Diagnosing the Mutations and the Effect Concentration Chromium Cr (VI) in KRAS gene in Exon1 for lung Cancer Patients in Najaf Governorate

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Abstract

The importance of the current study lies in investigating the mutations in the exon1 of the KRAS gene as oncogenes, the presence of mutations associated with lung cancer and the lack of response to chemotherapy. As well as to know the relationship between diagnosed mutations and concentration of chromium . These study was conducted at AL-Furat AL-Awsat oncology Center In Najaf for period from December / 2019 to April / 2020 , on the study included 60 patients who were diagnosed with lung cancer and 22 healthy people where used as control group . the diagnosis of mutation in both patients and healthy subjects has been studied and compared. Then revealing the relationship between mutations and health status to see the effect of mutations on them. The present results showed that there were many mutations found in KRAS gene exon1, 42 genetic mutations in the KRAS gene were detected in exon1 , and these mutations appeared in varying proportions in both patients people, as is evident in In exon1, the frequency of mutation were rs104894361 SNP were in 11(18.33%) , rs121913236 SNP 13 (21.7%) and rs104894366 were 18 (30%) , distribution in 24 patients . more frequency mutations appeared in four patients (2,17,27,42) (6.7%) where found three types of mutations (rs104894361 SNP , 21913236 SNP and rs104894366) those patients have highest recurrence of (1.330, 1.153, 1.224 -1.490) Respectively. While 10 patients (16.7 %) (21,46,9,34,35,30,12,37,14, 39) have only two mutations with distribution in three types also , and 10 patients(16.7 %) were have just one mutation. We found correlation between high Chromium (Cr) concentration in serum of patients with lung cancer compared to healthy. The results of the study showed that there were significant differences (P=0.0001) between the presence of mutations and concentration of chromium, which indicates a relationship between the of mutations and concentration of chromium in the serum the of patient of lung cancer. DNA was extracted from the blood samples by several DNA extracts and mutations were detected by sequencing analyzing after amplification by PCR technology.

***Key word**: KRAS mutations in exon1, Lung cancer, oncogenes, lung cancer in Najaf Governorate.*

Introduction

The occurrence of lung cancer is affected by environmental exposure and genetic or epigenetic susceptibility to disease development and progression[(1)] . Important factors associated with lung cancer development are occupational exposure to carcinogens (arsenic, asbestos, beryllium, cadmium, chromium, diesel umes, nickel, andsilica)[(2)] .According to the European Commission, based on socioeconomic, health, and environmental impact assessment, the strongest factors related to attributable cancer deaths include Cr(VI)[(3)] . Lung cancer is the most common cancer with high lethality Carcinogenic Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation is the most common gain-of-function modification in Western countries,

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accounting for 30 percent of lung adenocarcinomas and about 10 percent of Asian lung adenocarcinomas[4] . Lung cancer is one of the most dangerous malignant diseases that threaten human life. It accounts for about 26 percent of all cancers as the leading cause of cancer death worldwide and ranks as the most deadly cancer among males and the second deadliest cancer among females[5] . Gaseous pollutants, such as sulfur dioxide (SO2), ozone (O3), carbon monoxide (CO), and nitrogen dioxide (NO2) have also been tested for possible links to lung cancer[6] . The occurrence of lung cancer is affected by environmental exposure and genetic or epigenetic susceptibility to disease development and progression[7] . One recent study showed that deficiency of IL-17C would promote the growth and metastasis in lung cancer model[8]. In addition, activation of the tumor-associated microbiota and TLR signaling stimulates the expression of calcineurin and nuclear factor of activated T cells (NFAT) factors, which sustains the survival and proliferation of cancer stem cells[9] .Thus, IL-17C promotes tumor-associated inflammation and tumor proliferation [10] .

Aims of Study

To evaluate the of environmental factors as Concentration Chromium in serum of patients and healthy to found some correlations with SNP mutation in exon1 of KRAS gene.

Material & Methods

Sample collection

This study was conceived to test population consisting of 60 case with non-small cellular lung carcinomas 40Male and 20 females, They are from the central and southern regions of Iraq. Any subject with the following health problems was excluded from the current study:

The ages of the patients ranged between 15-77 years and 49.6 ± 10.9 year (mean ± SD).

The controles (healthy) subject groups of 15 males and female were included in the study as a control group. Their ages ranged between 22-78 years and 47.1 ± 14.9 year (mean ± SD), they were free of The symptoms and signs of any chronic diseases such as diabetes, heart disease, kidney disease or others were chosen to participate in thi's a study.

Atomic absorption spectrometer

Atomic absorption spectrometer 6300 model analysis was performed in the central laboratory (College of Pharmacy / University of Kufa). Cr was determined in liquid samples by the atomic absorption spectrometer by flame under standard conditions and analysis of titration solutions prepared by diluting the stock solution with 100 ppm Cr.

Sampling the blood

Blood was extracted by vein puncture and 1 milliliter and 5 milliliters of blood were taken separately from all individuals in this sample. It included one milliliter of blood collected in tube-containing EDTA and used for DNA extraction; 5 milliliters were collected immediately for blood culture processing.

Genotyping

Extracting DNA

Whole blood samples were collected from the patient group and the healthy control group in EDTA tubes. Then using ReliaPrepTM Blood gDNA Miniprep Program (Promega), the DNA was extracted from whole blood.

Amplification of the primers

The genes examined were amplified using specific prefixes. Amplification products and other properties of the intended genes are scheduled as in table (1)

Table 1: Primer sequences of the genes IL-12 and IL-33, annealing temperature (AT) and product size.

Forward primer	Ex1K1extF: 5′-AGGCCTGCTGAAAATGACTGAA-3′
Reverse primer	EX1K1extR: 5′-ACTCATGAAAATGGTCAGAG-3′
Annealing Temperature	61°C
Product size	208 bp

Thermo cycler amplification software

The PCR thermo cycler program which delivered the best results of IL-12 gene amplification.

Table2: Thermo cycler system for KRAS gene amplification with PCR

Type of Cycle	Temperature^{o}C	Time	No. of Cycles
Initial denaturation	94	5 min	1
Denaturation	94	1 min	35 x
Annealing	61	1 min	
Extension	72	1 min	
Final extension	72	5 min	1

Analysis Sequencing

The PCR products 80samples (60 patients and 22 control healthy) were sent to company macrogen (Koria), in ice bag by DHL. performed the DNA sequencing by AB DNA sequencing system, using Migax64 software & SLC Sequence Viewer software, for analysis sequencing to detected any mutations in the samples for current study by comparing the observed DNA sequences of local samples with the retrieved DNA sequences ,The analysis of sequence reveated the presence of three mutations in exon1 of the KRAS gene in Lung cancer. all of these detected SNP were found to be deposited in the dbSNP as past known genetic polymorphism. The variations in prediction capabilities can be due to the fact that different sets of sequences and alignments are used in each system. We used MutPred method to assess the degree of tolerance for each amino acid replacement based on physiochemical.

Statistical Analysis

Statistical analysis was performed using Mann Whitney test and Chi-square (χ^2) test to determine statistical differences between different groups using the Design Statistical Package for Social Sciences (SPSS 19). Probability ($P \leq 0.05$) was considered statistically significant.

Results & Discussion

Concentration of chromium in Patients and healthy subjects:

The results of the current study appeared as shown in Table (3), which shows a comparison between the healthy and the patients regarding chromium element concentration and age. Where the concentrations of the hexavalent chromium element (VI) in the serum for the patients ranged between (0.224-1.992) and was the average concentration (0.76737), while the concentrations of the chromium element in healthy people ranged between (0.09 - 0.231), the while Chromium concentration average was (0.13973), Where the results are shown difference significant (P=0.0001) at the level of significant (0.05) which indicates a relationship between the high chromium element concentration and the incidence of lung cancer, comparison as with the healthy. studied the effect of carcinogens on the risk of lung cancer in the general population. De Matteis et al they showed that patients appear to have an increased risk of lung cancer due to exposure to chromium, which is consistent with our study results. Patients from these families with high levels of chromium in the blood will have the option of lung CT scanning for early detection of disease. Analyzes of chromium levels may be an attractive option to identify patients with particularly early-stage disease.

Age: Whereas for the age where the ages of the patients ranged between (15-77), and the average The rate was (41.85), and the ages of healthy people ranged between (16-76) , and the average The rate was (41.18). Where the results showed that there was no significant difference. the was significant is (P = 0.904) for age between the healthy and the patients, This explains that the study samples were close between age the patients and the age of healthy. and this is due to the accuracy of choosing the study samples of the for the healthy sample compared to patients.

Table (3): Shows the Comparison of healthy and Patients subjects with respect to chromium concentration and age

Comparison of healthy and Patients subjects with respect to chromium concentration and age							
	Cr con. average	**N**	**Std. D**	**Minimum**	**Maximum**	**Mean Rank**	**p-value**
Patients	0.76737	60	0.311614	0.224	1.992	52.48	0.0001
Healthy	0.13973	22	0.037749	0.09	0.231	11.55	
	Age average	N	Std. D	Minimum	Maximum	Mean Rank	p-value
Patients	41.85	60	18.965	15	77	41.69	0.904
Healthy	41.18	22	17.778	16	76	40.98	

Chromium concentrations and mutations in the KRAS gene:

Table (4): Shows Relationship between different chromium concentrations and mutations in the (KRAS gene) in exon1 for Patients. The chromium concentrations were divided into three groups based on the difference in the concentration ratios. Where the concentration first group ranged from (0.2-0.8) and the second group was (0.81-1.4) and the third group, where the concentrations ranged between (1.4-1.2) and the highest concentration ratios in it compared to the previous groups.

Where the study found (11) patients in group (A) whose levels of chromium concentrations in the serum

ranged between (0.2-0.8).These patients had a mutation of the first type rs104894361 SNP, while group (B) included 6 patients with a type first mutation and the second rs121913236 SNP, rs104894361 SNP, while the third group (C) included (7) patients and among them (4) had three types of mutations rs121913236 SNP, rs104894366 SNP, rs104894361 SNP and had the highest rate of concentration of chromium in the serum, and this indicates that the high concentration of chromium leads to occur mutations in the gene of the (KRAS gene) In Exon1, which causes lung cancer. The results of the statistical analysis of this study showed a significant difference (*P*=0.001), which indicates the existence of a relationship between the high concentration of chromium and mutations in the gene (KRAS), compared with patients who did not show any mutation , this What reinforces the results of the study that with high concentrations of chromium is the reason for the occurrence of mutations in the gene (KRAS) in patients with lung cancer, and this study is the first to detect high chromium concentrations in the (KRAS gene) mutations in Exon1. Another limitation of our study is that, the high risk of lung cancer is generally recognized as being associated with occupational exposure to Cr(VI), but we did not have patient work histories available to include in this study. Nevertheless, our study may provide an avenue to begin to screen for lung cancer occurrence.

Table (4): Shows Relationship between different chromium concentrations and mutations in the KRAS gene in exon1 for Patients.

MUTATION * CONT								
			CONT			Total		
			A(0.2-0.8)	B(0.81-1.4)	C(1.4-1.9)		X2	P-value
KRAS MUTATION		Count	11	6	7	24	13.309	0.001
		% of Total	18.3%	10.0%	11.7%	40.0%		
		Count	29	7	0	36		
		% of Total	48.3%	11.7%	0.0%	60.0%		
Total		Count	40	13	7	60		
		% of Total	66.7%	21.7%	11.7%	100.0%		

Conclusions

1. The present results showed that there were three mutations found in KRAS gene exon1.
2. Forty two genetic mutations in the KRAS gene were detected in exon1 in all patients.
3. The frequency of rs104894361 SNP mutation in exon1,were 11(18.33%), while in rs121913236 SNP were 13 (21.7%) and in RS 104894366 were 18 (30 %).
4. more frequency mutations appeared in four patients (2,17,27,42) (6.7%) where found three types of mutations (rs104894361 SNP, rs121913236 SNP and rs104894366).

5. Those (2 , 17 , 27 , 42) patients have highest recurrence of concentration of chromium in the serum (1.330 , 1.153 , 1.224 -1.490).
6. There was a correlation between some mutations with others.
7. The DNA sequencing assay is one of the easiest and most effective ways to determine genetic mutations and more accurately.
8. The SNP rs reported in lung cancer patient appears to be promising and can be used as a predictive tool or a genetic prediction test tool for lung cancer if found positive in a larger sample size.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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