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Levels of Some Trace Elements in Sera of Patients with Lung Cancer and in Smokers

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ABSTRACT

Lung cancer is one of the most common leading causes of cancer mortality around the world. Many efforts have been made recently to use element contents in human body to aid for diagnosing various diseases including cancer. Accordingly, this study aimed to investigate the serum contents of elements Cr, Cu, Mn, Ni, Zn, Mg, and Fe of lung cancer patients and compared them with those found in smokers and non-smoking controls using atomic absorption spectrophotometry technique. The results obtained showed that there were significantly lower levels of Zn, Fe, and Mg (p<0.001) in patients and smokers than in non-smokers. The Zn and Mg levels of patients were significantly lower than in smokers. The serum levels of Cu, Mn, Ni, and Cr were significantly higher (p<0.001) in lung cancer patients than in smokers and controls. In addition, it was found out that the Cu/Zn ratio was significantly higher in patients and smokers than those of controls. The results suggest that adequate intake of Zn and Mg may have a protective role in the occurrence of lung cancer, while the high levels of Cu, Mn, Ni, and Cr may cause a threat of lung cancer. It is also possible to conclude that exposure to cigarette smoke leads to impair oxidant defense system. The data suggest that Cu/Zn ratio could be of great value in diagnosis and evaluating the lung cancer. In addition, it is possible to rely on trace elements to give indications to warn, especially the smokers, of the risk of lung cancer.

Key words: *Lung cancer, Trace elements, Cu/Zn ratio, Atomic absorption spectrometry, Smoking.*

1. INTRODUCTION

Cancer is a group of diseases that lead in most cases to death. It characterized by spread and uncontrolled growth of abnormal cells. It has been reported that 5-10% of cancer cases are due to genetics while 90-95% of cases are due to environmental agents, and therefore, cancer is considered an environmental disease [1,2]. It has been found that tobacco, diet and obesity, infections, radiation, stress, lack of physical activity, and environmental pollutants represent the common environmental agents that contribute to cancer death [3].

Lung cancer is the most important causes of cancer-related death worldwide [4]. In fact, it is one of the most important and frequent malign tumors, where it represents over 90% of lung tumors. The clinical signs in this disease appear extremely late in evolution. It has been found that less than a third of lung cancer cases being in a useful therapeutic stage with a survival rate of 6-18 months. Of these cases, only approximately 20% live more than 1 year. Lung cancer causes over 1.4 million of death per year registered at global level [5,6].

Most of studies concerning lung cancer are centered on the proved role of tobacco in carcinogenesis [7,8]. It was found that the risk of lung cancer is at least 20 times higher in smokers compared to non-smokers. This risk is demonstrated through the rate of deaths which occurs because of lung cancer, where it is recorded in non-smoker healthy subjects of approximately 8/100000 inhabitants, but of 138/100000 inhabitants recorded in cigarette smokers, on the whole, and of 266/100000 inhabitants in heavy smokers [9,10]. It has been unambiguously demonstrated that the risk of lung cancer increases with both duration of smoking and amount of smoking. Furthermore, it has been observed that the risk of lung cancer is higher in those who start smoking at a younger age [11].

Trace elements are essential components of biological structures and have several important roles in living organisms. They are dietary minerals that are needed in very small amounts for the

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suitable growth, development, and physiology of the body. They are essential for enzymes reactions and have important actions throughout most of the biological processes [12-14]. Although the trace elements are essential components for biological activities, the immoderate levels of them can be toxic for the body health. It has been found that the imbalances in the trace elements optimum levels may adversely affect biological processes and are associated with many fatal diseases, such as toxoplasmosis and many types of cancers [15,16]. Furthermore, it has been found that there is a close relationship between pathological accumulation of heavy metals in lung tissue and the process of tumor growth which may have an implication in the pathogenesis of lung cancer [17]. In fact, estimation of biochemical changes in some trace elements may be of epidemiological importance in the early diagnosis, prognosis, and therapy evaluation of lung cancer patients. Hence, they may be considered as biomarkers for cancer risk.

Cigarette smoking is one of the main causes of lung cancer. Cigarette smoke contains about 73 known carcinogens, including metals [18]. However, the metals levels in cigarette smokers and their relation with lung cancer risk have not been studied extensively.

Despite considerable efforts that have been made from a lot of researchers, there are still delays in the diagnosis and treatment of lung cancer. Accordingly, this study aimed to investigate a possible relationship between the levels of some elements that may be affected in lung cancer patients as well as in smokers. Moreover, therefore, study the perturbations that may occur in these variables in smokers, and possibility to utilize them as an early indication to predict the risk of developing lung cancer. To this end, the analytical technique atomic absorption spectrometry was used to determine the levels of elements (Cr, Cu, Mn, Ni, Zn, Mg, and Fe) in serum samples of lung cancer patients as well as in serum samples of smokers and non-smokers subjects.

2. EXPERIMENTAL

2.1. Subjects and Study Design

This study was achieved between October 2014 and June 2015 in poison center at Ghazi Hariri Hospital for Specialized Surgery/Medical City/Ministry of Health and the laboratories of Chemistry Department/College of Sciences/Mustansiriyah University, Baghdad, Iraq. The study includes three groups: (1) 40 male lung cancer patients group (their ages range 45-63 years with a mean age 53.4 years), (2) the control group (non-smokers group) comprised of 35 male healthy volunteers (their ages range 42-65 years with mean age 51.4 years), and (3) 38 male smokers group, their ages range from 20 to 25 years with mean age 22.37 years.

2.2. Sample Collection

Blood samples were collected from patients with lung cancer, control, and smokers. From each individual, 10 ml of blood were drawn by vein puncture using disposable syringes. The blood sample was placed in plastic disposable tube, and then, left to stand at room temperature for 20-30 min. Sera were separated from clotted blood by centrifugation at 5000 rpm for 10 min, and the obtained serum transferred immediately to another test tube. These samples were frozen at −20°C for subsequent analysis.

2.3. Method of Elements Assay

All standard elements and chemicals used were supplied by Sigma-Aldrich, Germany. Serum elements Cu, Zn, Mg, and Fe of all samples were measured using flame atomic absorption spectrophotometer (FAAS, Model AA646, Shimadzu Corporation, Kyoto, Japan), whereas the Cr, Mn, and Ni were measured using flameless atomic absorption spectrophotometer (GFAAS, Model 210VGP, Buck Scientific, USA) [19-21]. The serum samples were diluted with 10-fold deionized distilled water (nanopure water (18.3Ω)) for Cu, Zn, and Fe measurements. Determination of these elements was performed at wavelengths 324.7 nm, 213.9 nm, and 248.3 nm, respectively. For determination of Mg, the samples were diluted 50-fold with lanthanum chloride (LaCl2.7H2O), and the assay was performed at wavelength 285.2 nm. The determination of Ni, Mn, and Cr was carried out at wavelengths 238 nm, 379.5 nm, and 357.9 nm, respectively, by injection the serum samples to the graphite tube of the GFAAS. A standard calibration curve was prepared using the following concentrations (0.05, 0.1, 0.2, 0.4, 0.8, 1.6, and 2 μg/ml) of each element. Furthermore, to obtain optimal results, diluted hydrochloric acid was periodically aspirated to clean nebulizer system of the AAS, and in addition, the burner head was cleaned before every run.

2.4. Statistical Analysis

The statistical analyses were carried out using the Statistical Package for Social Sciences computer program (SPSS) version 20.0 for Windows (IBM SPSS Statistic software, IBM Corporation, New York, United States). One-way analysis of variance test was used to assessment the mean differences between three study groups. The result values were expressed as a mean ± standard deviation, and the upper and lower limits of data were recorded for each element. The statistical tests were considered to be statistically significant at $p<0.05$ with 95% confidence ınterval.

3. RESULTS AND DISCUSSION

3.1. Results

The collective results for quantitative analysis of the elements (Cr, Cu, Mn, Ni, Zn, Mg, and Fe) among three study groups are summarized in Table 1. These results indicated that the mean serum values of Cr,

Table 1: Serum levels of elements (Cr, Cu, Mn, Ni, Zn, Mg, and Fe) in patients with lung cancer, smokers, and non-smokers (control).

Element (µg/L)	Control (non-smoker)		Smokers		Patients		p value	p value	p value
	Mean±SD	Upper and lower limits	Mean±SD	Upper and lower limits	Mean±SD	Upper and lower limits	Non-smokers and patients	Non-smokers and smokers	Smokers and patients
Cr	0.148±0.038	0.163	0.236±0.028	0.245	0.343±0.077	0.371	<0.001	<0.001	<0.001
		0.134		0.227		0.318			
Cu	10.565±1.084	10.962	13.063±0.756	13.311	16.099±0.847	16.372	<0.001	<0.001	<0.001
		10.167		12.815		15.824			
Mn	1.25±0.306	1.363	1.926±0.575	2.112	3.03±0.382	3.156	<0.001	<0.001	<0.001
		1.134		1.739		2.909			
Ni	1.89±0.245	1.981	3.07±0.359	3.186	4.21±0.418	4.336	<0.001	<0.001	<0.001
		1.798		2.953		4.064			
Zn	9.64±0.612	9.867	7.41±0.767	7.659	5.72±0.736	5.949	<0.001	<0.001	<0.001
		9.417		7.156		5.471			
Mg	12.29±0.881	12.605	11.43±1.765	12.003	10.16±1.485	10.661	<0.001	0.015	<0.001
		11.969		10.859		9.656			
Fe	176.92±65.786	201.947	84.66±25.815	96.102	86.73±23.729	93.115	<0.001	<0.001	0.84
		151.899		73.211		80.285			

SD: Standard deviation

Cu, Mn, and Ni (0.343, 16.099, 3.03, and 4.21 μg/L, respectively) in lung cancer group were raised when compared to that of the control group (0.148, 10.565, 1.25, and 1.89 μg/L, respectively) (Figure 1). This increase was found to be highly significant ($p<0.001$). Furthermore, the Cr, Cu, Mn, and Ni values of lung cancer patients were higher than those of smokers (0.236, 13.063, 1.926, and 3.07 μg/L, respectively), and statistically, this increase were highly significant ($p<0.001$). Furthermore, the Cu, Ni, Mn, and Cr levels were noted to be higher in smokers in comparison to healthy control subjects ($p<0.001$) (Table 1 and Figure 1).

When Zn and Mg levels were compared between groups, the results indicate that the values measured in lung cancer patients (5.72 and 10.16 μg/L, respectively) were lower than those of healthy subjects (9.64 and 12.29 μg/L, respectively) (Figure 2), and statistically, the difference between them was highly significant ($p<0.001$). In addition, the results of lung cancer patients showed a presence of highly significant decrease in Zn and Mg values ($p<0.001$) compared with smokers group (7.41 and 11.43 μg/L, respectively), also the presence of highly significant decrease in Zn value ($p<0.001$), and significant decrease in Mg value ($p<0.05$) of smokers group compared with the values of control group (Table 1 and Figure 2).

Furthermore, the results obtained indicate that the Fe value of lung cancer patients (86.73 μg/L) was highly significantly lower ($p<0.001$) than the value measured for the healthy control subjects (176.92 μg/L) (Figure 3). The results also reveal no significant difference in levels of Fe between smokers and patients ($p>0.05$), but there was highly significant decrease ($p<0.001$) in Fe level of smokers (84.66 μg/L) compared with healthy control subjects (Table 1 and Figure 3).

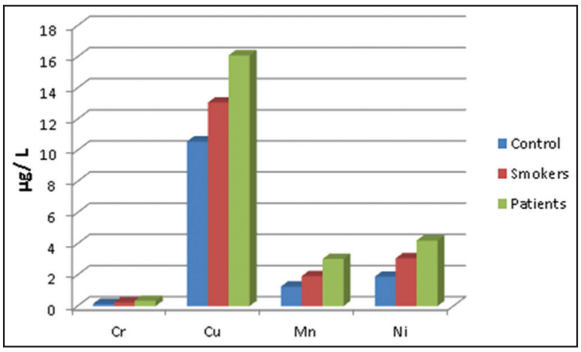

Figure 1: Diagram shows the mean values of serum Cr, Cu, Mn, and Ni levels (μg/L) in lung cancer patients and control group as well as smokers group.

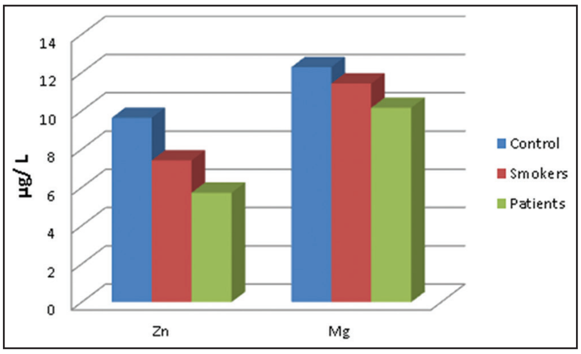

Figure 2: Diagram shows the mean values of serum Zn and Mg levels (μg/L) in lung cancer patients and control group as well as smokers group.

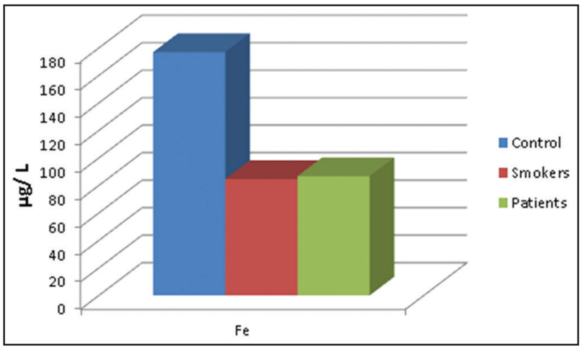

Figure 3: Diagram shows the mean values of serum Fe levels (μg/L) in lung cancer patients and control group as well as smokers group.

3.2. Discussion

We found that the element levels in serum of patients with lung cancer differ significantly compared with their levels in smokers and controls. Therefore, these variables could be of great values and significant factors that can be rely on in the early diagnosis and predict the risk of lung cancer.

It has been reported previously that the cumulative exposure to Cr was found to be associated with an increased lung cancer risk [22]. Another study has been indicated similar results for lung cancer tissues, where the results showed that Cr contents in lung tumors of lung cancer patients were significantly higher than those of normal lung tissue for the control subjects. This gave a clear indication that accumulation of Cr in lung tumors may play a crucial role in the development of lung cancer [23]. Moreover, it has been found that the serum level of Cr in the colon cancer patients was higher than those of healthy controls [24]. In fact, the findings of the present work give further evidence that Cr may play a role in etiology of lung cancer. However, the question remains how does that occur? Therefore, further comprehensive studies are needed to advance understanding of the relationship between Cr and its role in the risk of lung cancer.

Indeed, there are 55 carcinogens in cigarette smoke that have been evaluated by the International Agency for Research on Cancer (IARC) [25]. Cr is one of these carcinogens, where it was found that the Cr amount in mainstream cigarette smoke approximately 0.2-500 ng/cigarette [25,26]. Therefore, the increases, obtained in this study, in Cr levels in smokers may be related to the intake of this toxic trace element present in cigarette smoke, which lead with the continuation of smoking, to the risk of lung cancer.

The results obtained for Cu in this study are in good agreement with the published data of the previous studies [27-29] but disagree with the results obtained by Cobanoglu *et al.* [30], who found that the level of Cu was lower in lung cancer patients compared with the control. This study showed that high level of Cu as a result of smoking is associated with lung cancer. For this, it is possible that this element may play a role in the development and pathogenesis of lung cancer. It is important to note here that Cu is one of the elements that present in cigarette smoke, and therefore, this is may be the main reason of its high level that detected in serum of smokers. In fact, Cu is a redox-active transition metal that can participate in single electron reactions and catalyze the formation of free radicals, especially the undesirable OH radicals [31]. Hence, any increase in its levels, as a result of smoking, will lead to increase the formation of toxic free radicals, which in turn lead to increase the risk of lung cancer.

Manganese is an essential element that is required for health in trace amounts but becomes toxic at higher exposures. The previous studies [27,30] have been mentioned a high Mn level in lung cancer patients compared, which is in agreement with the findings of the present work. On the other hand, there is a study that has reported a decreased Mn level in lung cancer patients compared with the control [32]. Furthermore, serum level of Mn has been found elevated in colon cancer patients [24]. According to the findings of the present work, it is possible to conclude that high level of Mn may play a vital role in pathogenesis of lung cancer. However, further comprehensive studies are needed to advance our understanding of the relationship between Mn element and its role in cancers. In fact, Mn amounts in cigarette smoke ranging from 155 to 400 µg/g [33], and accordingly, cigarette smoke may be the reason of elevated of Mn level in serum of smokers.

It is well known that Ni has a carcinogenic effect [15], where exposure to Ni can lead to the formation of free radicals in human tissues, which in turn cause enhanced lipid peroxidation and modifications to DNA bases. It was found that the primary route for Ni toxicity in the body is depletion of glutathione and protein-bound sulfhydryl groups, which lead to increased production of reactive oxygen species, such as superoxide anion, hydrogen peroxide, and hydroxyl radical [34,35]. A previous study has shown that Ni contents in lung tumors were significantly higher than those of normal lung tissue [23]. Furthermore, it has been indicated that the hair samples of smokers contain a greater amount of Ni compared with non-smokers [36].

Cigarette smoking represents a great source of toxic elements for human body, where cigarette contains approximately 0.078-5 µg/g of Ni [25]. The findings of the present work reflect the great risk of smoking, where measurement of Ni in sera has shown significantly higher levels in smokers than non-smokers, which therefore lead to increase the percentage of free radicals in human body. Furthermore, the findings of the present work have shown that there were higher levels of Ni in lung cancer patients compared to healthy persons. These findings suggest that the Ni may be a plausible lung carcinogenic factor.

Most important to know that Zn plays an anticarcinogenic role through structural stabilization of DNA, RNA, and ribosome and has a protective effect against free-radical injury, where it functions as a cofactor of superoxide dismutase [37]. Furthermore, it has been found that Zn has an important role as a cellular growth protector, including the growth of neoplastic cells [32]. There is an agreement between the findings of this study and the previous studies, where it has been revealed that the serum Zn level was significantly lower in lung cancer patients than those of controls [15,27-30]. Moreover and for the subject of smoking, the previous studies have been demonstrated that serum levels of Zn were lower in smokers than in non-smokers [38,39]. In contrast, there is a study indicated that serum level of Zn was higher in smokers than in non-smokers [40].

It has been interpreted previously that Zn deficiency could be a risk factor for prostate cancer [41]. In the present study, the results suggest that the decrease in Zn levels may be, in similar way with prostate cancer, a risk factor for development of lung cancer. It was suggested that Zn may act as a protection against lung cancer, and therefore, low levels of Zn can facilitate the pathogenesis of lung cancer [30].

As mentioned before, cigarette smoke contain a lot of carcinogens and oxidizing substances that produce toxic free radicals. It was found that Zn has antioxidant-like properties and has the ability to stabilize macromolecules against excess production of free radical [42]. Hence, more of Zn will consume to eliminate or to remove free radicals, and this may elucidate the reason of low levels of Zn measured in smokers. Furthermore, the low levels of Zn in lung cancer patients may be due to its involvement in the antioxidant defense since the patients might be under higher oxidative stress. In fact, further studies are warranted to determine whether a change in serum

Zn levels may represent an independent risk factor for lung cancer and therefore a possible target for cancer prevention.

Indeed, the Cu/Zn ratio in serum samples has been utilized previously as a way of evaluation and prognosis assessment of cancer patients [43,44]. Similarly, in the present study, it was found out that the Cu/Zn ratio has significantly increased lung cancer patients as well as in smokers (0.28 and 0.18, respectively) when compared with control subjects (0.11).

The data obtained in this study for Mg values agree with that reported in the previous study, which found that Mg level measured in lung cancer patients was lower than those in control group [30]. Interestingly, serum levels of Mg have been found elevated in prostate cancer patients [45] and colon cancer patients [24]. Furthermore, a previous study also found a decreased serum Mg level in smokers compared to control, which is in agreement with the findings of the present work [46].

Mg is an essential element which plays a role in diverse cellular reactions, and several metabolic and physiological functions, where it contributes in most anabolic and catabolic functions [47]. In fact, Mg shares approximately 300 different enzymatic reactions which are involved in several functions such as DNA and RNA synthesis and protein synthesis [48]. Although the role of Mg in carcinogenesis is not clear so far, there is evidence that Mg deficiency could cause leukemia and other types of cancers [49]. In the current study, low Mg level in serum was detected in lung cancer patients compared with healthy persons. These findings give further evidence that Mg deficiency could generate different types of cancers. The main reason of low level of Mg which detected in smokers may be attributed to the participating of Mg in the antioxidant defense system by increasing the activity of glutathione peroxidase (the enzyme responsible of the reaction between glutathione and free radicals) [50].

In this study, results indicated that there was a significant decrease in Fe levels in patients and smokers compared to control group. These results agree with the findings reported by Diez *et al*. [29] but disagree with the findings of the study conducted by Cobanoglu *et al*. [30]. Furthermore, it has been reported that there was a decrease in Fe level in patients with prostate cancer [45], renal cancer [51], breast cancer [52], and testicular cancer [53] when compared with those of controls. In fact, studies that discuss the relationship between iron and lung cancer and/or smoking are very rare. Fe is a redox-active transition metal. It can participate in the formation of hydroxyl radicals from hydrogen peroxide and superoxide by Fenton and Haber-Weiss reaction [54]. Moreover, this reaction may involve in the harmful effects of smoking. The possible ways to produce damage in the DNA from cigarette free radicals involve contribution of iron in the reduction of hydrogen peroxide to the hydroxyl radical (the radical responsible for binding to and nicking DNA) [55].

The finding of our study suggests that smoking may make the body needs more of iron, and therefore, causes a decrease in iron stores, to makes it more available to use in conversion of H_2O_2 to the hydroxyl radical. In addition, cancer induction and development may also cause a decrease in iron stores or metabolically available iron. The presence of an association between lung cancer and iron is observed in the present study.

4. CONCLUSION

There is a definite correlation between trace elements content and many common diseases including cancers. The present work gives new evidence about the significance of trace elements in the early diagnosis and prognosis of lung cancer. The results of the current work indicated low levels of Zn, Fe, and Mg elements in serum samples of both lung cancer patients and smokers than in controls. In contrast, the results indicated high levels of Co, Cr, Ni, and Mn in lung cancer patients and smokers than in those of healthy controls. These findings give a first impression that adequate intake of Zn and Mg may have a protective role in the occurrence of lung cancer, whereas the high levels of the elements Ni, Cr, Cu, and Mn may cause a threat of lung cancer. The data suggest that Cu/Zn ratio could be of great value in diagnosis and evaluating the lung cancer patients as well as a good indicator of the weakening of the oxidant defense system in smokers.

The present work refers to the great relevance of monitoring the levels of trace elements particularly considering the conditions of increasing smoking habit among young people, where it is possible to rely on trace elements levels to give indications to beware, especially for smokers, of the risk of lung cancer. Therefore, the obtained data may be of value and may present new information for understanding the possibly vital role of trace elements in the pathogenesis of lung cancer.

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