of smokers group ($p<0.001$) compared with the control group. Also, the results indicated that in smokers group the mean value for vitamin C was highly significant lower ($p<0.001$) than in lung cancer patients group.
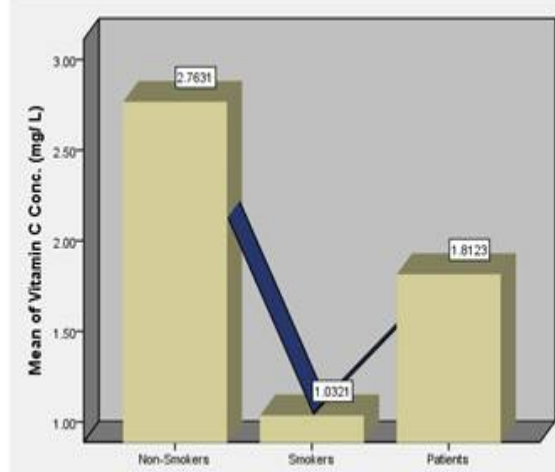

Fig. 2: Chromatogram shows a comparison between serum vitamin C concentration (mg/ L) in lung cancer patients and healthy non-smokers subjects (control group) as well as smokers group

The results obtained in this study are very similar to the results published previously from (Çalışkan-Can *et al.,* 2008)[11], who reported that the serum levels of vitamins C were significantly lower ($p<0.001$) in lung cancer patients than in healthy control group. But, the

results obtained are disagree with the findings of (Anthony H. M. *et al.,* 1982)[19], the concentration of vitamin C in patients with lung cancer was higher than those of normal persons.

Furthermore, it has been found that the results obtained, in the present work, for the smokers group were in a good agreement with the published values of the previous studies such as (Faruque M. O., 1995; Bolton-Smith *et al.,* 1991; Schectman G., *et al.,* 1989; Bridges R., *et al.,* 1990) [15, 17, 20, 21], who reported that serum vitamin C levels were significantly lower in cigarette smokers than non-smokers. There is a single study from (Yeung D. L. 1976)[13] showed that the vitamin C level does not affected via cigarette smoking. The concentration of vitamin C measured gave the same value (1.32 ± 0.07 mg/ 100 ml) from both smokers and non-smokers groups.

Vitamin C plays a significant role as free radical scavengers and antioxidant agents[22], it significantly decreases the adverse effect of reactive species such as oxygen species and nitrogen species that can cause oxidative damage to macromolecules such as lipids, DNA and proteins which are implicated in chronic diseases including cardiovascular diseases, neurodegenerative diseases and cancers[23]. It has also able to protect membrane and other hydrophobic compartments from such damage by regenerating the antioxidant form of vitamin E[24]. In addition, it was found that vitamin C has the ability to strengthen the immune system and fight cancerous cells. Lower level of this antioxidant vitamin might expose the prostate cancer patients to infections and the destructive effect of the free radicals released in the body [25].

Unlike vitamins A and C, the results obtained for vitamin D showed a clear increase in the serum concentration that measured for lung cancer patients and smokers when compared with those of control group. The mean values±SD measured of Vitamin D for control, lung cancer patients and smokers were (6.2896± 1.5867 mg/L), (10.9923±1.1602 mg/L) and (62.9128 ± 13.8542 mg/L) respectively, as shown in Table 1 and presented in Figure 3.

These results were observed to be increased significantly in patients with lung cancer when compared with those of control (non-smokers) group ($p<0.05$). They also indicated the presence of highly significant increase in concentration of vitamin D in smokers group when compared with the control group ($p<0.001$). In addition, the results obtained showed a highly significant decrease in the vitamin D level of lung cancer patients

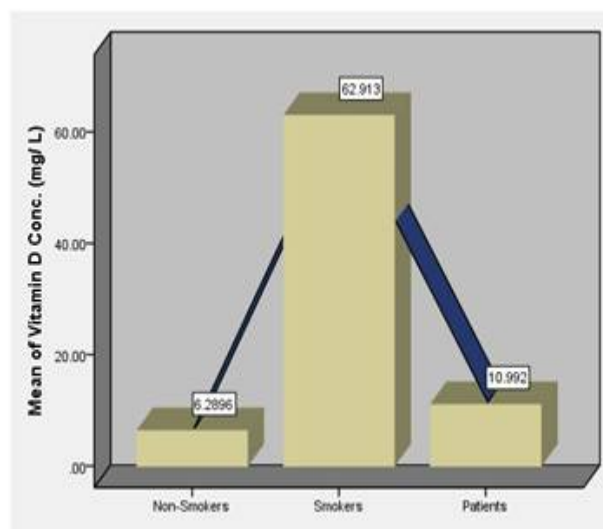($p<0.001$) compared with those of the smokers group.

Fig. 3: Chromatogram shows the mean values of serum vitamin C concentration (mg/ L) that measured for lung cancer patients and healthy (non-smokers) control group as well as smokers group

If fact, vitamin D is converted to its active form locally in the lung, therefore it may play an important role in lung health. It was reported by (Norton R and O'Connell MA, 2013)[26] that an adequate vitamin D status may be important for lung cancer prevention. While, Kilkkinen A. and co-workers [27] reported that there was no overall association between vitamin D and lung cancer risk. In addition, it has been reported from (Garland C. F. *et al.,* 2009)[28] that higher serum levels of the main circulating form of vitamin D, 25-hydroxyvitamin D (25(OH)D), are associated with substantially lower incidence rates of colon, breast, ovarian, renal, pancreatic and aggressive prostate cancers. Furthermore, it has been reported by (Giovannucci E.*et al.,*2006)[29] that low levels of vitamin D may be associated with increased cancer incidence and mortality in men, particularly for digestive-system cancers, suggesting that vitamin D has potent anticancer properties, especially against digestive-system cancers.

It has been found that the results obtained of the present work completely disagree with the results reported by (Kassi E. N. *et al.,*2014)[30], who found that vitamin D level was lower by approximately 4.3 ng /dl in smokers group compared to the non-smokers group. In this study, the high level of vitamin D that recorded for smokers group may be gives an indication for one of the risk of smoking that lead to accumulation this vitamin in the body and therefore causing serious health problems that should be avoid. Regarding with lung cancer, it

may be possible to suggest that if the vitamin D levels were already high before the cancer arose, this relative rise of vitamin D may have made the emergence of the cancer more likely.

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