Alterations in serum biochemical parameters of patients with lung cancer exposed to radiotherapy

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Abstract: In this study, the alterations in serum biochemical parameters of patients with lung cancer exposed to radiotherapy was investigated. For this aim, the levels of serum gamma glutamyltransferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (ALB), bilurubin (BLB), copper (Cu), sodium (Na) and potassium (K) were evaluated before and after radiotherapy. Serum enzyme, protein, Na and K levels were determined using an autoanalyzer. Serum Cu analysis was made with Atomic Absorbtion Spectrophotometre (AAS). Although we found significant increases in levels of GGT, BLB, Cu and K in patients, levels of AST, ALT, ALB and Na in patients showed significant decreases. The levels of serum AST and ALT fairly decreased after radiotherapy. The level of GGT in patients was significant higher than that in the controls before radiotherapy. However, GGT level showed again a distinctly decrease after radiotherapy. There was an inverse relationship among serum BLB, Cu and ALB values. Besides, serum Na levels showed significantly decrease in patients at the end of radiotherapy treatment compared to the controls and before radiotherapy, and K levels increased significantly following radiotherapy. In conclusion, the selected serum parameters are very sensitive and useful biomarkers for the study of the effects of radiotherapy.

Key words: Lung cancer, Enzyme parameters, Gamma radiation, Radiotherapy, Serum elements
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Introduction

Lung cancer is the most common cause of cancer death between men and women in Turkey (Ekinci and Ekinci, 2004). Nevertheless, approximately one million people worldwide die from this disease every year (Nagar *et al.*, 2003; Boyle and Dresler, 2005; Cavusoglu *et al.,* 2009; Cavusoglu and Yalcin, 2009). Surgery to remove cancerous tumors, chemotherapy and radiotherapy, either in combination or alone, are the common treatments, depending on cancer type and stage (www.cancerhelp.or.uk, 2008). Radiotherapy is the most common treatment for lung cancer. The purpose of radiotherapy is to kill or damage cancer cells. Positive effects of radiotherapy are temporary and usually limited to the area treated by radiation. Radiotherapy may cause also long term side effects on body's healthy tissues. The commonest side effects of radiotherapy are tiredness and feeling run down, sore throat and difficulty swallowing, cough, hair loss, chest pain, temperature and shivering, feeling sick and sore skin (Faulhaber and Bristow, 2005) (www.cancerhelp.or.uk, 2008). Radiotherapy uses high-energy gamma (denoted as γ) rays to kill cancer cells and shrink tumours. γ–radiation is a form of electromagnetic radiation, and its the major effect in cells is DNA breaks (Manda and Bhatia, 2003). γ–radiation induces single-strand and double-strand breaks in DNA which finished with mutations and chromosome aberrations (Natarajan, 2002). Additionally, γ-radiation can cause to enzyme inactivation which result in structural degradation, cross-linking, breakage of chemical bonds (Shacter, 2000).

The aim of this study is to examine the levels of serum ALB, BLB, Cu, Na and K and the activities of serum GGT, AST and ALT

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enzymes in patients with lung cancer following exposure to γ– radiation.

Materials and Methods

Patients and treatment: The present study was carried out on 14 male patients received treatment for lung cancer from November 2004 to July 2006 in Ankara University Andicen Polyclinic of Dr. Abdurrahman Yurtarslan Research Hospital. The patients were randomly selected. Their mean age was 53±4 years (range, 45-60 years). Small cell lung cancer was diagnosed in 11 (78.5%) patients, adenocarcinoma – in 2 (14%), large cell lung cancer – in 1 (7%) patient. Stage I cancer was diagnosed in 2 (14%), Stage II – in 4 (28.5%), and Stage III and IV – in 8 (57%) patients, depending on tumour stage. Histologically, small cell lung cancer was predominant (11 cases). All patients were heavy smokers had smoked more than 20 cigarettes per day for at least 20 years. Seven patients were current cigarette smokers. All patients had respiratory dysfunction. The tumors were located in the upper, lower and middle lobes of lungs. Chemotherapy was not given at the same time with radiotherapy at any point during radiotherapy treatment. Control group was consisted of 10 non-smoker subjects (mean age was 52±3 years) without any health problems and not exposed to radiotherapy.

Ethical standards: This study was carried out after obtaining approval of the local ethical committee (Protocol date: 27.10.2005) of Abdurrahman Yurtarslan Research Hospital and favorable to the guidelines set by the world health organization (WHO, Geneva, Switzerland). Each patient signed an informed–consent form before participating in the study.

Radiation treatment: Radiation procedure was carried out using a "ATC cobalt 60 SSD=80 cm". The dose equivalents *were calculated* and compared to the recommended by International commission on radiation protection (ICRP). Totally 14 patients were treated with γ-radiation for 5 weeks. 2 Gy/fraction per day for 5 day week^{-1} with a total dose of 50 Gy was applied to patients. Radiation was applied on thoracic region for 30 min at room temperature. Biochemical analyses were performed using serum samples of cancer patients exposed to γ–radiation.

Sample collection and analysis: Blood samples were taken from all the 14 patients immediately before and after radiotherapy treatment. Blood was collected from arm vena of each patient. Control group blood samples were also taken at the same day and same method as of the cancer patients. Peripheral blood smears were prepared for determine morphologically damages of blood cells. Whole-blood smears were stained with "May Grunwald-Giemsa" and were covered with cover glass. Each slide was examined under a light microscope (LM). Blood slides were also prepared for scanning electron-microscope examinations, and examined with a Jeol JSM-5600 scanning electron microscope. Damaged cells in each smear and slide were photographed at a magnification of X 2000 for SEM, X 500 for LM. For serum isolation, samples in nonheparinized tubes were centrifuged at 5000 rpm for 10 min. Then supernatant were removed and concentrations of ALB, BLB, GGT, AST, ALT, Na and K in isolated serum were determined by an autoanalyzer (Japan, Olympus AU600) using commercial test kits (Saraswathy and Usharani, 2007). The serum *Cu* analysis was made with atomic absorbtion spectrophotometre (AAS, Perkin-Elmer Model).

Statistical analysis: For the statistical analysis, data were analysed using the SPSS for Windows software, Version 10.0 (SPSS Inc., Chicago, USA). Statistically significant differences between groups were compared using analysis of variance (ANOVA) and Duncan test. The data are displayed as means ± standard deviation (SD) and p-values less than 0.05 are considered significant.

Results and Discussion

Blood analysis: In the control and treatment groups, the average levels of serum ALB, BLB, GGT, AST, ALT, Na, K and Cu with the statistical significance of standard deviations and the differences between the groups, are given in Table 1. There was a statistically significant decrease ($p < 0.05$) in serum level of ALB according to the values of the control group at pre-radiotherapy, and this reduce trend continued as an effect of radiation after radiotherapy treatment ($p < 0.05$). On the contrary, the levels of the BLB and Cu after radiotherapy were found to be increased significantly when compared with the controls and pre-radiotherapy ($p < 0.05$). The serum GGT level (48.21±30.09) in patients with lung cancer was approximately 3-times higher than the control levels (13.30±3.89 mg dl^{-1}) at pre-radiotherapy ($p < 0.05$). It showed again a decrease after radiotherapy application. But, this small decrease was statistically insignificant ($p > 0.05$). Although this decrease in serum GGT concentration after radiotherapy was still significantly higher than the control group levels ($p < 0.05$). We also found a significant decrease in the levels of AST and ALT during the radiotherapy period. The levels of AST and ALT significantly decreased in cancer patients at pre-radiotherapy when compared with the controls ($p < 0.05$), and they continued to decrease in cancer patients during radiotherapy ($p > 0.05$). We also observed a significant decrease in serum *Na* levels and a significant increase in serum K levels in patients after radiotherapy. At the end of radiotherapy, the average serum concentrations of *Na* in all subjects were about 1.59-fold lower and K levels were about 1.61-fold higher than in the controls.

Microscopic observations: The microscopic observation of the cells in blood samples collected after radiation period showed that γ–radiation induced morphological anomalies such as dense-vacuolization, membrane defects, cytoplasmic granulation, cellular death, deformity and hemolysis. Hemolysis (Fig. 1a) and deformity (Fig. 1b) damages were higher than the other types of morphological damages.

Reference values for human were displayed as 3.5–5.2 g dl^{-1} for ALB, 0.2–1.0 mg dl^{-1} for BLB, 0–55 U l^{-1} for GGT, 10–37 U l^{-1} for ALT, 10–37 U l^{-1} for AST, 1.2–1.4 mg l^{-1} for Cu, 135-145 mmol l^{-1} for *Na* and 3.5–5.5 mmol l^{-1} for K. In this study, we observed a decrease in level of serum ALB after radiotherapy. This decrease may be derived from the modification of protein structure of ALB by γ–radiation or may be resulted from disturbed protein synthesis in the liver, which is controlled by steroid hormones. For example, Gaber (2005) reported a decrease in the molecular weight of bovine serum ALB after exposure to γ-radiation. He showed that γ–radiation causes disruption of the ordered structure of ALB molecule, as well as degradation, cross-linking and aggregation of ALB. As is known, one of the important functions of ALB is transport Cu and BLB in blood circulation. Therefore, radiation-induced damage may be reduce amount or ligand binding ability of ALB (Naligan, 2008). As a result, these situations can be result an increase in levels of substances as BLB and Cu which transported by ALB. Pedersen *et al*. (1977) reported decrease of BLB binding affinity due to conformational alteration in structure of human serum ALB after photooxidation. In our study, the increased BLB and Cu concentrations correct this knowledge. The findings showed of the study that there was a significant increase in Cu and BLB levels of all subjects after radiotherapy. BLB is also a waste product that results from the breakdown of hemoglobin molecules. The amount of BLB in blood circulation may be increased with reasons which induced destruction of red blood cells such as radiation, chemical agents and hemolytic anemia (or hemolytic disease of the newborn). Especially, the radiation-induced temperature rise may be cause cell membrane damages, may be alteration their permeability or may be cause to hemolysis (Weenberg and Hence, 1986; Philip, 1997). Therefore, another reason for the increase in BLB concentrations may be hemolysis observed in microscopic analysis.

The Na levels *significantly* decreased whereas K levels significantly increased in serum of all patients exposed to radiation when compared with the controls and pre-radiotherapy. This result may be explained with damage γ–radiation on Na/K pump in cell membranes. As is known, radiation and radiation products (as free radicals and oxidative stress) can induce increase in membrane permeability (Mense *et al*., 1997). This situation causes to partially

Table - 1: Alterations in serum biochemical parameters of patients with lung cancer during radiotherapy

Reference values of parameters	Control group (n: 10)	Cancer patients (n: 14)		Change after radiotherapy
		Pre-radiotherapy	After radiotherapy	
ALB (3.5-5.2 g dl^{-1})	4.50±0.45[a]	3.81±0.43[b]	3.49±0.26[c]	Within range
BLB (0.2-1.0 mg dl^{-1})	0.34±0.84[b]	0.35±0.12[b]	0.57±0.19[a]	Within range
GGT (0-55 Ul^{-1})	13.30±3.89[b]	48.21±30.09[a]	34.43±25.63[a]	Within range
AST (10-37 Ul^{-1})	22.20±5.98[a]	14.86±3.84[b]	14.07±4.60[b]	Within range
ALT (10-37 Ul^{-1})	21.90±6.21[a]	14.71±5.08[b]	11.86±3.94[b]	Within range
Cu (1.2-1.4 mg l^{-1})	1.42±0.13[c]	1.92±0.17[b]	2.77±0.19[a]	Higher
Na (135-145 mmol l^{-1})	139.50±1.78[a]	140.57±1.55[a]	87.79±5.74[b]	Low
K (3.5-5.5 mmol l^{-1})	4.56±0.50[b]	4.24±0.46[b]	7.36±0.58[a]	Higher

All values are the mean±SD. Statistical significance between means was performed using one-way analysis of variance (ANOVA) followed by Duncan as a post ANOVA test ($p < 0.05$). GGT, gamma glutamyltransferase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALB, albumin; BLB, bilurubin; Cu, copper; Na, sodium and K, potassium
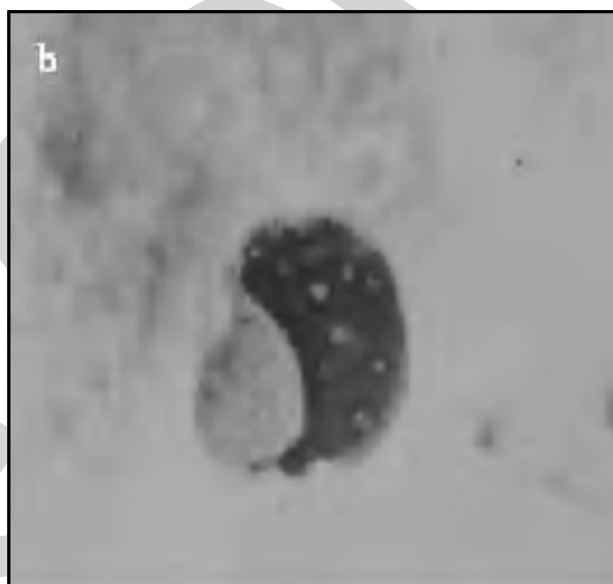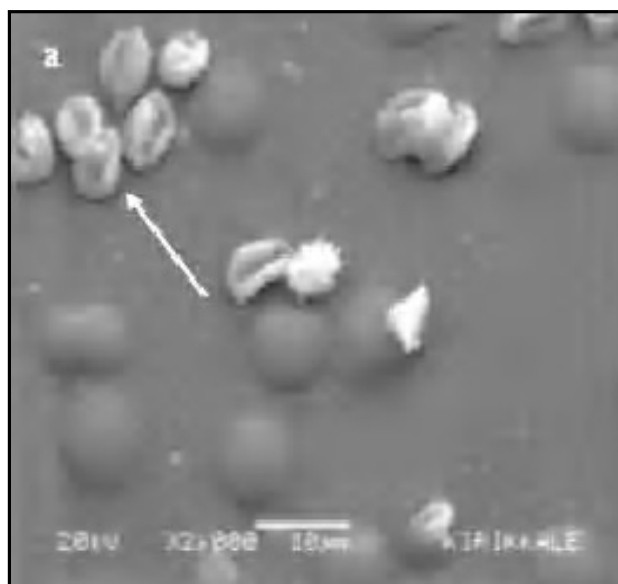
Fig. 1: (a) Electron microscopic image of erythrocyte. Arrow shows hemolysis, magnification, X 2000. (b) Light microscopic image of white blood cell. Showing cell deformation, magnification X 500

inhibition of Na/K transport in cells as erythrocytes. As a result, exposure to γ–radiation of cells induces changes in cation transport, and these changes in the transport activities may be increase or decrease concentrations in serum of the Na and K cations. In our study, findings showed that there was a significant increase in serum level of K and a decrease in serum level of Na. This information is in agreement with similar data reported by other authors so far. For example, Brugnara and Churchill (Brugnara and Churchill, 1992) investigated the effects of irradiation on cation content and membrane transport of red blood cell exposed to 20 Gy radiation. As a result, they observed a significant increase in external K and internal Na, and a decrease in internal K relative to the control units.

A higher GGT level was detected in serum samples taken from patients pre-radiotherapy compared to the controls. This finding can be cleared with the relationship balance between GGT activity and cancer. It is known that GGT activity is induced in numerous human carcinomas (Fiala *et al.*, 1979; Taniguchi *et al.*, 1985). This situation may be explained the high GGT activity observed in patients with lung cancer pre-radiotherapy. Moreover, a decrease in serum

GGT levels of all subjects was observed after radiotherapy. Although this decrease in serum GGT level was still significantly higher than the control group levels. The decreases may have resulted from some physicochemical changes such as generation of radicals, chemical bond weakening and hydration induced by γ–radiation. This circumstance changes the structure of the enzyme and inhibits the activity of the enzyme (Sedghi, 2005). This information have been noted in the studies from animals and humans exposed to radiation. For example, Altinas *et al.* (Altinas *et al.*, 2007) reported a decrease in serum GGT and ALP levels during the whole period of UVC radiation exposure in mice.

The rise in serum AST and ALT activities were observed at the end of 1th week of radiotherapy. It is difficult to explain the changing mechanism of serum ALT and AST parameters. It is likely that, these increases could be related to cell destruction. As is known, AST and ALT are synthesized by hepatocyte cells and they are sensitive and specific enzymes for liver disease (Senturk *et al.*, 2004). The rising in serum of AST and ALT levels may be commented as an indicator of liver disease. Although these enzymes are

expressed at a highest level in liver, they are also found in other tissues such as kidney, muscle and heart (Bellinger and Sloman, 1991; Minuk, 1998). In humans, AST and ALT levels rise during periods of chronic alcoholism, hepatocellular carcinoma and tissue injury (Garba and Gregory, 2005). Hence, a simultaneous increase in serum AST and ALT levels at the end of first week of radiotherapy may be probably related with γ–radiation-induced liver, heart and epithel tissue injury. But, serum AST and ALT levels significantly reduced at the end of radiotherapy period. The important fall in serum AST and ALT values could be related to the inhibitory effect of γ–radiation on enzyme activity. It was reported that radiation, directly or indirectly, causes damage in structure of hydroxyl, carboxyl and sulphydryl groups in structure of organic compounds as protein and enzyme. It also causes inactivation of ezymes or alteration of functions (Altinas *et al.*, 2007).

The results obtained in our study on GGT, AST and ALT are in agreement with those previous studies. For example, Kula *et al.* (1999) investigated alterations in serum biochemical parameters of steelworkers exposed to electromagnetic field. As a result, they reported a significant decrease in the level of total protein and in the activities of GGT, AST and malate dehydrogenase enzymes. In another study, El-Missiry *et al.* (2007) researched alterations in the levels of GGT, AST and ALT enzymes in serum of rats after exposure to different doses of γ–radiation. As a result, they observed a significant increase in the levels of these enzymes in mice exposed to γ-radiation. In a similar study, Arun *et al.* (2008) examined the levels of serum AST and ALT enzymes in 92 patients with head and neck cancer and 71 patients with cervix cancer receiving radiotherapy. As a result, they determined that AST and ALT values were much higher in all the malignant cases when compared with the healthy individuals. The values decrease and approach normal levels during radiotherapy progresses and, in 92% of head and neck cancer cases with no disease activity, the AST and ALT were normal or near normal.

In conclusion, the most changes in serum biochemical parameters of patients with lung cancer were found with exposure to α-radiation. We consider that these differences between the radiation-treatment and control groups may reflect a adaptation to harmful stimulate or a general response of the organism against to γ-radiation. Therefore, side effects of radiotherapy applications on healthy cells must be minimized or alternative methods should be developed.

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