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Evaluation of the Concentration of Ubiquitin C Protein (UBC) in Patients of Lung Cancer and Comparing with Healthy Subjects

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A B S T R A C T

Ubiquitin protein has a conserved amino acid sequence, found in all eukaryotes and it is important in many of cellular functions .The increase or decrease in the ubiquitin level lead to causing many diseases including cancer .The aim of this study is to measure the concentration of ubiquitin c protein in blood serum of lung cancer patients and healthy control subjects by using ELISA technique. The Mean ± SD for ubiquitin c protein was (7.51 ± 5.76) in blood serum of the normal healthy subject and Std. Error mean was (1.82). While the Mean ± SD was (10.99 ± 4.56) for ubiquitin c protein in serum of lung cancer patients and Std. Error mean was (1.44) .The results showed no statistically significance the P value (0.15) between the protein concentration in the serum of patients with lung cancer compared to the serum concentration of healthy normal subjects.

1. Introduction

Ubiquitin is composed of 76 amino acids protein described by Goldstein G. in 1975 with a molecular mass of about 8.5 kDa, [1]. Ubiquitin is found either as a free ubiquitin protein (mono ubiquitin) or as a chain of ubiquitin (poly ubiquitin). Moreover, it is conjugated to other proteins by isopeptide linkage, between carboxyl terminal glycine residue of ubiquitin and the side chain of lysine on the proteins, also to be joined by isopeptide bond with lysine in another molecules of ubiquitin to build poly ubiquitin chains [2]. The ubiquitin protein plays an important role with proteasome for destruction and removing harmful, unwanted, misfolded proteins and for the proteins that have unnecessary highly increasing. Ubiquitin is also involved in protein modification, and regulation of signal pathways, in spite of not fully completed understanding on several ubiquitin modifications [3]. Ubiquitination, is a post translational modification (PTM) lead to protein destruction, which involves attaching ubiquitin protein to the target protein (misfolded protein) that will be delivered to

proteasome which is a complex protein that is responsible for degradation of unwanted proteins into small peptides, thus this type of PTM is important for regulation of apoptosis, DNA repair, gene transcription, and many other cellular processes [4,5]. Researchers found that, unusual expressions or disorders of components of the Ubiquitin Proteasome System (UPS) can effect human health and cause various diseases like neurodegenerative disorders, inflammations and different types of cancer by increasing the development of tumors or by inhibition its progress [6,7].

Ubiquitin is considered as naturally blood protein, representing up to 0.1-5% of total proteins concentration in eukaryotic cells [8]. ELISA technique provides a useful and reliable technique to estimate the concentration of ubiquitin c protein from serum of blood samples. A sandwich ELISA has been used in this study to measure levels of poly ubiquitin c (UBC) protein [9].

2. Materials and Methods

The study group included 30 patients of lung cancer with ages (22-73) years. Patients samples were collected at Al-Amal National Hospital for Cancer Treatment in Baghdad province/Iraq. The blood samples of healthy control group consist of (20) samples were obtained from healthy men and women, age ranged from (22-73) years. Samples that have possibility to have kidney problems were excluded the exclusion was done according to the abnormal elevation in serum creatinine in the study samples. Three milliliters (3ml) into gel tube to be used for measuring the creatinine concentration in addition to ubiquitin c protein concentration in samples by using qualitative ELISA kit. The blood that transferred into gel tube was separated by using centrifuge at (3000 rpm) for (10) minutes to obtain the serum. Creatinine level was determined by using commercially available creatinine kit (Cat. No. CRE1202)/ Mindary/China and Mindary BS-230 automated Clinical Chemistry Analyzer. The concentration of ubiquitin c protein were determined in this study with high sensitivity and specificity assay for detection of Human UBC by using Human Ubiquitin C (UBC) ELISA Kit (catalog no. MBS287857) according to the instruction of the manufacture .The type of Elisa is the quantitative Sandwich immunological procedures in which the Ag-Ab reaction is monitored by enzyme measurements.

3. Statistical Analysis

Statistical analysis was performed using SPSS to evaluate significant variance between the means. $P < 0.05$ was considered statistically significant and used to compare between means in this study.

4. Results

In this study, serum creatinine concentration was measured for all cancer patients as well as for healthy control subjects of the present study (9) lung cancer patients of which kidney dysfunction suspects were excluded. These subjects showed high level of serum creatinine that was over the normal range (Ref Range) therefore; the ubiquitin c protein concentration was not measured in their serum as shown in Table 1. The concentration of serum creatinine for the healthy control subjects is shown in Table 2. The concentration of ubiquitin c protein has been significantly elevated in serum of lung cancer patients highly than healthy control subjects as shown in Tables 1 and 2.

The Mean ± SD and Std. Error Mean for ubiquitin c protein in serum of lung cancer patients and the healthy control subjects is listed in Figure 1. The results showed no significant differences between protein concentrations in the serum of patients with lung cancer compared to the serum concentration of healthy control subjects. Table 3. The results of the current study agreed with published works [10,11] in which the measurement of creatinine levels is an excellent way to indicate the kidney function. kidney diseases and dysfunctions could lead to a significant increase in amount of ubiquitin c protein in blood as well as in case of cancer disease, therefor; using creatinine test was to exclude patients with high level of creatinine which considered out of normal range to distinguish the increase of the ubiquitin c protein level in serum is caused by cancer not by kidney dysfunction [12].

Our results agreed with studies [13,14] which demonstrated that the poly ubiquitin is elevated in patients with lung cancer disease and also agreed with [15] that demonstrated the elevation of poly ubiquitin (UBC) in cancer cells despite of showing differences in stress between cancer types. The higher concentrations of ubiquitin c protein in serum of cancer patients than healthy subjects indicated the important role of this protein for regulation cellular functions and for degradation of unwanted proteins and any errors in expression would effect on the produced protein these effects are

appeared as miss folding, corruption, that make the ubiquitin c protein unable to do his functions in cells and cause diseases including cancer. These results agreed with studies [16-18] that showed an elevation of poly ubiquitin concentration in many types of cancer.

Table 1: The results of serum creatinine concentration level and serum ubiquitin c protein concentration level in lung cancer patients

No.	Gender Male=M; Female=F	Age/year	Type of cancer	Level of creatinine (mg/dl)	Level of ubiquitin c protein (µg/ml)
1	M	57	Lung Cancer	0.81	12.22
2	M	22	Lung Cancer	1.1	11.13
3	F	65	Lung Cancer	0.83	2.40
4	M	37	Lung Cancer	0.95	6.04
5	M	70	Lung Cancer	1.24	10.95
6	M	57	Lung Cancer	1.2	18.40
7	M	67	Lung Cancer	0.99	12.59
8	M	47	Lung Cancer	0.76	14.59
9	M	58	Lung Cancer	1.04	7.86
10	F	65	Lung Cancer	0.89	13.68
11	F	47	Lung Cancer	0.53	3
12	M	57	Lung Cancer	1.2	5.40
13	M	70	Lung Cancer	0.98	8.89
14	F	63	Lung Cancer	0.7	10.09
15	F	56	Lung Cancer	0.65	13.01
16	F	43	Lung Cancer	0.63	6.07
17	M	65	Lung Cancer	0.87	9.30
18	F	55	Lung Cancer	0.54	3.33
19	F	43	Lung Cancer	0.9	5.88
20	M	73	Lung Cancer	1.25	14.02
21	M	62	Lung Cancer	1.03	6.01
22	M	56	Lung Cancer	1.5	
23	M	75	Lung Cancer	5.19	
24	F	71	Lung Cancer	1.02	
25	M	66	Lung Cancer	3.08	
26	M	68	Lung Cancer	2.02	
27	M	43	Lung Cancer	1.9	
28	F	64	Lung Cancer	1.35	
29	M	34	Lung Cancer	1.65	
30	M	56	Lung Cancer	1.43	

The excluded samples for cancer patients with serum creatinine concentration out of normal range are listed from (22-30). Normal range (Ref Range) of serum creatinine, Adult, Male 0.80-1.30 mg/dL; Adult, Female   0.50-0.90 mg/dL

Table 2: The results of serum creatinine concentration level and serum ubiquitin c protein concentration level in healthy control subjects

No.	Healthy subjects	Gender Male=M; Female=F	Age/year	Level of creatinine (mg/dl)	Level of ubiquitin c protein (µg/ml)
1	Healthy Control	F	22	0.78	1.22
2	Healthy Control	M	34	1.3	15.68
3	Healthy Control	M	53	1.2	0.77
4	Healthy Control	F	42	0.61	2.22
5	Healthy Control	F	68	0.54	10.04
6	Healthy Control	M	48	0.77	16.68
7	Healthy Control	M	52	0.67	8.22
8	Healthy Control	F	38	0.69	7.59
9	Healthy Control	F	27	0.56	9.86
10	Healthy Control	F	35	0.81	2.77
11	Healthy Control	F	38	0.7	2.68
12	Healthy Control	F	37	0.82	3.45
13	Healthy Control	M	55	1.02	0.89
14	Healthy Control	M	26	1.15	5.50
15	Healthy Control	F	44	0.88	7.78
16	Healthy Control	F	54	0.64	6.45
17	Healthy Control	M	56	0.99	10.9
18	Healthy Control	F	46	0.58	12
19	Healthy Control	M	62	1.25	9.7
20	Healthy Control	F	47	0.51	2.33

Normal range (Ref Range) of serum creatinine, Adult, Male 0.80-1.30 mg/dL; Adult, Female 0.50-0.90   mg/dL
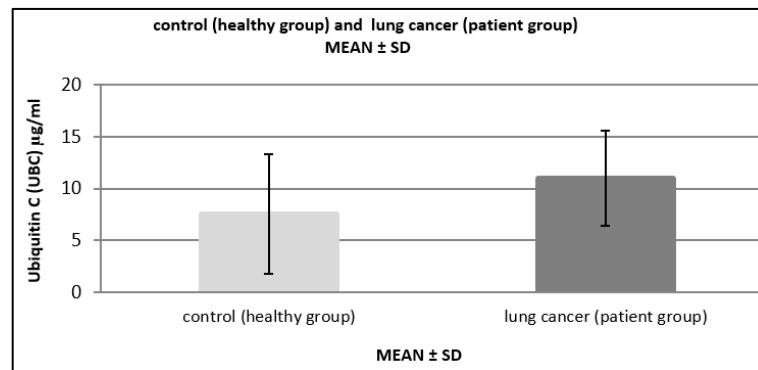
Figure 1: Mean ± SD value of ubiquitin c protein concentration in serum of healthy control subjects and lung cancer patients

Table 3: P value is non-significant (p<0.05) which is mean there is non-significant difference in MEAN and SD between control (healthy group) and lung cancer (patient group). NS[*]: Non-Significant

		Mean ± SD	Std. Error Mean	P VAUE
Ubiquitin C (UBC) µg/ml	Control (healthy group)	7.51 ± 5.76	1.82	0.15 NS[*]
	Lung cancer (patient group)	10.99 ± 4.56	1.44	

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