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Cytogenetics and molecular study of P53 gene in liver cancer diagnosed in Erbil city using de novo sequencing method

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Abstract:

Liver cancer is the sixth most frequent cancer and the second leading cause of cancer death through the world. The first objective of the present study is to know the chromosomal aberrations in peripheral blood of liver cancer patients. The present study was carried out on 50 patients who were suffering from liver cancer in Erbil city and 25 healthy individuals as a healthy group in both sex and different age groups. The second objective of the present study is to know the mutations in TP53 tumor suppressor gene, so molecular study was carried out on 18 patients who were suffering from liver cancer in Erbil city and 1 healthy individual as a control group. Detection a mutation in TP53 gene in peripheral blood cells may be helpful to the diagnosis of liver cancer, especially to the accurate staging of liver cancer. Additionally, cytogenetic study followed by molecular analysis of recurring chromosome changes has facilitated the identification of crucial ontogenesis and tumor suppressor genes. The mechanism of liver carcinogenesis involve multiple endogenous and exogenous genetic alterations, so many factors contribute to its development such as genetic factors, Hepatitis Band C virus infections, smoking habit and alcohol habit. Other factors was also studied included patient gender and age. The results of the present study suggest that highest value of chromosomal aberrations was (Dicentric chromosome) which occurred in males at fifth age group (65-74) , also shown that most patients are males at age group(65-74), most of them have smoking , alcohol habit and Hepatitis B viral infection . From the study of TP53 gene, we observed mutation in exon 7 which was deletion of nitrogen base G , just before the coding region , which represent splice- site mutation.

Keywords: *Liver Cancer, chromosome aberrations, P53 tumor suppressor gene, Erbil City*

Introduction:

Liver cancer is also known as hepatic cancer , primary liver cancer is globally the sixth most frequent cancer, and the second leading cause of cancer death (1) .The Commonest ten Cancers death registered in Iraq during 2014 in which liver cancer represent 284 cases males and 229 cases were females (2) , while in Erbil City represent 350 case during 2012-2016 (3).

Higher rates of liver cancer occur where hepatitis B and C are common, including East – Asia and sub- Saharan Africa (1). Liver cancer is seen more in men than in women, the average age at diagnosis of liver cancer is 63 year and the gender difference in cancer susceptibility is one of the most consistent findings in cancer epidemiology (4).

The main risk factors for liver cancer include viral infection with hepatitis virus (HCV) or hepatitis B virus (HBV) is common cause of liver cancer in the world today, accounting for 80% of hepatocellular carcinoma (5). The viruses cause cirrhosis occurs within the liver. HCC usually arises after cirrhosis (6). Many genetics and epigenetics changes are formed in liver cells during HCV and HBV infection, which is a major factor in the production of the liver tumours. The viruses induce malignant changes in cells by altering gene methylation, affecting gene expression and promoting or repressing cellular signal transduction. The virus action pathways can prevent cells from undergoing a programmed form of cell death (apoptosis) and promote viral replication and persistence (7)

Alcohol intake correlates with risk of HCC, and the risk is far greater in individuals with an alcohol-induced cirrhotic liver (8). There were also relationship between smoking

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and liver cancer, there are over 4000 chemicals in cigarette smoke, more than 50 of them are known to be carcinogens (9).

Epidemiological prospective studies have shown that increased chromosomal aberrations (CAs) in peripheral blood lymphocytes may predict cancer risk (10). Chromosomal abnormalities are a defining feature of solid tumors. Such cytogenetic alterations are mainly classified into structural chromosomal aberrations and copy number alterations, giving rise to aneuploid karyotypes. The increasing detection of these genetic changes allowed the description of specific tumor entities and the associated patterns of gene expression (11).

The liver cancer genome contains multiple types of somatic alterations, including mutations (such as single nucleotide substitutions, and small insertions and deletions), changes of gene copy numbers (copy number loss, gain and amplification) and intra-chromosomal and inter-chromosomal rearrangements (large deletion, inversion, tandem duplication and translocation (12).

Mutations in the p53 gene have been found in many types of cancer and may be the most common mutation observed in human cancers , p53 (also known as protein 53 or tumor protein 53) is a tumor suppressor protein that in humans is encoded by the TP53 gene (13). P53 is a crucial in multicellular organisms, where it regulates the cell cycle and thus, functions as a tumor suppressor that is involved in preventing cancer. As such, p53 has been described as "the guardian of the genome" because of its role in conserving stability by preventing genome mutation. The p53 has several biological effects involving cell-cycle arrest, DNA replication and repair, proliferation, apoptosis, angiogenesis inhibition, and cellular stress response (14). In humans, p53 is encoded by the TP53 gene located on the short arm of chromosome 17 (17p13.1). The gene spans 20 kb. (15).The name p53 is in reference to its apparent molecular mass: it runs as a 53-kilodalton (kDa) protein on SDS-PAGE (13).

TP53 protein is present at very low levels in normal cells but under certain stress, cells are able to up regulate their p53 levels by a post-transcriptional mechanism (16). DNA sequencing is the process of determining the precise order of nucleotides within a DNA molecule. It includes any method or technology that is used to determine the order of the four bases—adenine, guanine, cytosine, and thymine in a strand of DNA. The advent of rapid DNA sequencing methods has greatly accelerated biological and medical research and discovery (17).

Materials and Methods :

Ethics statement:

A special questionnaire was used in order to record direct response of patients through several visits to Rezgari Hospitals in Erbil city , between March 2015 to July 2015 . Each patient was provided with a questionnaire form, which includes some fields of information to be filled about the patient including (Name, Address, Gender, Age, smoking habit , Alcohol habit and Hepatitis viral infection). Furthermore, the questionnaire forms were filled in through direct interviews with patients and their relatives as well as using medical records available in the hospital with the help of doctors and staff.

1- Blood sampling:-

Five ml of blood were collected from 50 patients, using sterile disposable syringes. Then, the blood was put in tube one for chromosomal study (Lithium Heparin) tube , while tube two used for molecular study in which five ml of blood were collected from 18 patients, using sterile disposable syringe, then it was collected into anticoagulant ethylene diamine tetra acetic acid (EDTA) tube .

2- Blood culture and harvesting for chromosomal study

About one ml or 6 – 7 drops of heparinized blood was cultured in 5 ml of RPMI - 1640 culture medium, then supplement 0.3 ml of Phytohemagglutinin . Culture tubes were incubated at 37 C° for 72 hours, after 71 hours of incubation 0.2 ml of colchicines was added to the culture tube with mild shaking and then incubated at 37 C° for next 1 hour. After many steps of centrifugation and adding of fixatives, 3 to 4 drops of cell suspension were dropped evenly from appropriate distance (typically 30 cm) on to a wet chilled and grease free slide, then the slide was dried at room temperature. The slide was stained with freshly prepared giemsa stain (1 giemsa stain : 4 Sorensone buffer solution) for 2-3 minutes. The slide was washed by Sorensone's buffer and left to dry at room temperature. Excess buffer was removed by slanting the slide on filter paper. Microscopic examination was performed using Olympus Microscope with ocular lens 10X and adjective lens 100X for chromosomal examination.

3- Molecular study of P53

Extraction of DNA by Genomic DNA Extraction (Wizard Genomic DNA Purification Kit cat#A1120) was don. This kit was from Promega company in USA which obtained by bio tech company in Sulaimania City. Samples will be tested to determine the concentration of DNA, by Nanodrop (Binder ,Germany) which present in Salahaddin university research center . Also samples run on agarose gel electrophoresis in order to determine the integrity. DNA amplification was done by PCR (PTC-200PCR system- BIO-RAD), California.

DNA amplification by PCR: A-PCR amplification system

GC buffer I	13.5ul
dNTP (2.5 mM)	2 ul
Forward primer (10mM	1ul
Reverse primer 10mM	1ul
DNA	2ul
rTaq	0.2 ul
H2O	6.3ul

B-PCR reaction procedure(Programe)

98	5min	
98	20sec	Denaturation
55	20sec	Annealing
72	30sec	Extension
30cycles		
72	5min	
12	forever	

After thawing the sample from the ice, centrifuging and fully mixing we took appropriate sample for testing.
PCR product was purified according to Millipore 96-well purification plate Instruction. add 100ul ddH2O to 96-well plate, mix with the PCR product and let stand for 10min at room temperature. Pump for 10 min till dry with Vacuum Pumps. And add 40ul ddH2O to 96-well plate, dissolving for 10 min at room temperature. Pipette the purification product to a new plate.

PCR product sequencing: PCR products are analyzed by a 96- capillary automated sequencer (ABI PRISM-3100 Genetic Analyzer, Applied Biosystem) based on the sanger method. The sequence was done in Tehran (poiya Gene center).

Sequencing reaction system : DNA(Purified PCR product) 3µl 30-50 ng; Primer(3 pM) 1 µl; Bigdye 0.5 µl; ddH2O 0.5 µl ; Total 5 µl. Sequencing reaction procedure(Programme start with 95 2min;95 10s ;51 10s; 60 190 s;25 cycles and end 12 forever.

● PCRprimer.

Forward primer	TCCTTGGCTTTTGAAAATAAGCTCC
Reverse primer	TTTACTTTGCACATCTCATGGGGTT
Product length	1000 bp.

●Sequencing primer:

Forward primer	ACCCCATGAGATGTGCAAAGTAA
Reverse primer	TTGGTCTCCTCCACCGCTTC

Statistical Analysis

Performed, using SPSS version 18 software application to study the chromosomal aberrations in different diseases on different age and sex groups, while for molecular study of p53 gene PCR products and sequencing blast in NCBI(ational Center for Biotechnology Information).

Results and Discussion:

The study was done to know the chromosomal aberrations in peripheral blood of liver cancer patients also to know the mutations in TP53 tumor suppressor gene, detection a mutation in TP53 gene in peripheral blood cells may be helpful to the diagnosis of liver cancer, especially to the accurate staging of liver cancer.

Figure (1) shows that the highest number of patients with liver cancer was males (76 %) while female represent (24 %).Also it shows that the highest number of patients is at age interval (65-74) year, represent (30%) who are males. The gender difference in cancer susceptibility is one of the most consistent findings in cancer epidemiology. There are differences in cancer incidence between males and females (4). Both estrogens and androgens have been recognized as modulators of immune response and determinant of gender differences in diseases susceptibility (18).

Hepatocellular carcinoma (HCC), was the most common liver cancer occurs mainly in men. Similar gender disparity was seen in mice given a chemical carcinogen diethylnitrosamine (DEN), they propose that estrogen-mediated inhibition of IL-6 production by Kupffer cells reduces liver cancer risk in females, and these findings may be used to prevent HCC in males. Production of a protein that promotes inflammation appears to be linked to the higher incidence of liver cancer in men than in women. Overall, men are three to five times more likely to develop HCC than women (19).

In figure (2) show that most patients having Hepatitis B viral infection represent (55%) while hepatitis virus C represent (30%) , and non infection with Hepatitis represent(15%). Worldwide, the most common risk factor for liver cancer is chronic (long-term) infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), these infections lead to cirrhosis of the liver and are responsible for making liver cancer to be the most common cancer in many parts of the world, in United States, infection with hepatitis C is the more common cause of HCC, while in Asia and developing countries, hepatitis B is more common. People infected with both viruses have a high risk of developing chronic hepatitis, cirrhosis and liver cancer (20).

One risk factor for the development of HCC is chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), which increases the risk of HCC by approximately 20-fold (21).

Figure (3) we presented that most patients having alcohol habit represent (75%) while fewer are non- alcohol habit

represents (25%). Our results suggest that alcohol consumption and cigarette smoking may have minor age- and sex-specific effects on the development of primary liver cancer. Epidemiological studies found that alcohol consumption can increase the risk for cancers of the upper aero-digestive tract, stomach, large bowel (i.e., colon and rectum), liver and breast, with higher levels of consumption leading to greater increases in risk (22). Cirrhosis is histological development of regenerative nodules surrounded by fibrous bands in response to chronic liver injury that leads to portal hypertension and end stage liver disease, thus liver cirrhosis appears to be an important step in the development of liver cancer (23).

While in figure (4) we shows that most patients in our study having smoking habit represent (70%) while other are non- smoker habit represent (30%). Smoking causes a variety of adverse effects on organs that have no direct contact with the smoke itself such as the liver. It induces three major adverse effects on the liver: direct or indirect toxic effects, immunological effects and oncogenic effects (24)

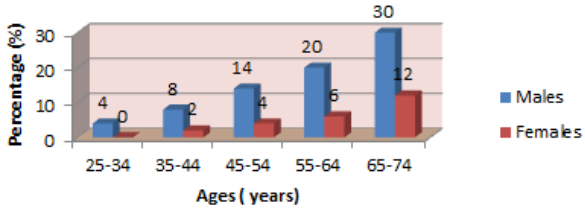


Figure 1: Distribution of patients with liver cancer according to sex and age group.

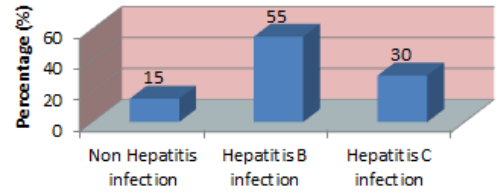


Figure 2: Distribution of patients with Liver cancer according to hepatitis viral infection.

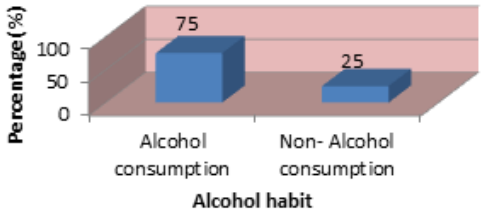


Figure 3: Distribution of patients with liver cancer according to alcohol habit.

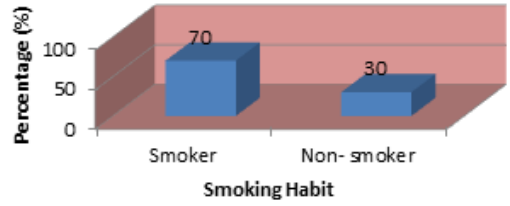


Figure 4: Distribution of patients with liver according to smoking Habit.

The study of chromosomal aberrations (CAs) in patients who were suffering from liver cancer, in both sexes at different age groups, from the (50) blood samples only (8) samples shows normal chromosomes, while (42) samples show CAs. The results of the present study represent different types of chromosomal aberrations in peripheral blood lymphocytes ofliver cancer patients, including both sexes and different age groups .The values in tables represent the mean of chromosomal aberrations, the (C1 and C2) represent control and patients respectively. In case of study age groups, we obtained five age groups for those patients who under chromosomal study, included (A1, A2, A3, A4, A5) represent age group (25-34 year), (35-44year),(45-54 year),(55-64 year),(65-74 year) respectively, while study of both sex, males and females represent (S1 and S2) respectively.

We presented in table (1) the highly significant effect at (P< 0.01) of both states(healthy and patients) on different chromosomal as well as chromatid aberrations like (dicentric chromosome and chromatid interchange :quadriradial and centromeric break), as shown in Figure (5 b, c and d) .

While the differences between the mean values were clear shown in table (2), were the value of L.S.D. at both levels(P< 0.05 and P< 0.01) as follows (0.202, 0.290),(0.202, 0.290),(0.224, 0.322) respectively. Ages, have highly significant effect at (P< 0.01) on all types of CAs, were the value of L.S.D. are (0.320, 0.460) (0.320,0.460) and (0.355,0.508) respectively . Also sex have highly significant effect at (P< 0.01) on all types of CAs, were the value of L.S.D. are (0.202, 0.290) (0.202, 0.290) and (0.224, 0.322) respectively.

It was clear from Table (2) that in case of both states the highest value of CAs was dicentric chromosome (5.933 ±0.803) which occurred in liver cancer patients. In case of age the highest value of CAs was dicentric chromosome (6.583±1.803) which occurred at fifth age group 65-74 year. In case of sex the highest value of CAs was dicentric chromosome (4.266±0.869) which occurred in males. The ef-

fects of interaction between states, age, and sex on CAs, the results shown that the highest value of CAs was (dicentric chromosome) (14.666±0.333) , which occurred in liver cancer patients who are males at fifth age group 65-74 year . Physiological, hormonal and genetic differences between males and females affect the prevalence, incidence and severity of diseases and responses to therapy.

The incidence of liver metastases was higher in patients with primary colorectal cancer with genetic changes including gains at 8q and 20q might be useful to identify patients at high risk for developing liver metastases which analyzed by comparative genomic hybridization (25) .

Table 1 : Analysis of variance to study the chromosomal aberrations in patients who were suffering from liver cancer in Erbil City(in both sexes at different age groups).

		Mean square(MS)		
Source of variation	**d.f**	**Dicentric chromosome**	**Chromatid inter-change (quadriradial)**	**Centromeric break**
Cases (healthy, C1:Patients,C2)	**1**	360.150 **	205.350 **	54.150 **
Age (A)	**4**	55.475 **	18.942 **	12.017 **
Sex (S1,S2)	**1**	36.817 **	18.150 **	20.417 **
Case/ Age(C/A)	**4**	58.025 **	12.975 **	10.900 **
Case/ Sex(C/S)	**1**	33.750 **	28.017 **	25.350 **
Age/ Sex(A/S)	**4**	4.608 **	2.358 **	10.917 **
Case/ Age/Sex (C/A/S)	**4**	4.292 **	2.392 **	8.767 **
Error	**40**	0.217	0.217	0.267
Total	60			

Table 2 : Mean± S.E to study chromosomal aberrations presented in patients under study who were suffering from liver cancer in Erbil City (in both sexes at different age groups).

Factors	**Chromosomal aberrations**		
	Dicentric chromosome	**Chromatid interchange (quadriradial)**	**Centromeric break**
Control(C1)	1.0330.089±	0.666±0.102	0.866±0.104
Patients(C2)	**5.933±0.803**	**4.300±0.470**	**2.766±0.499**
L.S.D P< 0.05	**0.202**	**0.202**	**0.224**
P< 0.01	**0.290**	**0.290**	**0.322**
A1(25-34) year	1.416±0.193	1.083±0.259	0.833±0.112
A2 (35-44) year	1.666±0.256	1.083±0.416	1.500±0.261
A3(45-54) year	3.166±0.767	3.166±0.851	1.000±0.000
A4(55-64) year	4.583±1.183	3.333±0.810	3.000±0.000
A5(65-74) year	**6.583±1.803**	3.583±0.941	2.750±0.871
L.S.D P< 0.05	**0.320**	**0.320**	**0.355**
P< 0.01	**0.460**	**0.460**	**0.508**
S1(Male)	**4.266±0.869**	3.000±0.000	2.400±0.537
S2 (Female)	2.700±0.518	1.900±0.322	1.233±0.103

L.S.D P< 0.05 P< 0.01	**0.202 0.290**	**0.202 0.290**	**0.224 0.322**
C1A1	1.000±0.258	0.333±0.210	0.666±0.210
C1A2	1.000±0.258	0.333±0.210	0.833±0.307
C1A3	1.166±0.166	0.500±0.223	1.000±0.000
C1A4	1.166±0.166	1.166±0.166	1.000±0.000
C1A5	0.833±0.166	0.666±0.210	0.833±0.166
C2A1	1.833±0.166	1.833±1.666	1.000±0.000
C2A2	2,333±0.210	1.833±0.703	2.166±0.166
C2A3	5.166±0.980	5.833±0.542	1.000±0.000
C2A4	8.000±1.211	5.000±0.991	5.000±1.505
C2A5	**12.333±1.054**	6.500±0.670	4.666±1.358
L.S.D	**0.452 0.649**	**0.452 0.649**	**0.502 0.722**
C1S1	1.066±0.153	0.333±0.125	0.866±0.165
C1S2	1.000±0.09	0.866±0.133	0.866±0.133
C2S1	**8.733±1.106**	5.733±0.492	4.133±0.855
C2S2	3.133±0.576	2.866±0.616	1.400±0.190
L.S.D	**0.286 0.411**	**0.286 0.411**	**0.317 0.455**
A1S1	1.133±0.333	1.000±0.365	0.666±0.210
A1S2	1.500±0.223	1.166±0.401	1.000±0.000
A2S1	2.000±0.365	1.166±0.760	1.333±0.494
A2S2	1.333±0.333	0.500±0.223	1.666±0.210
A3S1	4,333±1,358	3.666±1.498	1.000±0.258
A3S2	2.000±0.447	2.666±0.918	1.000±0.258
A4S1	5.833±2.166	4.500±1.431	4.666±1.666
A4S2	3.333±0.918	2.166±0.542	1.333±0.210
A5S1	**7.833±3.058**	4.166±1.720	4.333±1.498
A5S2	5.333±2.092	3.000±0.000	1.166±0.307
L.S.D.	**0.452 0.640**	**0.452 0.640**	**0.502 0.722**
C1A1S1	0.666±0.333	0.333±0.333	0.333±0.333

C1A1S2	1.333±0.333	0.333±0.333	1.000±0.000
C1A2S1	1.333±0.333	0.000±0.000	0.333±0.333
C1A2S2	0.666±0.333	0.666±0.333	1.333±0.333
C1A3S1	1.333±0.333	0.333±0.333	1.333±0.333
C1A3S2	1.000±0.000	0.666±0.333	0.666±0.333
C1A4S1	1.000±0.000	1.333±0.333	1.000±0.000
C1A4S2	1.3333±0.333	1.000±0.000	1.000±0.000
C1A5S1	1.000±0.000	0.333±0.333	1.000±0.000
C1A5S2	0.666±0.333	1.000±0.000	0.666±0.333
C2A1S1	2.000±0.000	1.666±0.333	1.000±0.000
C2A1S2	1.666±0.333	2.000±0.000	1.000±0.000
C2A2S1	2.666±0.333	3.333±0.333	2.333±0.333
C2A2S2	2.000±0.000	0.333±0.333	2.000±0.000
C2A3S1	7.333±0.333	7.000±0.000	0.666±0.333
C2A3S2	3.000±0.000	4.666±0.333	1.333±0.333
C2A4S1	10.666±0.333	7.666±0.333	8.333±0.333
C2A4S2	5.333±0.333	3.333±0.333	1.666±0.333
C2A5S1	**14.666±0.333**	8.000±0.000	7.666±0.333
C2A5S2	10.000±0.000	5.000±0.000	1.666±0.333
L.S.D	0.640 0.920	0.640 0.920	0.710 1.020

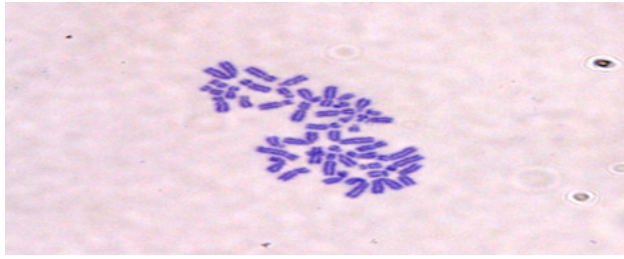


Figure (5 –a): Normal distribution of human chromosome (1000 X, Giemsa stain).

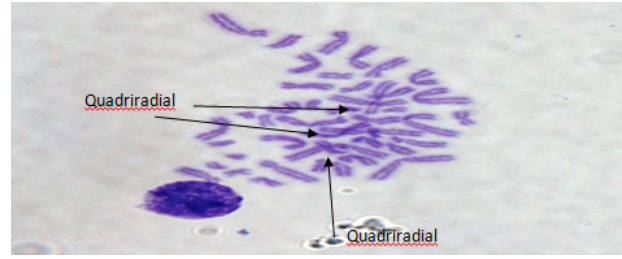
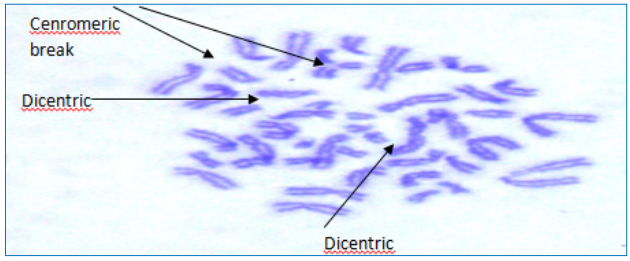


Figure (5-b): Chromosome aberrations in lymphocytes of patients who were suffering from liver cancer (1000 X, Giemsa stain).



Figure(5-c) : Chromosome aberrations in lymphocytes of patients who were suffering from liver cancer (1000 X, Giemsa stain).

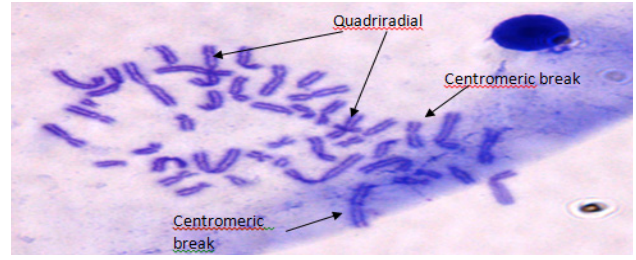


Figure (5-d) : Chromosome aberrations in lymphocytes of patients who were suffering from liver cancer (1000 X, Giemsa stain).

For molecular study we selected the TP53 gene for analysis because loss of wild-type p53 protein reduces its efficient involvement in apoptosis and is associated with reduced tumor sensitivity to chemotherapy and radiotherapy as well shorter patient survival

In TP53 we selected the exons which represent (E5, E6, E7 and E8,), because high susceptibility to be mutated in cancer cases. The Nucleotide sequence was read by Bio Edit Program and use of Blast in NCBI (National Center for Biotechnology Information) to compare with other isoforms of TP53in Gene Bank (http: // blast. Ncbi.mlm.nih.gov / BLAST. Cgi. Program= blastn & BLAST – PROGRAM= mega blast- type= BLAST search). For our results to be certified also by manual using the normal sequence of TP53 Exons (5-8) and codons (Amino acids) which obtained in (IARC-TP53 DATA BASE, Ref sequence: Swiss Prot # P04637) International Agency for Research on sancer. Extracted DNA was run on agarose gel electrophoresis as shown in figure (6). DNA amplification was done by PCR (PTC-200 PCR system- BIO-RAD, California and gelectrophoresis for PCR product was done as shown in figure (7).

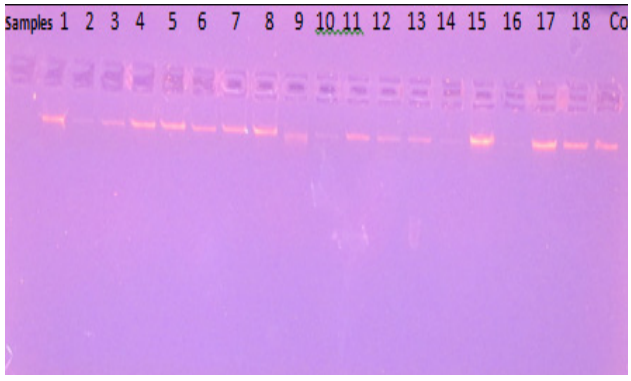
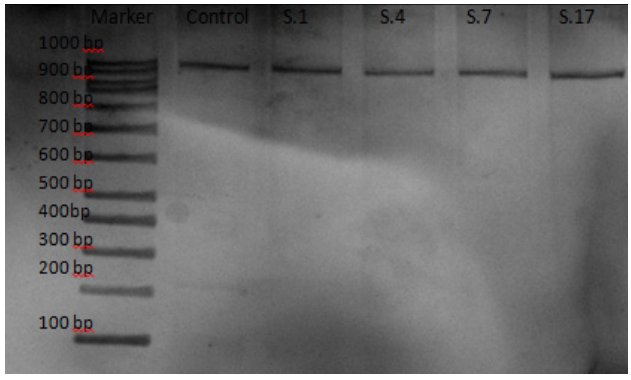


Figure (6) Electrophotogram of genomic DNA 1 % gel (5 sample)



Figure(7) : Electrophoresis condition: 2.2ul product +1 % gel, Marker:100bp, 200bp300bp,400bp,500bp,600bp,700bp,800bp,900bp,1000bp. Concentration of 750bp fragment is 30ng/1.5uL

In liver cancer we study TP53 for only eighteen patients and one control as shown in figure (6) , the failure of fourteen extracted DNA samples cannot be amplified maybe caused by samples status (defect DNA), patients case because many of them were in very severe case and take high dose of chemotherapy may leaded to happened high rate of mutation in TP53 exones specially 5-8, this result was confirm by second round PCR, for those reasons only five samples were amplified and sequenced as shown in figure (7). Samples included (2 males 45 and 46 year s old , 2 females 45 and 72 years old and 1 control . The exons (E5, E6, E7 and E8) of TP53 were studied.

In all patients the sequence of Exon 5 was as follows:-

GGGGCGTAACGAAGGGGAGGCACGGCTAGGCTGCCGTCTTCCAGTTGCTTTATCTGTTCACTTGTGCCCTGACTT
TCAACTCTGTCTCCTTCCTCTTCCTACAGTACTCCCCTGCCCTCAACAAGATGTTTTGCCAACTGGCCAAGACCTGCCCT
GTGCAGCTGTGGGTTGATTCCACACCCCCGCCCGGCACCCGCGTCCGCGCCATGGCCATCTACAAGCAGTCACAGCAC
ATGACGGAGGTTGTGAGGCGCTGCCCCCACCATGAGCGCTGCTCAGATAGCGATGGTGAGCAGCTGGGGCTGGAGAG
ACGACAGGGCTGGTTGCCCAGGGTCCCCAG.

The blast in NCBI :- Query is our results , while Subject is sequence in NCBI

```
Query  79   ACTCTGTCTCCTTCCTCTTCCTACAGTACTCCCCTGCCCTCAACAAGATGTTTTGCCAAC  138
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  467  ACTCTGTCTCCTTCCTCTTCCTACAGTACTCCCCTGCCCTCAACAAGATGTTTTGCCAAC  408

Query  139  TGGCCAAGACCTGCCCTGTGCAGCTGTGGGTTGATTCCACACCCCCGCCCGGCACCCGCG  198
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  407  TGGCCAAGACCTGCCCTGTGCAGCTGTGGGTTGATTCCACACCCCCGCCCGGCACCCGCG  349

Query  199  TCCGCGCCATGGCCATCTACAAGCAGTCACAGCACATGACGGAGGTTGTGAGGCGCTGCC  258
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  348  TCCGCGCCATGGCCATCTACAAGCAGTCACAGCACATGACGGAGGTTGTGAGGCGCTGCC  289

Query  259  CCCACCATGAGCGCTGCTCAGATAGCGATGGTGAGCAGCTGGGGCTGGAGAGACGACAGG  318
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  288  CCCACCATGAGCGCTGCTCAGATAGCGATGGTGAGCAGCTGGGGCTGGAGAGACGACAGG  229

Query  319  GCTGGTTGCCCAGGGTCCCCAG  340
            |||||||||||||||||||||
Sbjct  228  GCTGGTTGCCCAGGGTCCCCAG  207
```

Green color represent start and end of E 5 of TP53, while red represent the mutated part of this exon and also other regions of TP53. The result shows that all nucleotide in exon 5 in all patients were normal as shown in Figure (8) .

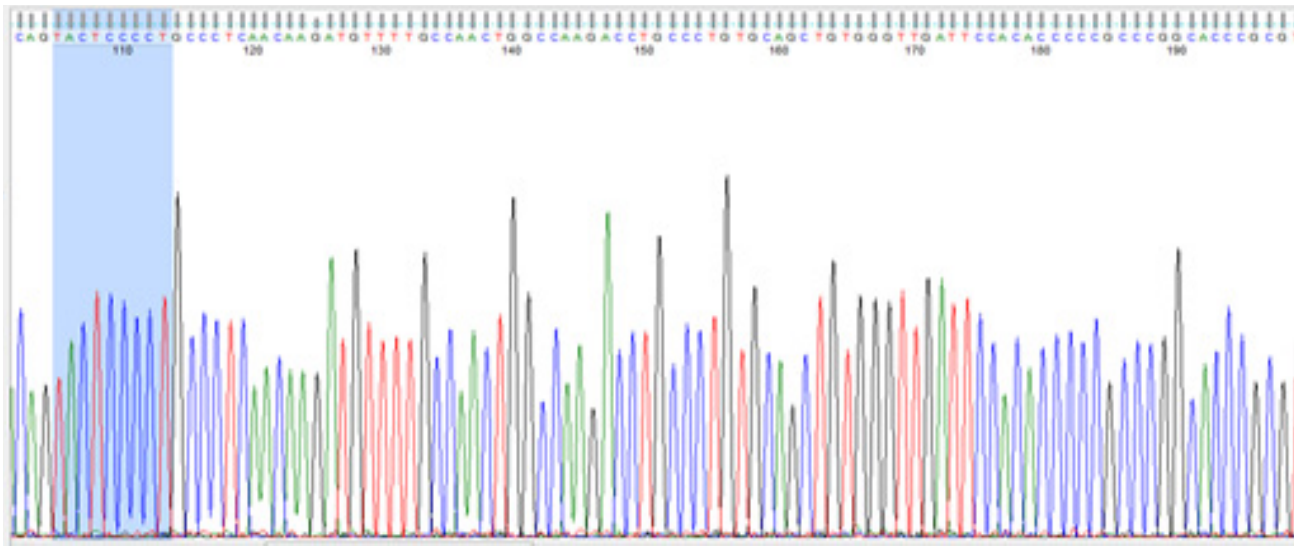


Figure (8): The sequence of TP53 - Exon 5- in liver cancer patients.

While in all patients the sequence of Exon 6 were as follows:-

GCCTCTGATTCCTCACTGATTGCTCTTAGGTCTGGCCCCTCCTCAGCATCTTATCCGAGTGGAAGGAA
ATTTGCGTGTGGAGTATTTGGATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCTATGAGCCGCCT
GAGGTCTGGTTTGCAACTGGGGTCTCTGGGAGGAGGGGTTAAGGGTGGTTGTCAGTGGCCCTCCGGGTGA
GCAGTAGGGGGGCTTTCTCCTGCTGCTTATTGGACCTCCCA.

The blast in NCBI :- Query is our results , while Subject is sequence in NCBI

```
Query  29   GGTCTGGCCCCTCCTCAGCATCTTATCCGAGTGGAAGGAAATTTGCGTGTGGAGTATTTG  88
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  761  GGTCTGGCCCCTCCTCAGCATCTTATCCGAGTGGAAGGAAATTTGCGTGTGGAGTATTTG  820

Query  89   GATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCTATGAGCCGCCTGAGGTCTGG  148
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||| |||
Sbjct  821  GATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCTATGAGCCGCCTGAGGT-TGG  879
```

The red color represent a gap which are addition of nitrogen base C in intron after the coding region of E6 represents as single nucleotide polymorphism (SNP), as shown in figure (9), while all nucleotide in exon 6 in all patients were normal as shown in Figure (10)

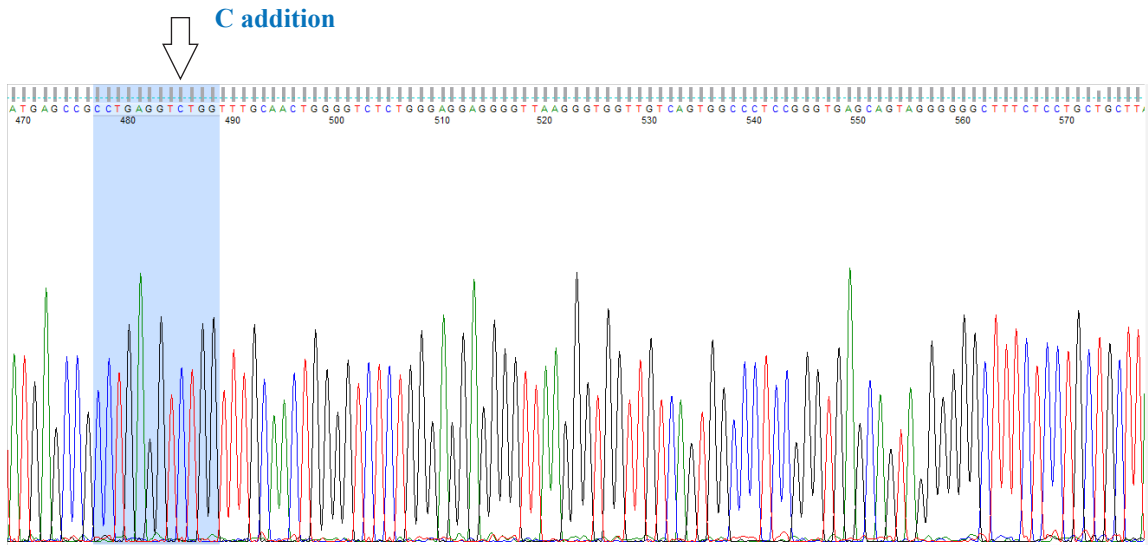


Figure (9): The sequence of TP53 - Exon 6 in liver cancer patients.

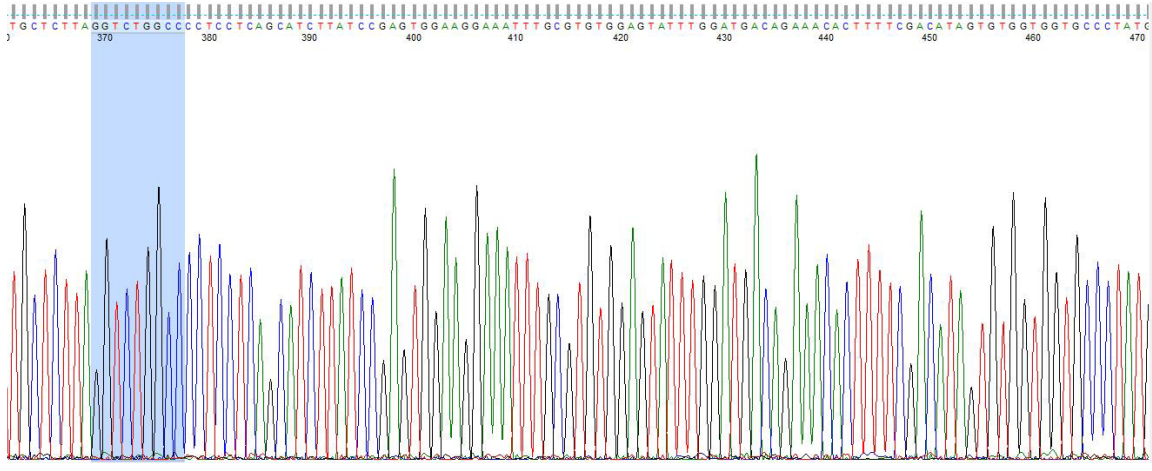


Figure (10): The sequence of TP53 - Exon 6 in liver cancer patients.

In all patients the sequence of Exon 7 were as follows:-

TTGAGGGGTTGGTGTGAAATGGGGTCTCTGGGGGGAGGGGTAAAGGGGGTTTTTCAGGGCCTCTCGGGGAGACCAAATGGGGG
GCTTTTCCCTCGTGTTTTATGTACTCTCCCTTAACCCCCTGAGTGGTGCAAAAAAAGTGGGTTTATCTTTTTCCCCATTGAAAAAAAT
AAAAGCTTCACGGGGTTAAGGGCCTCCCCGTTTGGCTGGGGGCAATGGGTCAATCCCGTTATCCCCACACTTTGGGGGGCCCAAG
CCGGCGGATCCCGAGGGTGGGGAATTGGGACCCTCCTGGGTAACGGGGAAACCCCCTTTTTTTTGAAAAAAAAAAAAAAAAATTA
ACCCGGGGGGGGGTTGGGCCCCCGTAGTTCCCCCTTCTTGGGGGGGTGGGGAAAGAGAAAGGGGGGAACCTGGGGGGGGGGG
GTTTCCGTGAGCTGAGATCCCCCCCCTTCCCTCCCCCCTGGGGGGCAGAGGGGGATTTCCTTTTAAAAAAAAAAAAAAAAGGCCTC
CCCTGCTTGCCACAGGTCTCCCCAAGGCGCACTGGCCTCATCTTGGGCCTGTGTTATCTCCTAGGTTGGCTCTGACTGTACCACCAT
CCACTACAACTACATGTGTAACAGTTCCTGCATGGGCGGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCA
GGTCAGGAGCCACTTGCCACCCTGCACACTGGCCTGCTGTGCCCCAGCCTCTGCTTGCCTCTGACCCCTGGGCCCACCTCTTACCGA
TTTCTTCCATACTACTACCCATCCACCTCTCATCACATCCCCGGCGGGGAATCTCCTTACTGCTCCCACTCAGTTTTCTTTTCTCTGGC
TTTGGGACCTCTTAACCTGTGGCTTCTCCTCCACCTACCTGAGCTGAGCCAGG.

The blast in NCBI :- Query is our results , while Subject is sequence in NCBI

```
Query  569  CCT-AGGTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGC  627
            ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  549  CCTGAGGTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGC  608

Query  628  ATGGGCGGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG  683
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  609  ATGGGCGGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG  664
```

The results shows deletion of nitrogen base G in region just before coding region of E7 so it's called splice- site mutation and affected the transcription factor of this exon and failure of transcription and translation, this type of mutation only occur in patients (1, 2 and 4), as shown in Figure (11) while patient 3 shown normal sequence .

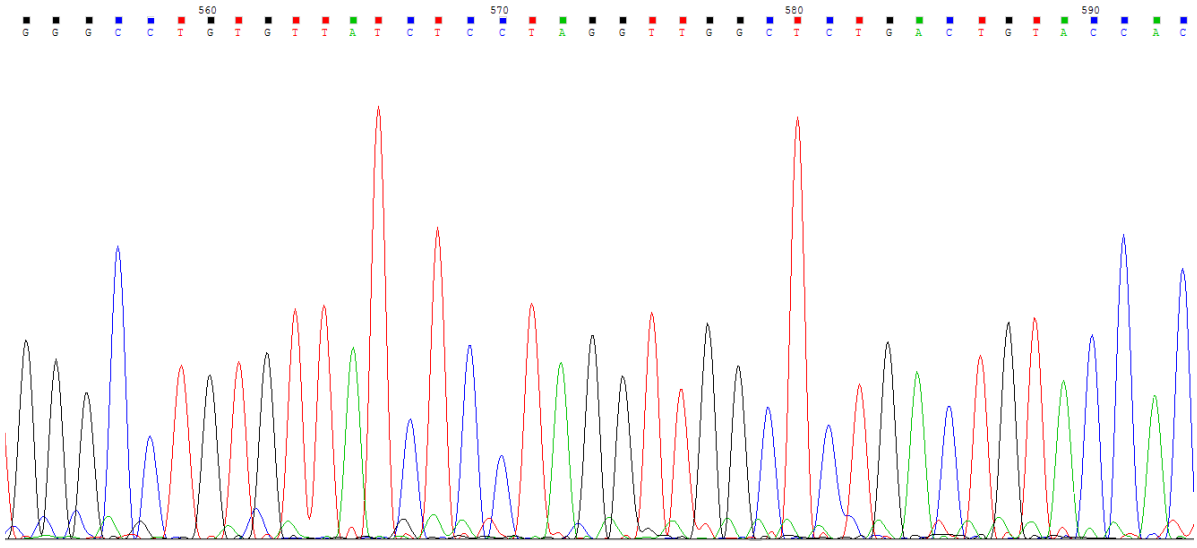


Figure (11): The sequence of TP53 - Exon 7 in liver cancer patients.

In all patients the sequence of Exon 8 were as follows:-

GGGACTAGTACTGGAGCTGGAGCTTAGGCTCCAGAAAGGACAAGGGTGGTTGGGAGTAGATGGAGC
CTGGTTTTTTAAATGGGACAGGTAGGACCTGATTTCCTTACTGCCTCTTGCTTCTCTTTTCCTATCCTGAGT
AGTGGTAATCTACTGGGACGGAACAGCTTTGAGGTGCGTGTTTGTGCCTGTCCTGGGAGAGACCGGCGCA
CAGAGGAAGAGAATCTCCGCAAGAAAGGGGAGCCTCACCACGAGCTGCCCCCAGGGAGCACTAAGCGA
GGTAAGCAAGCAGGACAAGAAGCGGTGGAGGAGACCAAGGGTGCAGTTATGCCTCAGATTCACTTTTAT
CACCTTTCCTTGCCTCTTTCCTA.

The blast in NCBI :- Query is our results , while Subject is sequence in NCBI.

```
Query  139  AGTGGTAATCTACTGGGACGGAACAGCTTTGAGGTGCGTGTTTGTGCCTGTCCTGGGAGA  198
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  663  AGTGGTAATCTACTGGGACGGAACAGCTTTGAGGTGCGTGTTTGTGCCTGTCCTGGGAGA  722

Query  199  GACCGGCGCACAGAGGAAGAGAATCTCCGCAAGAAAGGGGAGCCTCACCACGAGCTGCCC  258
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  723  GACCGGCGCACAGAGGAAGAGAATCTCCGCAAGAAAGGGGAGCCTCACCACGAGCTGCCC  782

Query  259  CCAGGGAGCACTAAGCGAG  277
            ||||||||||||||||||
Sbjct  783  CCAGGGAGCACTAAGCGAG  801
```

The nucleotide sequence in coding region show normal sequences as shown in figure (12)

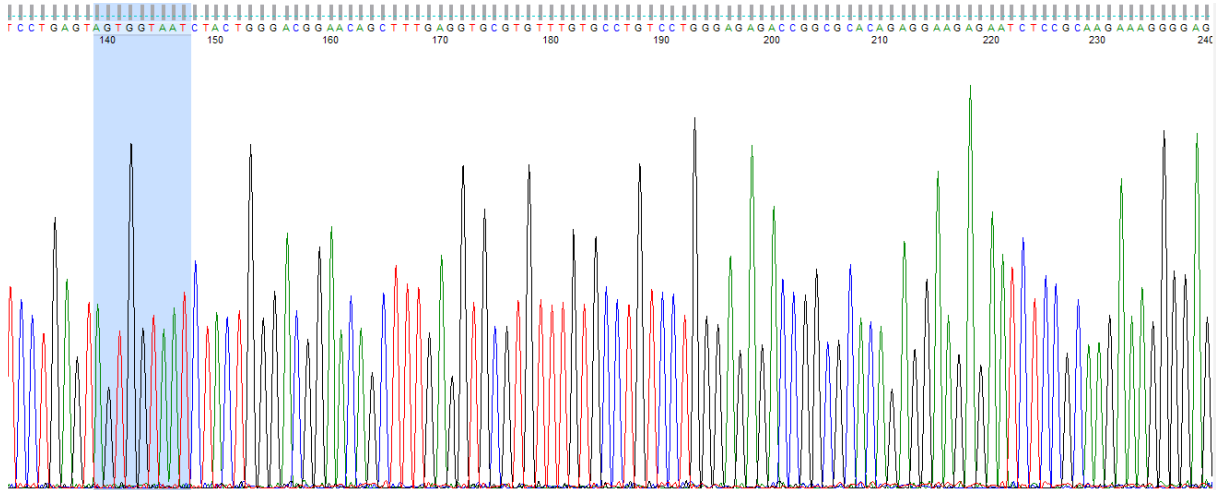


Figure (12): The sequence of TP53 - Exon 8 in liver cancer patients.

Gene mutations play a key role in transforming normal cells into cancerous cells; they directly or indirectly suppress the normal function of tumor suppressor genes or enhance transforming activity of oncogenes , numerous gene mutations have been identified in liver cance, one of the most commonly mutated genes is TP53, which encodes p53 protein. Tumor suppressor p53 plays a fundamental role in the regulation of the cell cycle and apoptosis, and its inactivation is central to the pathogenesis of many human cancers, including liver cancer(15) .

Specific mutations of the p53 tumor suppressor gene in hepatocellular carcinoma (HCC) have been reported from several parts of the world. The most frequent mutation of the p53 gene in HCC is base substitution at third base G to T of codon 249 in exon seven resulted in missense mutation leading to change of amino acid arginine to serine, whereas at codone 250 , it results in a change of proline to serine (26). In 1991, two reports showed that of all the mutations in the p53 gene in hepatocellular carcinoma, there was a predominance of the G:C to T:A transversions at the third base of codon 249 (Arg to Ser) in patients from Mozambique and China (27) .

Mutations of P53 are the most frequently detected genetic alteration in human cancer which is found in more than 50% of all types of human cancers (28) . Numerous gene mutations have been identified in liver cancer, one of the most commonly mutated genes is TP53, which encodes p53 protein. Tumor suppressor p53 plays a fundamental role in the regulation of the cell cycle and apoptosis, and its inactivation is central to the pathogenesis of many human cancers, including liver cancer (29). Single-nucleotide polymorphisms may fall within coding sequences of genes, non-coding regions of genes, or in the intergenic regions (regions between genes), SNPs within a coding sequence do not necessarily change the amino acid sequence of the protein that is produced, due to degeneracy of the genetic code while SNPs that are not in protein-coding regions may still affect gene splicing, transcription factor binding, messenger RNA degradation, or the sequence of non-coding RNA (30).

Coclusions

From the results of the present study, the following conclusions could be considered:

1- Chromosome aberrations was observed in patients who were suffering from liver cancer, included (Dicentric chromosome , chromatid interchange quadriradial and centromeric break). The highest value of CAs was (Dicentric chromosome) which occurred in liver cancer patients who are males at fifth age group (65-74).

2- Most patients who were suffering from liver cancer are males at age group(65-74), most of them have smoking ,alcohol habit and hepatitis viral infection .

3- From the study of TP53 gene in patients who are suffering from liver cancer in Erbil City, we observed mutation in exon 7 which was deletion of nitrogen base G ,just before the coding region , represent splice- site mutation .

References:

1. Jemal A., Bray F., Center M.M., Ferlay J., Ward E. and Forman D. (2011). Global cancer statistics. Cancer journal for clinicians 61 ;2: 69–90.
2. Iraqi cancer board – Iraqi cancer registry center – Baghdad – Iraq-2012.
3. General directorate of health – Hawler-2012-2016.
4. Drak M.T and Karpuzoglu, E.(2012). Gender differences in cancer susceptibility: an inadequately addressed issue. Frontiers in Genetics, Review Article.vol.3 article 268/ 1
5. Arzumanyan A., Reis HM .and Feitelson MA. (2013). Pathogenic mechanisms in HBV- and HCV-associated hepatocellular carcinoma. Nature reviews. Cancer.13 ; 2: 123–35.
6. Kew MC. (2013). Hepatitis viruses (other than hepatitis B and C viruses) as causes of hepatocellular carcinoma: an update. Journal of viral hepatitis,20 (3) : 149–57.
7. Jeong SW., Jang JY. and Chung RT. (2012). Hepatitis C virus and hepatocarcinogenesis. Clinical and molecular hepatology 18 (4) 347–56.
8. Fattovich G., Stroffolini T., Zagni, I and Donato F. (2004). Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology 127 ;5 Suppl 1: S35–50
9. Chuang SC., La Vecchia C and Boffetta P. (2010). Liver cancer: descriptive epidemiology and risk factors other than HBV and HCV infection. Cancer letters.286 ;1: 9–14.
10. Sona V. , Zdenka P.,Ludovit M., Zdenek S., Hana Z .,Sylvie S.,Elena K., Erika H. and Sona V. (2015). Structural chromosomal aberrations as potential risk markers in incident cancer patients. Mutagenesis, 31(3).
11. Grade M., Difilippantonio M.and Camps J.(2015) Patterns of Chromosomal Aberrations in Solid Tumors.Cancer Research.200:115-42
12. Shibata T. and Aburatani H. (2014) . Exploration of liver cancer genomes. Nature Reviews Gastroenterology & Hepatology .11: 340–349 .
13. Isobe M., Emanuel B.S., Givol D., Oren M. and Croce C.M.(1983).Localization of gene for human p53 tumor antigen to band 17p13. Nature. 320 ;6057:84-85.
14. Bell S.Klein C. Muller L. Hansen S. and Buchner S.(2002). P53 contains large unstructured regions in its native state. J Mol Bio. 322: 917-927.
15. Bai L. and Zhu W. (2006). P53: Structure, Function and Therapeutic. Applications . Journal of Cancer Molecules. 2;4: 141-153.
16. Atalay, R. C. and Ozturk, M. (2000). P53 mutations as fingerprints of environmental carcinogens. Pure Appl. Chem. 72;6: 995- 999.
17. Pettersson E, Lundeberg J, Ahmadian A (February 2009). "Generations of sequencing technologies". Genomics 93 (2): 105–11. .
18. Klein S.L.(2012) Immune cells . journal articles. Endocrinology. 153: 2544–2550.
19. Naugler WE., Sakurai T., Kim S., Maeda S., Kim K., Elsharkawy AM., Karin M. (2007). Gender disparity in liver cancer due to sex differences in My D88-dependent IL-6 production . Science. 6;317(5834):121-4- 23.
20. American Cancer Society. Cancer Facts & Figures (2015). Atlanta,: American Cancer Society.
21. Parkin D.M., Bray F., Ferlay J. and Etal (2002). Global cancer statistics, CA: A Cancer Journal for Clinicians 55:74–108, 2005.
22. Bagnardi V., B langiardoM., Lavecchia C., and C orrao G. A.(2001). Meta analysis of alcohol drinking and cancer risk. British Journal of Cancer .85:1700–10
23. Detlef S. and Nezam H.(2010).Liver cirrhosis.Lncet.8: 371(9615) : 838-851.

24. E l -Zayad I AR. (2006) .Heavy smoking and liver. World J .Gastroenterol 12;38: 6098-6101.
25. Aragane, H., Sakakura, C., Nakanishi M., Yasuoka R., FujitaY., Taniguchi H., Hagiwara A., Yamaguchi T., Abe T., Inazawa J. and Yamagishi H. (2001) .Chromosomal aberrations in colorectal cancers and liver metastases analyzed by comparative genomic hybridization. It J. Cancer .94; 5: 623-9.
26. Katiyar S., Dash B., Guptan R., Sarin S. and Das B.(2000.) TP53 tumor suppressor gene mutation in hepatocellular carcinoma patients in India ; cancer .l88 ;7: 1565-1573.
27. Staib F. Hussain. S.P., Hofseth L.J., Wang X.W. and Harris C.C. (2003). TP53 and liver carcinogenesis. Hum Mutat. 21: 201-216.
28. Mirzayans R. and Murray D.(2008). Pharmacological Modulation of p53 function in cancer therapy. Current signal transduction Therapy. 3; 183-194.
29. Cetin-Atalay ,R. and Ozturk M.(2000). P53 mutations as fingerprints of environmental carcinogens .Pure Appl. Chem, 72; 6: 995–999
30. Varela A.M. and Amos M. (2010). Heterogeneous distribution of SNPs in the human genome: Microsatellites as predictors of nucleotide diversity and divergence. Genomics 95(3): 151–159.

دراسة وراثة خلوية و جزيئية للجين P53 في سرطان الكبد المشخصة في مدينة اربيل باستخدام طريقة تعاقب جديدة

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قسم علوم الحياة/ كلية التربية/ جامعة صلاح الدين/ أربيل

الخلاصه:

مرض سرطان الكبد هو السادس الاكثر انتشارا من انواع السرطانات و المسبب الثاني من السرطانات المودية للموت في العالم ان الهدف الاول من الدراسة الحالية هي معرفة التشوهات الكروموسومية في الدم المحيطي لمرضى سرطان الكبد. ان الدراسة الحالية اجريت على(50مريض) مصابين بسرطان الكبد في محافظة اربيل و (25شخص من اصحاء) كمجموعة سيطرة، والهدف الثاني من هده الدراسة هي معرفة الطفرات في الجينة الكابتة للورم TP53 . هناك كثير من العوامل التي تساعهم في تطور المرض منها عوامل وراثية، عدوى بالفايروس التهاب الكبد نوع (B,C) ، التدخين والمشروبات الكحولية و عوامل اخرى يتضمن جنس المريض وعمره.من الدراسة الحالية تمت الاستنتاج بان اعلى قيمة للتشوهات الكروموسومية هي التشوه (كروموسوم ثنائي السنترومير) التي وجدت في الذكورفي المجموعة العمرية الخامسة (65-74) سنة، وايضا وجد بان معظم المرضى هم ذ كور في عمر(65-74) سنة ومعظمهم كانوا مدخنين ولديهم عادة شرب الكحول ومصابين باللتهاب الكبد الفايروسي نوع B.ومن الدراسة للجينة الكبتة للورم TP53تم ملاحظة حدوث طفرة في الاكسون السبع للجينة وهي من نوع النقص في القاعدة النتروجينية كوانين Gفي المنطقة قبل المشفرة وتمثل (Splice site mutation).