Serum Trace Elements and Immunoglobulin Profile in Lung Cancer Patients

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ABSTRACT

Objective: The purpose of the study was to compare serum concentrations of trace elements (zinc [Zn], copper [Cu], and manganese [Mn]) and immunoglobulins (IgG, IgA, and IgM) of lung cancer patients with those of healthy subjects and to determine the relationships between trace elements and immunoglobulin levels with their nutritional status and socioeconomic factors.

Methods: The study was conducted in 45 lung cancer patients and 50 age- and gender-matched healthy volunteers. Flame atomic absorption spectroscopy method was employed to analyze serum trace element concentrations, and turbidimetry method using immunoglobulin kit was used for the estimation of serum immunoglobulin levels. Independent sample *t*-test, Pearson's correlation analysis, regression analysis, and one-way analysis of variance (ANOVA) were performed to analyze the data.

Results: Compared to the control subjects, lung cancer patients had significantly ($P < 0.05$) lowered body mass index (BMI). Independent sample *t*-test depicted that serum concentrations of Zn and Mn were decreased while serum concentration of Cu increased significantly in the lung cancer patient group. In the case of immunoglobulins, only IgG were found to be decreased significantly ($P < 0.05$) in the lung cancer patients compared to the control subjects. The concentrations of Zn, Cu, and Mn were 0.028 ± 0.007 mg/L, 1.91 ± 1.1 mg/L, and 0.011 ± 0.15 mg/L, respectively, in lung cancer patients, and 1.14 ± 0.27 mg/L, 1.15 ± 1.09 mg/L, and 0.44 ±

0.59 mg/L, respectively, in the healthy controls. Immunoglobulin G concentration was found to be 14.96 ± 3.92 g/L in lung cancer patients and 20.56 ± 8.02 g/L in healthy volunteers. The concentrations of serum IgA and IgM were found to be unchanged. Correlation analysis did not indicate any significant relationship among the parameters but multiple regression analysis showed a significant relationship between BMI and serum IgG level of lung cancer patients that was further justified by a one-way ANOVA test.

Conclusion: Serum concentrations of Zn and Mn were decreased, but serum concentration of Cu increased significantly in the lung cancer patient groups. In the case of immunoglobulins, only IgG were found to be decreased significantly ($P < 0.05$) in lung cancer patients compared to the control subjects.

INTRODUCTION

Lung cancer is the most common malignancy in the world. Its overall 5-year survival rate is only 15%, compared to 61% for colon cancer, 86% for breast cancer, and 96% for prostate cancer.[1-3] It accounts for approximately 6% of all deaths in the United States each year and is the leading cause of cancer death for both men and women.[4] There are 2 major types of lung cancer: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Non-small cell lung cancer accounts for approximately 75% of all lung cancers diagnosed.[5] The radiographic findings and clinical presentation provide a presumptive differentiation between SCLC and NSCLC. Massive lymphadenopathy and direct mediastinal invasion are well-recognized phenomena in SCLC.[6,7] A mass in or adjacent to the hilum is a particular characteristic of SCLC and is seen in about 78% of cases.[6,7] In the case of NSCLC, the method of achieving a diagnosis is usually dictated by the presumed stage of the disease. Non-small cell lung cancer is further classified into squamous and large cell carcinomas and adenocarcinoma.

When advanced, lung cancers are difficult to treat and existing therapies often do not offer long-term disease control. The poor prognosis is largely due to lack of sufficient screening and early diagnostic tools to physicians. Currently the screening and early diagnosis of lung cancer relies mainly on chest x-ray, low-dose computed tomography, bronchoscopy, sputum cytology, and tumor markers including carcinoembryonic antigen (CEA), cytokeratin-19 fragments (Cyfra21-1), carbohydrate antigen 19-9 (CA19-9), squamous cell carcinoma antigen (SCCAg), and neuron-specific enolase (NSE).[8] All these methods, however, lack adequate sensitivity and/or specificity.[9-12] Thus, there is an urgent need to search for more specific methods that would provide more specific information for screening and early diagnosis of lung cancer. Because of the marked heterogeneity of lung cancer, a panel of biomarkers for screening and diagnosis would be most appropriate.[12]

The biological role of trace elements, especially copper (Cu) and zinc (Zn), in different physiologic and pathologic conditions has been extensively investigated in many diseases.[13,14] Changes in serum Cu and Zn levels have been found in lymphoproliferative disorders, ovarian, breast, lung, and gastrointestinal cancer, sarcomas, and some other types of malignant tumors.[15-20] In many studies, it was found that 3-dimensional active conformations of many proteins are maintained by trace elements. Some metal ions play important role to form the 3-dimensional structure of proteins.[21] Trace elements at optimum levels are required for numerous metabolic and physiological processes in the human body as well.[21] Zinc and Cu are

Table 1. Socioeconomic Status and Chronic Energy Deficiency (CED) Data of Lung Cancer Patients (n = 45) and Controls (n = 50).

	Lung Cancer Patients			Controls		
Parameter	**n**	**%**	**Mean ± SD**	**n**	**%**	**Mean ± SD**
Education						
Illiterate	20	44.44		18	36	
Secondary (vi-x class)	12	26.67		9	18	
Higher secondary	8	17.78		15	30	
Graduate and above	5	11.11		8	16	
Occupation						
Service	15	33.33		16	32	
Small business	18	40		22	44	
Housewife	12	26.67		12	24	
Monthly income in US$						
0-50	14	31.11	109.53 ± 70.44	12	24	97.53 ± 60.62
51-100	16	35.56		14	28	
101-150	6	13.33		9	18	
151-200	6	13.33		8	16	
201-300	3	6.67		7	14	
Age in years						
25-40	13	28.89	52.33 ± 12.03	15	30	452.86 ± 13.21
41-50	12	26.67		12	2	
51-60	14	31.11		15	30	
61-80	6	13.33		8	16	
Smoking behavior						
Non-smoker	5	11.11		14	28	
Partial smoker	22	48.89		15	30	
Habitual smoker	18	40		21	42	
Marital status						
Married	35	77.78		35	70	
Unmarried	10	22.22		15	30	
BMI* (kg/m^2)						
15.5-18.4 (CED)	13	28.89	19.79 ± 2.58	4	8	25.53 ± 4.54
18.5-25.0 (Normal)	30	66.67		19	38	
>25.0 (Obese)	2	4.44		27	54	

*$P < 0.05$.

important cofactors for several enzymes that play a role in maintaining DNA integrity.[22] Manganese (Mn) plays an important role as a cofactor for various enzymes. It is involved in glycosyltransferase and other enzymes, including those needed for chondroitin sulfate synthesis and connective tissue metabolism.[23] Therefore, imbalances in the optimum levels of these trace elements may adversely affect biological processes and are associated with many fatal diseases, such as cancer, and hepatitis.[24] For instance, many studies found that patients with human squamous cell carcinoma of the esophagus had a significantly lower level of serum Zn compared with age-matched healthy

Table 2. Serum Trace Elements Concentration of Lung Cancer Patients (n = 45) and Controls (n = 50).

Trace Elements (mg/L)		Patients N	%	Mean ± SD	Controls n	%	Mean ± SD	*P* Value
Zn	<0.03	32	71	0.028 ± 0.007			1.14 ± 0.27	0.000
	0.03-1	13	29		18	36		
	>1				32	64		
Cu	<0.03			1.91 ± 1.1	13	26	1.15 ± 1.09	0.000
	0.03-1	11	24		17	34		
	>1	34	76		20	40		
Mn	<0.03	39	86.67	0.011 ± 0.15	24	48	0.44 ± 0.59	0.000
	0.03-1	6	13.33		13	26		
	>1				13	26		

controls ($P < 0.05$).[25] Extensive studies have also been done on the role of Cu in cancer prognosis. It was found that serum Cu level was increased in bronchogenic carcinoma.[26] The serum level of Mn also changes in various cancerous diseases.[27]

During development of cancer, the ability of the immune system to identify and destroy nascent tumors and thereby to function as a primary defense against cancer has been debated for many decades. Recent findings now offer compelling evidence that particular immune cell types, effector molecules, and pathways can sometimes collectively function as extrinsic tumor suppressor mechanisms.[28] Therefore, during cancer prognosis, levels of immunoglobulins and complements are altered markedly to compensate for the changing environment of the cancer cell. Circulating immune complexes are detectable in patients with carcinomas of the head and neck, stomach, rectum, external genitals, lungs, and in patients with Hodgkin's disease or melanomas.[29] Immunoglobulin levels are found abnormal in all the aforementioned conditions. The serum level of IgG was found to be increased in patients with brain tumors while those of IgA and IgM remained unchanged.[30] Previous studies revealed that lung cancer patients have defective immune responses, and different factors might contribute for the development of the disease.[31,32]

In light of the above investigations, the present study was designed to investigate the serum levels of trace elements (Zn, Cu, and Mn) and immunoglobulins (IgA, IgG, and IgM) to distinguish lung cancer patients from a healthy population. The study also investigated the correlation among concentrations of trace elements and also determined the relationship of trace element and immunoglobulin levels in lung cancer patients with their nutritional status and socioeconomic factors.

MATERIALS AND METHODS

Study Subjects

The study group consisted of 45 newly diagnosed lung cancer patients (25 males and 20 females). The patients were randomly recruited from Ahsania Mission Cancer Hospital, Dhaka Medical College Hospital, Holy Family Red Crescent Hospital, and Bangabandu Sheikh Mujib Medical University, Dhaka. The control group consisted of 50 healthy subjects (25 males and 25 females). Both study and control groups were of the same socioeconomic status with similar food habits. The mean age was 52.33 ± 12.03 years (range 25-80

Table 3. Serum Immunoglobulins Concentration of Lung Cancer Patients (n = 45) and Controls (n = 50).

Immunoglobulins (g/L)		Patients n	Patients %	Patients Mean ± SD	Controls n	Controls %	Controls Mean ± SD	*P* Value
IgG	<15	8	18	14.96 ± 3.92	11	22	20.56 ± 8.02	0.021
	16-20	17	38		18	36		
	>20	20	44		21	42		
IgA	2-4	6	13	5.06 ± 1.88	8	16	4.50 ± 1.40	0.265
	5-6	30	67		31	62		
	>6	9	20		11	22		
IgM	<3	14	31	5.33 ± 2.24	19	38	4.49 ± 2.44	0.762
	3-5	23	51		22	44		
	>5	8	18		9	18		

years) in the lung cancer patients group and 52.86 ± 13.21 years (range 25-80 years) in the control group. The diagnosis of lung cancer patients was confirmed histologically by biopsy. Regarding patients, both SCLC and NSCLC patients were identified as lung cancer patients. The study subjects were briefed about the purpose of the study, and written consent was taken from each of them. Ethical approval was obtained from the Bangladesh Medical Research Council (BMRC).

Socioeconomic and smoking information were collected in a questionnaire. A routine physical check-up including organ activity, weight, nutritional condition, and blood pressure was preformed on all of the patients by an oncologist. Socioeconomic information was recorded at the time of admission into the hospital. Anthropometric data (height and weight) and information on smoking habit were collected during hospitalization under the direct supervision of a lung cancer specialist. All subjects had to go through clinical examination to determine existence of other diseases such as viral infections, liver disease, etc, that might alter trace elements and immunoglobulin level. Pathological tests such as complete blood count, thyroid function test, renal impairment test, and liver function test were also performed for each subject. The selection criteria for the patients were as follows:

1. Patients who had not gone through any treatment (surgery, radiation therapy, or chemotherapy)
2. Patients who did not suffer from any major illness in the past
3. Patients who had not taken long course of any mineral supplement for the last 4 to 6 months

The control subjects were healthy individuals with sound physical health.

Blood Collection

A 5-mL venous blood sample was collected from the antecubital vein of each of the lung cancer patients and healthy volunteers in a metal-free sterile tube, between 8 to 9 AM, and after an overnight fasting. Standard precautions for trace element determination were taken; samples with signs of hemolysis were discarded. The blood was then allowed to clot and centrifuged for 15 minutes at 3000 rpm to extract the serum. The serum was aliquoted into eppendorf tubes and stored at -80°C for analysis of trace elements and immunoglobulins. Blood collection and serum separation were carried out in a dust-free environment.

Table 4. Correlation Between Different Parameters in Lung Cancer Patients (n = 45).

		Zn	Mn	Cu	IgG	IgM	IgA
BMI (kg/m^2)	R	0.019	0.048	0.106	0.024	0.055	-0.032
	P	0.900	0.752	0.490	0.877	0.720	0.836
Income	R	-0.009	-0.023	-0.156	-0.073	-0.247	-0.082
	P	0.955	0.881	0.308	0.635	0.102	0.594
Age	R	0.134	0.203	-0.166	0.261	0.192	0.045
	P	0.380	0.181	0.275	0.083	0.207	0.767

R = Pearson correlation; *P* = significance (2-tailed).

Analysis of Trace Elements

The trace element (Zn, Cu, and Mn) levels in both patients and controls were determined by using flame atomic absorption spectrometry (Varian SpectraAA 220) according to the method of Akyol et al.[33] Serum samples were diluted by deionized water by a factor of 30. Different concentrations (0.5, 1.0, 2.0, 5.0, and 10.0 mg/L) of trace elements were used for calibration of standard graphs. Absorbance was read at 324.7 nm, 213.9 nm, and 279.5 nm for Cu, Zn, and Mn, respectively, in the atomic absorption spectrometer. To verify the assay accuracy and to maintain quality, the standard solutions were run for every 10-test sample. A software package (SpectrAA software) was used to calculate concentrations of Cu, Zn, and Mn. Variation coefficients were 1% for Mn and Zn and 4% for Cu. Extensive precautions were taken for both collection and subsequent handling of serum in order to avoid or minimize trace elements contamination.

Immunoglobulin Profiling

The serum immunoglobulin (IgG, IgA, and IgM) levels in both patients and controls were determined by turbidimetry method using an immunoglobulin kit (Chronolab, Switzerland). In this method, anti-human antibodies were mixed with samples containing IgG, IgA, and IgM that formed insoluble antigen-antibody complexes. These complexes caused an absorbance change depending upon the immunoglobulin concentration that was quantified by a calibrator. The serum was diluted with saline (1:4), and 10 μL of the diluted serum was pipetted into microtitre plate. Separate microtitre plate was used for each of the immunoglobulins (IgG, IgM, and IgA); 5 μL, 10 μL, 25 μL, 50 μL, and 75 μL calibrator protein were also pipetted into marked wells of each of the microtitre plate for calibration. Then, 230 μL of tris-buffer (pH 7.5) was added to each serum-containing well of the 3 plates. The tris-buffer was added to calibrator containing wells to make a total volume of 240 μL. The plate content was mixed well with the help of a vortex mixer. Diluted respective anti-human IgG, IgM, and IgA (1:1 diluted with saline) then were added to the wells of their respective microtitre plates. The plates were incubated for 2 minutes (as specified in the kit procedure) to react the anti-human immunoglobulin with the test serum and calibrator protein. After proper mixing, absorbance was taken at 550 nm for IgG and IgA and at 405 nm for IgM. Calculation was done with the help of calibration curves for each immunoglobulin.

Statistical Analysis

The SPSS software package (Version 11.5, SPSS Inc., Chicago, Illinois, USA) was used to analyze the data. Descriptive statistics were used for all variables. Values were expressed as percentage, mean, and standard deviation. Comparison of trace elements and immunoglobulins of lung cancer patients and controls were performed by cross-table variables and independent sample *t*-test. Correlative analysis was performed to find correlation of body mass index (BMI) and socioeconomic factors on the serum trace element and immunoglobulin concentrations. Multiple regression analyses and one-way analysis of variance (ANOVA) were also performed to determine the extent of contribution of socioeconomic factors and BMI to affect the level of trace elements and immunoglobulins. All comparisons were 2-tailed, and P values of <0.05 were considered significant.

RESULTS

Table 1 shows the socioeconomic information of the lung cancer patients and control subjects. It was shown that the majority of lung cancer patients were literate (56%) with various professions having average monthly income of US$109.53 ± 70.44, average age 52.33 ± 12.03 years, and 78% were found to be married. The mean BMI of patients was 19.79 ± 2.58 kg/m^2, which was significantly ($P < 0.05$) lower than that of the control subjects (25.53 ± 4.54 kg/m^2). The vast majority (89%) of the patients were smokers.

Serum trace elements levels are presented in Table 2. The concentrations of Zn, Cu, and Mn were 0.028 ± 0.007 mg/L, 1.91 ± 1.1 mg/mL, and 0.011 ± 0.15 mg/L, respectively, in the lung cancer patients, and 1.14 ± 0.27 mg/L, 1.15 ± 1.09 mg/L, and 0.44 ± 0.59 mg/L, respectively, in the healthy controls. Trace elements analysis indicated that the concentrations of serum Zn and Mn level were decreased significantly ($P < 0.05$) in lung cancer patients compared with control patients, while the concentration of serum Cu level was increased significantly ($P < 0.05$) in lung cancer patients compared with control subjects. Serum concentrations of immunoglobulins are presented in Table 3. Serum concentrations of IgG, IgA, and IgM of lung cancer patients were found to be 14.96 ± 3.92 g/L, 5.06 ± 1.88 g/L, and 5.33 ± 2.24 g/L, respectively, and 20.56 ± 8.02 g/L, 4.50 ± 1.40 g/L, and 4.49 ± 2.44 g/L, respectively, in control subjects (Table 3). Serum analysis indicated that only IgG concentration was decreased significantly ($P = 0.021$) in lung cancer patients while the concentrations of IgA and IgM were not changed significantly ($P > 0.05$).

Correlation of serum trace elements and immunoglobulins in lung cancer patients with their socioeconomic factors are presented in Table 4. No significant correlation was found among the parameters. We also did not find any significant relationship between serum trace element concentrations ($P > 0.05$).

Multiple regression analysis was also performed using the serum concentration of trace elements and immunoglobulins with socioeconomic factors. A significant relationship was found only with IgG level of patients as a dependent variable and BMI as an independent variable. Multiple linear regression analysis found that the IgG level had changed significantly with BMI ($R^2 = 0.815$; $t = -3.567$; $P = 0.03$). Finally one-way ANOVA test was done with the same parameter and a significant relation was found between BMI and IgG level of the patients ($F = 7.147$; $P = 0.006$).

DISCUSSION

The role of trace elements in various cancers has been the subject of surmise, and, surprisingly, reports of different authors are often conflicting and contradictory. A major reason for these discrepancies is the difficulty in analyzing trace elements and the problems that exist in collecting specimens without contamination.[34] Likewise, here, we have analyzed the serum concentrations of Zn, Cu, and Mn in lung cancer patients. Our analysis of serum trace elements indicated a significant decrease in the serum concentration of Zn and Mn and an increase of serum Cu concentration in lung cancer patients compared with those of the control subjects. Previous reports also found a decreased serum Zn level and increased Cu level in lung cancer patients compared to controls, which is in agreement with our findings.[35-37] Serum Cu has also been found elevated in bronchogenic carcinoma and squamous cell carcinoma of the larynx.[26] Interestingly, in another study, Diez and colleagues[37] found a higher level of Mn and lower level of Zn in lung cancer patients than those found in control samples. Low serum Zn level has been observed in serum of patients suffering from bronchus and colon cancer, but not in other forms of cancer.[38] Conversely, one of the previous studies found higher concentration of Zn in cancerous lung tissue as well.[27] Zinc acts as a cellular growth protector, including growth of neoplastic cells, and its deficiency was demonstrated to be involved in several stages of malignant transformation.[39] Trace elements play an important role in maintaining 3-dimensional structures of many proteins such as thymidylate synthetase, dihydrofolate reductase, p53, p16, K-ras, among others,[21] so it may be suggested that altered level of trace elements in the cancer patients may induce the deformation of various protein structure. It is further noted that the deficiency of trace elements might be a risk factor for the development of some cancers.[40]

Serum immunoglobulin analysis depicted that only IgG level was decreased significantly in the lung cancer patients, while IgA and IgM concentrations remained unchanged. Previous studies suggest that in pulmonary carcinoma, the concentrations of immune complexes and IgG correlate significantly.[29] Viramontes et al[41] found decreased IgA and IgG concentration in lung cancer patients. Previous reports also suggested defective immune activity in cancer patients.[31,32] Some investigators reported a positive correlation between the extent of metastatic breast cancer and the serum level of various immunoglobulins, particularly IgA.[42] Though our study suggested decreased level of serum IgG in lung cancer patients, elevated levels of serum IgG, IgA, or IgM antibodies were frequently observed in cancers of epithelial origin, including carcinomas of breast, colon, and liver.[43] These tumor-reactive immunoglobulins have been interpreted as humoral responses of the host to cancer growth.[44] In fact, by Serex method, it has been firmly established that these tumor-reactive antibodies are capable of binding to normal and tumor-associated antigens, including those of cell surface and intracellular proteins.[45]

From sociodemographic data, it was found that the mean BMI of lung cancer patients was significantly ($P = 0.000$) lower than that of the control subjects, which is well predicted (Table 1). An elevated risk of lung cancer associated with lower levels of BMI has been reported in case-control and cohort studies, which supports our finding.[46] Regression analysis also found a significant relationship between BMI and IgG level in cancer patients, which is further fortified by a one-way ANOVA test. Serum concentration of IgG and BMI of lung cancer patients were reduced significantly compared to healthy volunteers. Again, a significant relationship was also found between BMI and IgG level of lung cancer patients. Therefore, it is inconclusive whether the disease affects the serum level of IgG.

Further studies are suggested to elucidate the relationship between trace elements and immunoglobulin level in lung cancer patients that can be used as a tool for the diagnosis of lung cancer patients.

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