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Detection one of DNA repair gene for lung cancer in sample of Iraqi patients

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Abstract: The DNA sequence can be changed and damage by environmental agents such as mutagenic chemicals and certain types of radiation. The correction of DNA sequence errors in all types of cells is important for survival. DNA gene repair is an important way to repair this damage. One of DNA repair genes is the *Ref-1* gene, However *Ref-1* gene like other genes contains mutations which are found in different sites. In present study, whole blood was collected from 20 individuals distributed to two groups:- Ten lung cancer patients from various provinces and both gender and different age groups and Ten volunteers from healthy people as control group. DNA was extracted with ReliaPrep Blood gDNA Miniprep System and the results of *Ref-1* by PCR gave positive for all samples. then analysis PCR product of 5 samples (patients and normal), results compared with sequencing of original Ref-1gene in NCBI showed similarity between sequences of normal samples, while there were differences in the sequences of samples lung cancer patients. Results of sequence analysis *Ref-1* showed that were 11 mutation in 5 patients samples with gene *Ref-1*and show single mutation in some samples .Mutations lead to changes in the genetic code and in amino acids that is important reasons of an increase in the gene expression changes in *Ref-1*gene.

Keywords: Lung cancer , Ref-1 gene , PCR ,NCBI .

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Introduction:

Cancer is one of the most important health problems of the current era and also a leading cause of death among populations. Cancer can simply be defined as a malignant tumor or malignant neoplasm, It includes a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. It can also be defined as a group of disorders that are characterized by uncontrolled division of cells and the ability of these abnormal cells to spread. Abnormal dividing cell may spread by direct growth into adjacent tissues through invasion, or by implantation into distant sites by metastasis(1) Cancer may affect people at all ages, but the risk tends to increase with age certain preventive measures to cancer include stop smoking, and avoiding carcinogenic factors(2). Lung cancer, also known as lung carcinoma is a malignant lung tumor characterized by uncontrolled cell

growth in tissues of the lung. If left untreated, this growth can spread beyond the lung by the process of metastasis into nearby tissue or other parts of the body. Most cancers that start in the lung, known as primary lung cancers.(3) .Signs and symptoms which may suggest lung cancer include:Respiratorysymptoms: coughin g, coughing up blood, wheezing, or shortness of breath

Systemic symptoms: weight loss, weakness, fever, or clubbing of the fingernails .Symptoms due to the cancer mass pressing on adjacent structures: chest pain, bone pain, superior vena cava obstruction, or difficulty swallowing. (4).

Cancer develops following genetic damage to DNA and epigenetic changes. These changes affect the normal functions of the cell, including cell proliferation, programmed cell death (apoptosis) and DNA repair. As more damage accumulates, the risk of cancer increases. (5). Smoking is by far the leading risk factor for lung cancer. About 80% of lung cancer deaths are thought to result from smoking. The risk for lung cancer among smokers is many times higher than among non-smokers. The longer smoke and the more packs a day smoke, they lead to greater risk .

Cigar smoking and pipe smoking are almost as likely to cause lung cancer as cigarette

Smoking menthol cigarettes might increase the risk even more since the menthol allows smokers to inhale more deeply .

Secondhand smoke can increase the risk of developing lung cancer. Secondhand smoke is thought to cause more than 7,000 deaths from lung cancer each year (6).

Types of lung cancer:

There are 2 main types of lung cancer:

- Non-small-cell lung carcinoma (NSCLC) (7).
- Small-cell carcinoma (8).

Ref-1 gene

Apurinic/apyrimidinic (AP) sites occur frequently in DNA molecules by spontaneous hydrolysis, by DNA damaging agents or by DNA glycosylases that remove specific abnormal bases. AP sites are pre-mutagenic lesions that can prevent normal DNA replication so the cell contains systems to identify and repair such sites. This gene encodes the major AP endonuclease in human cells if have mutation does not work (9).

Materials and Methods:

This is a case control study which was conducted spanning from December 2016 to May 2016. Apart from subject collection, all molecular studies were conducted in the molecular lab of the Department of Biotechnology /Department of applied science /Technology University. The total number of participants in the study was 20 individuals ,study groups included ten Lung cancer patients and ten apparently healthy individuals of different age. They were volunteers. This group served as a control.

Table (1): Primers used in the study

Primer	Sequence (5′ →3′ direction)
Forword	CCAGCTGAACTTCAGGAGCT
Reverse	CTCGGCCTGCATTAGGTACA

Blood Sampling:

From each participant, 2 ml of peripheral whole blood was aspirated from the left cubital fossa veins, directly into an EDTA containing tube.

DNA extraction:

DNA was successfully extracted from all samples by ReliaPrep Blood gDNA Miniprep System. Promega/USA Concentration and purity of DNA samples were measured by using the nanodrope–spectrophotometer (Quantus Fluo rometer) promega\ USA.

Primers:

Primers used in the study were design their sequences are shown in table (1):

Primer preparation and optimization:

A primer working solution was prepared from the lyophilized primers after dissolving in nuclease free water according to the manufacture to make a stock solution with a concentration of 100 µM for each primer and stored .

A working solution with a concentration of 10 µM was prepared by diluting 10µL of primers stock solution in 90 µL of nuclease free water and stored until use.

Amplification of Ref-1 gene using PCR:

Thaw primer solutions and template nucleic acid. with Taq PCR Master Mix and mix by vortexing briefly to avoid localized differences in salt concentration. Then prepare a reaction mix according to Table (1) . place the PCR tubes in the thermal cycler and start the cycling program. A typical PCR cycling program is outlined in Table (1) Note: After amplification, samples can be stored overnight at 2–8°C, or at –20°C for longer storage.

Table (2):Thermal profile of *Ref-1 gene*

Step	Ref-1		
	Temp. ($_o$C)	Time	No. of cyc
Initial denaturation	95	5 min	1
Denaturation	95	30 Sec	35
Annealing	60	30 Sec	
Extension	72	1 min	
Final extension	72	7 min	1

Gel Electrophoresis of PCR products:

Agarose gel was prepared (8 grams of agarose melted in 100 ml Tris-Borate-Buffer (TBE) and add Loading buffer, and set to run voltage at 70V.

Results and Discussion:

Age distribution:

Twenty individuals participated in the present study, including 10 lung cancer patients. Their ages ranged from 30-70 years.Their age group distribution showed the highest frequency 4 patients to be in the age group of 50 - 59 years. This was following by 3 patients in the age group of 60- 70 years and 2 patients in age group of 30-39 years Table (3) . age differences between different studies reflect sample size, sample type and geographical factors. It is highly linked to timing of sample collection, since this is not a wide range study and was conducted upon a short period of time .However, age group distribution reflects a generalized increase of cancer in middle age group patients .In the past, cancer was through to be a disease of old age patients (10). Increase exposure to carcinogens especially environmental pollutions had let to the appearance of cancer in younger age groups than it was originally present. And also included 10 persons as control .Their age ranged from 30 to 70 years . The predominant age group was 30 -39 years, table (3).

Table (3): Distribution of study samples according to age groups

Age group (year)	Healthy (no.= 10)	Patients (no.= 10)
30-39	4	2
40-49	3	1
50-59	1	4
60-70	2	3

Sex distribution:

Among the 10 lung cancer patients, 7 were males and 3 were females, while from the apparently healthy individuals 6 were males and 4 were females. Frequency distribution of study groups according to sex is shown in Table (4). Individuals in all study groups were randomly selected so that the difference in sex distribution does not reflect any significance.

Table (4): Distribution of study groups according to sex .

Gender	Healthy (no.= 10)	Patients (no.= 10)
Male	6	7
Female	4	3

Results of DNA Extraction:

DNA was successfully extracted from all samples .The concentration of total DNA ranged from 100 to 198 ng/ µl in patient samples. In control group it ranged from 90 to 189 ng / µl The purity DNA samples ranged from 1.67 to 1.94 ng/ µl in patients group The range was from 1.52to 1.96 ng/ µl , in control group.

There was no difference between the concentration of DNA of the study groups, there was no difference between the DNA purity of study groups as well. A good yield with a high concentration of DNA depends on the extraction conditions whereby strict aseptic techniques must be used The use of tips and microfuge tubes and decontamination of the working field by the use of UV light and suprase, all these could be considered working aids in reaching the best result.

Ref-1 gene amplification by PCR:

The *Ref-1 gene* was performed by polymerase chain reaction technique (PCR) using set of primers that amplify the gene.

All samples (patients and normal) showed positive results for the gene presence after analyzing on agarose gel as illustrated in figure (1).

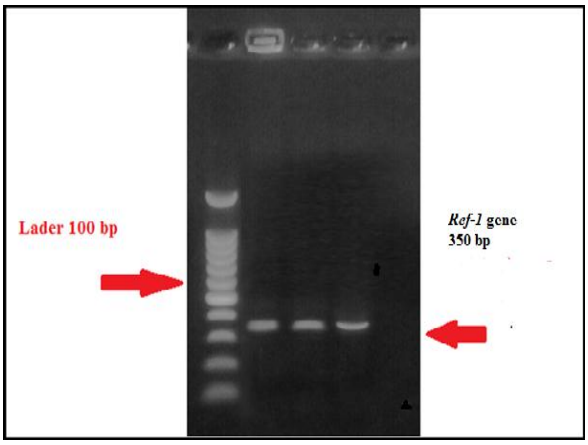

Figure (1): Agarose gel electrophoresis of PCR products of Ref-1 gene visualized under U.V. after staining with ethidium bromide .

Lane 1:- 100bp DNA ladder marker.

Lane 2:- product of Ref-1 gene**.**

Sequence of *Ref-1 gene:*

To identify and study the different between the sequences of Ref-1 gene in two groups in this study, send 20 µl of 10 samples (5 samples of patients and 5 samples of normal) of the product of the PCR reaction with the specific primers of gene Ref-1 gene to Macrogen company in the United States , and after obtaining the results compared to the results, with original sequencing of gene *Ref-1* in the Internet (http: NCBI Reference Sequence) Figure (2).

To confirm comparing of samples sequences of the study were used software program (Bio Edit Pro. version: 7.0.0), which is available at the web site (http://www.mbio.ncsu.edu/bioedit/ bioedit.html). Figure (3) Results were compared with the original sequence of the gene.

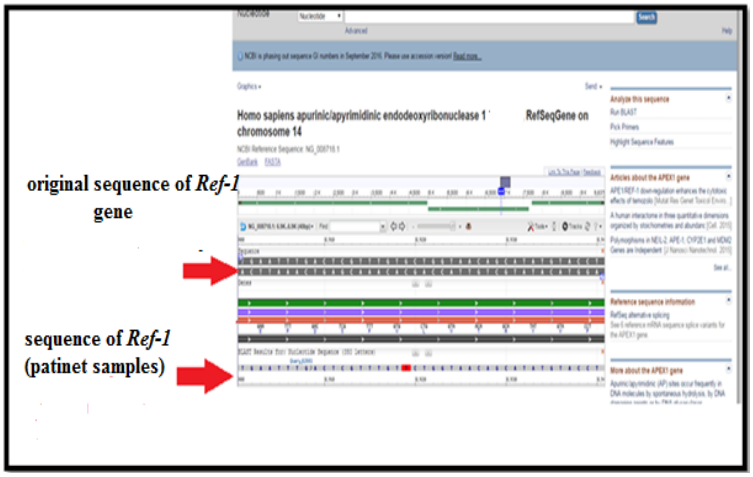

Figure (2): Comparison of the results of sequencing *Ref-1* gene with the original sequence of the same gene in NCBI Reference Sequence.

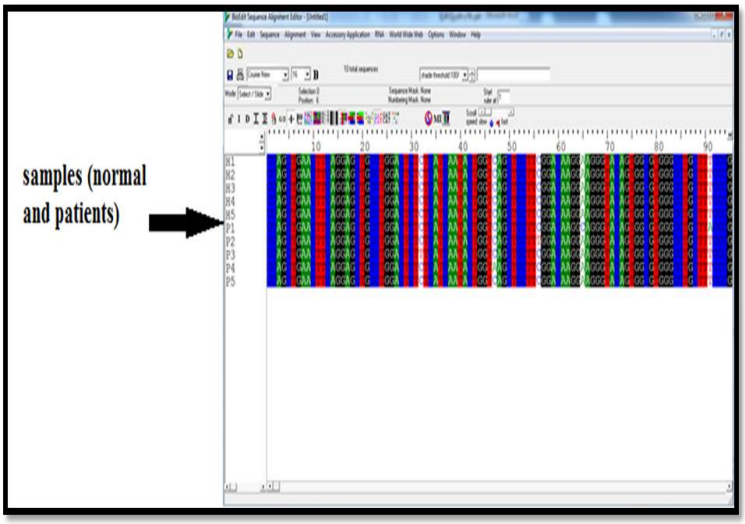

Figure (3): Results of software BioEdit program

Table (6): Results of gene sequence analysis Ref-1 gene.

No. of sample	Wild type	Mutant type	Site	Change in amino acid	Type of mutation	Effect
6	GAA	GCA	65	E / A	Substitution	Missense
	TCC	ACC	90	S / T	Substitution	Missense
	CGC	CGA	96	R / R	Substitution	Silent
	GTG	GTT	316	V / V	Substitution	Silent
7	TCG	TTG	56	S / L	Substitution	Missense
	ATT	GTT	290	I / V	Substitution	Missense
	TCG	TCC	310	S / S	Substitution	Silent
8	TCT	TTT	32	S / F	Substitution	Missense
	TCG	TCT	310	S / S	Substitution	Silent
9	TCA	TAA	47	S/ Stop codon	Substitution	Nonsense
10	CAG	CGG	98	Q / R	Substitution	Missense

Summarized the results of gene sequence analysis *Ref-1* in the table (6) showed that there were 11 mutation in 5 patients samples with gene *Ref-1 gene* and show that there is a single mutation in some samples, and more than a mutation in the other samples, and this shows that the type and location of mutations that were found could lead to the difference in the effect of mutations. Some of these mutations lead to changes in the genetic code and then change in the amino acids at the translation that is the most important reasons of an increase in the gene expression changes in *Ref-1* gene . This results are agreed with China M. *et al* 2005 They found evidences that mutation in *Ref-1* gene is associated with an altered risk of lung cancer.and also agreed with(11). They found the effect of smoking and presence of mutation in Ref-*1* gene are association with risk of lung cancer

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