Point Mutation of p53 Gene Exon 7 as a New Early Diagnostic Marker of Hepatocellular Carcinoma

Sanaa M. Abdelaal[1], Mahmoud Elbendary[2], Mustafa Neamatallah[3], Emad Al-Shehat[4] and Mamdouh Elsherbiny[5]

Departments of Medical Microbiology & Immunology[1], Tropical Medicine[2], Medical Biochemistry[3] and Gastroenterology[4], Faculty of Medicine - Mansoura University and Medical Oncology, National Cancer Institute - Cairo University

ABSTRACT

Background:One of the characteristics of hepatocellular carcinoma (HCC) is the selective mutation resulting in serine substitution at codon 249 of the p53 tumor suppressor gene, and it has been identified as a "hotspot" mutation in hepatocellular carcinomas occurring in populations exposed to aflatoxins and with high prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV).

Objective: To evaluate whether this "hotspot" mutation could be detected in cell free DNA circulating in plasma of patients with hepatocellular carcinoma and cirrhosis, and tried to determine the significance of the detection of this molecular biomarker.

Subjects and Methods: Blood samples were collected from 105 subjects. Fourty two patients with hepatocellular carcinoma, 35 cirrhotic patients and 28 healthy control. DNA was extracted from the patient's plasma. The 249^{ser} p53 mutation was detected by restriction digestion analysis after PCR amplification.

Results: A total of 34 of the 105 subjects (32.4%) had p53 mutation, 22 from 42 subjects (52.4%) with hepatocellular carcinoma, 10 from 35 subjects (28.6%) with cirrhosis, and 2 from 28 subjects (7.1%) were healthy controls. The adjusted odds ratio (OR) for having mutation was 7.33 (95% confidence intervals (CI) 1.87-28.75) for HCC cases compared to controls which was extremly significant statistically (P<0.0001). Eight out of the 34 positive mutation were $HbsAg^+$ (23.5%) "5 from HCC and 3 from cirrhotic patients" while 17 out of 34 positive mutation were positive Anti-HCV (50%) "13 from HCC and 4 from cirrhotic patients".

Conclusion: These data show that the 249^{ser} p53 mutation in plasma is strongly associated with HCC. We found this mutation was also detected in plasma DNA of cirrhotics and healthy controls but at a much lower frequency. We consider that these findings, together with the conventional methods of HCC diagnosis, will give more information in ealry diagnosis of HCC, and 249^{ser} p53 mutation may be a new early diagnostic marker for HCC.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide, it is the fifth most common cancer (5.4% of all new cancer cases)[1,2].

The major risk factors for HCC include chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, to which 80% of the HCC cases are attributed worldwide[3]. Other known risk factors, such as aflatoxin B_1 (AFB_1) dietary exposure or heavy alcohol consumption, are capable of inducing HCC alone, but they also synergize with each other and chronic viral hepatitis[4].

Studies indicate that people who live in areas where potential AFB_1 exposure is high are 3 times more likely to develop HCC. Those who test positive for HBV are about 7 times more likely to develop HCC, when both criteria are met, people are 60 times more likely to develop this disease[5], while coinfection with HCV and HBV result in a 130 fold risk increase[6].

The tumor suppressor gene p53 is found to be involved in the carcinogeneses of diverse types of cancer. Some domains of the p53 proteins are highly conservated, which also reflect their significance for the function of the p53 protein[7]. If point mutations occur in these sites, the peptides that are translated from these templates will affect the correct folding of the p53 protein. Hence, the cell cycle suppressing function of p53 will be affected, which will result loss of cell proliferation control[8,9].

Codon 249 (exon 7) of the p53 is a hotspot for point mutation in HCC, and most of the point mutations are G→ T changes at the third base (Arginine → Serine), and others are G→ C (Arginine → Serine)[10], The point mutation of p53 exon 7 is highly correlated with aflatoxin B_1 (AFB1) intake[11] and HBV infection[12]. However, analysis of liver tissue from patients with HCC in areas of low aflatoxin intake shows a different mutational spectrum[13].

Exposure to vinyle chloride (VC) is another risk associated with typical p53 mutation[14]. Typical A:T to T:A transversions in codon 179 (exon 5) and 255 (exon 7) have been described in patients suffering from hepatic angiosarcoma after VC exposure[15].

Mutations in p53 can be detected by conventional methods e.g. single strand conformational polymorphism (SSCP) and direct DNA sequencing. Methods based on hybridization

of DNA or RNA with multiple defined oligonucleotides or cDNA probes attached to a sloid glass or nylon matrix have been developed and are referred to as oligonucleotide microarrays or gene Chips[16].

Point mutations of codon 249 will lead to the loss of the *Hae III* restriction site in the tumor's genomic DNA (AGGCC → AGTCC)[17], Therefore, PCR-RFLP should be a expedient and convenient method to detect such point mutation.

We adopted this approach to evaluate the presence of 249^{ser} p53 mutation in plasma from HCC cases, cirrhotic patients and healthy controls, and this mutation if present could be regarded as a new biomarker in HCC earlier diagnosis

.

SUBJECTS AND METHODS

Subjects

One hundred and five subjects (79 males and 26 females) were recruited in this study from patients attending National Cancer Institute, Cairo University, Tropical Medicine Unite and Gastroenterology Center, Mansoura University. The subjects were classified into 3 groups:

Group A Fourty two HCC patients (33 males and 9 females),

Group B Thirty five cirrhotic patients (26 males and 9 females). Diagnosis of cirrhosis was made on the basis of a consistent clinical evaluation, laboratory diagnosis, radiological studies, and liver biopsy when available. All patients showed signs of portal hypertension and they were being evaluated clinically to define the presence or absence of ascites and/or hepatic encephalopathy. The severity of liver disease was staged according to the **Child-Pugh classification**[18]. Diagnosis of HCC was made by the ultrasound and a combined consideration of the history, physical examinations, □-fetoprotein (AFP) levels and noninvasive imaging procedures [Computed tomography and magnetic resonance image (MRI)].

Group C Twenty eight subjects served as controls were recruited from the outpatient clinics among individuals with no history or clinical findings suggestive of liver disease, and have the same distributions of age, gender and recruitment site with the HCC and cirrhotic cases (Table 1).

Samples

From each patient and control, 5 ml blood samples were taken. Blood samples anticoagulated with EDTA were processed immediately after collection, plasma was transferred to a plain tube and stored at-70°C.

Test for viral markers

Anti-HCV was detected using HCV 3rd generation EIA kit from Abbott (Wiesbaden, Delknheim, Germany) following the manufacturer's instructions.HBV surface antigen (HBsAg) and HBV core antibody (HBcAb) were detected using the Abbott Auszyme and Corzyme Kits (Wiesbaden, Delknheim, Germany). HCV RNA was detected in sera by RT-PCR.

DNA extraction

DNA was extracted from 200 □l of plasma using a QIA Amp kit (Qiagen Hilden, Germany) according to the manufacturer's protocol. The DNA was eluted from silica column with 50 □l of Nuclease-Free Water.

PCR

Primers used for PCR amplification were as follows. P1(up) (5'-ctt gcc aca ggt ctc ccc aa-3'), P2(down) (5'-agg ggt cag cgg caa gca ga-3'). The expected size of the product was 254 bp, and this fragment was located in the exon 7 of p53 gene[10].

PCR amplification was performed in a 50□l volume with perkin ELmer cetus (Norwalk, conn.) thermal cycler. Reaction mixture contained two □ l of each DNA preparation, 1□ l of sense primer, 1□l of antisense primer were added to 46□ l of master mix containing 3□ l Mg cl2 solution, 5□ l X PCR buffer [100 mM Tris Hcl (pH 8.3), 500 mM Kcl/□ l]. 1□ l dNTPs mixture (10 mM/□ l), 0.4 □ l Ampli Taq DNA polymerase (5 units/□ l) all in 36.6 □ l DNAase free water (The PCR reagents and primer from Fermentas).

The thermo-cycling conditions were initial denaturation step at 94°C for 5 min, and 40 cycles of 94°C for 30 sec, 60°C for30 sec, 72°C for 30 sec, and finally 72°C for 10 min. The amplification products (254 bp) were visualized by staining with ethidium bromide, after electrophoresis on 2% agarose gel.

Mutation detection by restriction analysis

The 254 bp of DNA fragment, which is derived from exon 7 of p53 gene, was submitted to restriction enzyme *Hae III* digestion (Roche Diagnostics, Germany). The restriction enzyme digestion reaction system was as follows: 1□l *Hae III*, 2 □ l 10 x buffer, 5□ l DNA PCR amplified fragment, 12□ l dd H2O (20□ l total volume). These reaction systems were submitted to 37°C water incubation for 4 hours. Enzyme *Hae III* cleaves a GG/CC sequence at codons 249-250, generating 92 bp, 66 bp and several small fragments from the 254 bp DNA product of the PCR reaction. If there is a mutation at codon 249-250 resulting in an uncleaved, 158 bp fragment, and this features will be distinguished from that of the normal samples on 2% agarose gel stained with ethidium bromide. Absence of the band at 254 bp (full-length-PCR products) provides a control for complete digestion of the PCR product. The presence of the uncleaved 158 bp fragment indicates that there are mutations in the corresponding samples.

Statistical analysis

Statistical analysis was done by using SPSS (statistical package for social science program version 15, 2007 on Windows XP). The qualitative data were presented in the form of number and percentage. The quantitative data were expressed in the form of mean, standard deviation and range. Chi-square test was used to study difference of qualitative data. One way ANOVA test was used for comparison between quantitative data of the three groups. Significance * was considered at P value less than 0.05. Highly significance ** was considered when P value is less than 0.01. Extremely significance *** was considered when P value is less than 0.001. Insignificance was considered when P value is more than 0.05.

RESULTS

The study population comprised 105 subjects classified into 3 groups: **Group A**, 42 HCC patients (33 men, 9 women) of mean age 50.76 ± 11.08 years (range 32-75), **Group B**, 35 cirrhotic (26 men, 9 women) of mean age 51.03 ± 10.67 years (range 35-70). Twenty eight healthy subjects (20 men, 8 women) of mean age 49.68 ± 12.63 years (range 25-80) served as a control group, **Group C**. HCC cases, cirrhosis cases and controls were of similar age and gender distribution (Table 1).

The clinical and laboratory characteristics of the 105 subjects are shown in table (2).

Anti-HCV was detected in the sera of 30 HCC patients (71.4%), 22 of cirrhotics (62.9%) and non of the control. There were 7 HCC subjects (16.7%), 4 cirrhotic subjects (11.4%) and non of the control with serological evidence of infection with HBV. Serum AFP levels were more than 400 ng/ml in 36 HCC subjects (85.7%), 25 of cirrhotic subjects (71.4%) having levels below 400 ng/ml (Table 3).

The electrophoresis on 2% agarose gel shows that the 254 bp specific DNA fragments amplified between p1 and p2 are at the appropriate location according to the DNA molecular weight marker (Figure 1).

Restriction enzyme have been used to detect the mutation in codon 249 exon 7. The PCR product is subjected to digestion with *Hae III*, this enzyme cleaves a GG/CC sequence between codon 249 and 250 to generate two fragments at 92 and 66 bp (Fig. 2). Samples with mutations in codon 249 will result in an uncleaved 158 bp fragment.

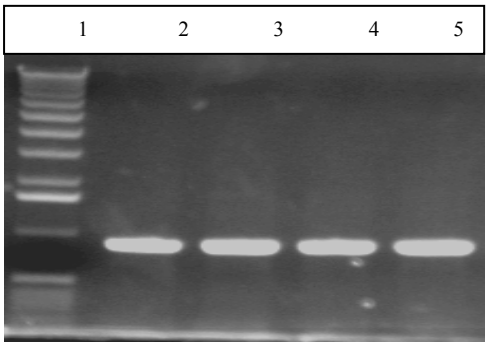

Figure (1): The electrophoresis map of PCR products. Lane 1: DNA Molecular weight marker. Lane 2-5: PCR products of partial samples.

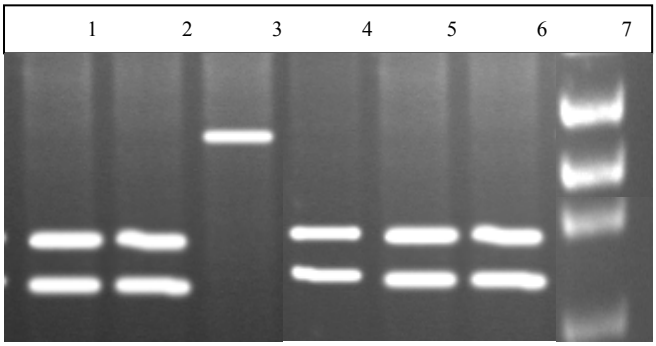

Figure (2): RFLP photo for the digestion of exon 7 PCR product with *Hae III*. Lanes 1 and 2 and 4-6 are wild-type samples, Lane 3 is a mutant sample and Lane 7 is the DNA marker.

A total of 34 of 105 subjects (32.4%) have 249^{ser} p53 mutation, including twenty two of 42 HCC patients (52.4%), ten of 35 cirrhotic patients (28.6%), and two of 28 control (7.1%). There was extreme significance difference between group A versus group C (P<0.0001)***, and significance difference between group B versus group C (P<0.03)** as regard incidence of mutation (Table 4).

As regard incidence of mutation in relation to viral infection, 8 out of 34 positive mutation were $HBsAg^{+}$ (23.5%) (5 HCC and 3 cirrhotic patients) while 17 out of 34 positive mutation were positive Anti-HCV (50%) (13 were HCC patients and 4 were cirrhotics) a 5.569 fold increased risk of p53 mutation in HBV infection (OR) 5.569; 95% CI 1.576-19.678 (Table 5).

Table (1): Gender and age of the studied patients and control

		Group (A)	Group (B)	Group (C)	P value
		HCC	Cirrhotics	Control	
Gender (m/f)		33/9	26/9	20/8	
Age	Mean ± SD	50.76 ± 11.08	51.03 ± 10.67	49.68 ±12.63	0.887
	Range	(32 – 75)	(35 – 70)	(25 – 80)	

P = 0.887

Table (2): Clinical and laboratory characteristics of studied cases:

Characteristics	Patients (N = 77)	Control subject (N = 28)
Ascites	63	Non
Haematemsis and melena	36	Non
Hepatic encephalopathy:		
Manifested	11	NA
Non	66	NA
Child-Pugh class:		
A	15	NA
B	22	NA
C	40	NA
Bilirubin (mean + SD)	3.5 + (101)	0.6 + 0.1
ALT	65 + 12	23 + 5.5
AST	56 + 14	27 + 6.8
Albumin	2.2 + 0.8	3.8 + 0.8

NA : not applicable

Table (3): Incidence of HBV, HCV infection and alpha-fetoprotein of the studied patients and control

	Group A (42)		Group B (35)		Group C (28)	
	Positive	Negative	Positive	Negative	Positive	Negative
Hepatitis B surface antigen	7 (16.7%)	35 (83.3%)	4 (11.4%)	31 (88.6%)	0 (0%)	28 (100%)*
Hepatitis C virus antibody	30 (71.4%)	12 (28.6%)	22 (62.9%)	13 (37.1%)	0 (0%)	28 (100%)**
Serum alpha-fetoprotein	36 (85.7%)	6 (14.3%)	25 (71.4%)	10 (28.6%)	0 (0%)	28 (100%)

* $X^2 = 5.027$ P = 0.081

** $X^2 = 38.022$ P = 0.0001

Table (4): Incidence of mutant p53 tumor suppressor gene among different groups

	Group A (42)		Group B (35)		Group C (28)	
	Positive	Negative	Positive	Negative	Positive	Negative
P53 mutation	22	2o	10	25	2	26
% within group	52.4	47.6	28.6	71.4	7.1	92.9
% within mutation	64.7	28.1	29.4	35.2	5.9	36.6
% of total	20.9	19.04	9.5	23.8	1.9	24.8

A vs C $X^2 = 15.26$ OR = 7.33 CI (95%) 1.87 - 28.75 P<0.0001

B vs C $X^2 = 4.63$ OR = 4.0 CI (95%) 0.953 - 16.79 P<0.03

Table (5): p53 mutations in different groups according to viral infection

		HBV			HCV		
		+ve	-ve	Total	+ve	-ve	Total
P53 positive Mutation	Count	8	26	34	17	17	34
	% within mutation	23.5%	76.5%	100%	50%	50%	100%
	% within viral infection	72.7%	27.7%	32.4%	32.7%	32.1%	32.4%
	% of total	7.6%	24.8%	32.4%	16.2%	16.2%	32.4%
P53 negative Mutation	Count	3	68	71	35	36	71
	% within mutation	4.2%	95.8%	100%	49.3%	50.7%	100%
	% within viral infection	27.3%	72.3%	67.6%	67.3%	67.9%	67.6%
	% of total	2.9%	64.8%	67.6%	33.3%	34.3%	67.6%
	Total count	11	94	105	52	53	105
	% within mutation	10.5%	89.5%	100%	49.5%	50.5%	100%
	% viral infection	100%	100%	100%	100%	100%	100%
	% of total	10.5%	89.5%	100%	49.5%	50.5%	100%

For HBV infection

X^2 9.135 OR 5.569 95% CI 1.576-19.678 P<0.003

For HCV infection

X^2 0.005 OR 1.014 95% CI 0.673-1.529 P = 0.946

DISCUSSION

Chronic hepatitis is characterized by increased regenerative cell proliferation, a process that makes cells more susceptible to gene mutations. Increased DNA synthesis *per se* is not sufficient to induce carcinogenesis unless genetic alterations, induced by various factors, appear and gradually accumulate[19].

One of the genes, most extensively studied in animal and human hepatocarcinogenesis, is the p53 tumour suppressor gene, and the development of human tumors is tightly correlated with its deletion, rearrangement and point mutation. p53 allele deletion and point mutation can be detected in many kinds of tumor tissue and 175, 248, 249, 273 and 282 point mutation are very commonly found[20,21,22].

The most striking example of a specific mutation in the p53 gene is a G → T transversion in the third base of codon 249, which has been detected in 10-70% of HCCs from area with a high exposure to AFB_1 reflecting DNA damage caused by AFB_1 metabolites in HCC[23].

Codon 72 polymorphism of the p53 gene has also been implicated in HCC and it has been suggested that it may have an impact on the clinical outcome of the disease[24].

P53 encodes a nuclear protein that controls normal cell proliferation, DNA repair and apoptotic cell deah[25].

The wild-type p53 protein is a nucleic phosphoprotein of 53,000 Daltons. Its quantity in serum is minimal and its half-life is about 20 min. Therefore, it is very difficult to be detected under normal condition[26]. However, expsure of cells to a variety of stress factors, results either in an increased rate of synthesis and stability of wild p53,or mainly in the production of a mutated protein with a longer half-life[27]. The detection of mutant p53 protein in healthy subjects indicates that as the p53 suppressive gene mutates the probability of developing cancer increase[28].

Frequent inactivation of p53 protein in most tumors indicate that the p53 protein plays a role in the development of human tumors and that the p53 gene is a susceptible target of carcinogens[29].

Many studies on the association between p53 gene mutation and tumors show that an abnormal expression of mutant p53 protein is probably linked to carcinogenesis of several types of human cell. Therefore, it is very important for early diagnosis of tumors to detect mutant p53 in the human body as early as possible[30].

Kirk et al. (2000)[31] reported for the first time the detection of codon 249 p53 mutation in the plasma of liver tumor patients from Gambia. Although the mechanisms accounting the presence of this circulating DNA are uncertain, there is some evidence that the DNA, of up to 21 Kb, are released from the tumor as a glyconucleoprotein complex, and may protect the DNA from degradation by nuclease[32]. DNA circulating in the plasma can be successfully retrieved and used as surrogate material to analyze for genetic alterations present in the original tumor[33].

This study detected p53 mutation in plasma of HCC, cirrhotics and healthy subjects. We found 34 of 105 (32.4%) subjects had 249^{ser} p53 mutation, including 22 of 42 (52.4%) HCC patients, 10 of 35 (28.6%) cirrhotic patients and 2 of 28 (7.1%) control. The difference of incidence of p53 mutation between HCC and control group

was extremely significant (P<0.0001) and consequently a 7.33 fold increased risk of liver cancer development (OR) 7.33 (95% CI: 1.87-28.75). We found also a significant difference between cirrhotics versus control group as regard 249^{ser} p53 mutation (P<0.03) and consequently 4.0 fold increased risk of cirrhotics development (OR) 4.0 (95% CI: 0.953-16.79).

Several other studies that examine p53 mutations in plasma of HCC patients reported rates similar to our finding; for example Huang et al. (2003)[10] reported that, the 249^{ser} p53 "hotspot" mutation was detected in 40% of HCC cases and a much lower prevalence was observed in cirrhotics (20%) and in controls (7%). The adjusted OR for having the mutation was 22.1 (95% CI: 3.2-91.7) for HCC cases compared to controls. Park et al. (2001)[34] reported that 45% of their subjects had a p53 mutation in tumor tissue. Other, for example Edamoto et al. (2003)[35] reported 13-15% HCC subjects positive for p53 mutation, but there was a wide array of etiologies including excess alcohol intake, in contrast to our study where HCV and HBV predominated.

Egypt has the highest prevalence of HCV in the world with antibodies to HCV (anti-HCV) being present in 12-25% of the population[36]. A chronic liver disease clinical center reported that almost 5% of patients with chronic liver disease had HCC[37]. Results from National Cancer Institute in Egypt show that the OR for the association of HCV with HCC was 27.2 (95% CI: 5.5-27.7) for rural males, and 9.3 (95% CI: 4.2-20.5) for females[38].

The molecular mechanism of HCV-induced hepatoceullar carcinoma is still unknown. P53, the major cellular defense against tumor development, has been implicated in the INF-a/b-based antiviral defense. Several of the HCV proteins such as core, NS3 and NS5A, have been shown to interact with p53, however, the exact mechanism is unknown[39].

Qadri et al. (2002)[40] hypothesized that HCV-NS5A protein binds to p53 in vivo leading to inactivation of p53 function and increasing the mutation frequency of important cellular genes (which increase the probability of neoplastic transformation).

Also other core protein, NS3 have seen to have a regulatory effect on cellular promoters, to interact with a number of cellular proteins, and to be involved in programmed-cell death modulation[41].

Recent reports suggest a mutagenic role for the HCV that could enhance mutation of several proto-oncogenes and anti-oncogenes, including p53[42].

In this study there was no significant association between p53 mutation and HCV infection (P = 0.946), out of 42 HCC subjects, 35 cirrhotics, 30 subjects (71.4%) and 22 subjects (62.9%) respectively were positive for anti-HCV. The prevalence of mutation within each group was 52.4% (22/42) for HCC subjects, 28.6% (10/35) for cirrhotic subjects and 7.1% (2/28) for control group. We found 13 of the 22 HCC positive mutation (59.1%) and 4 of 10 cirrhotic positive mutation (40%) were positive for anti-HCV. Neverthless virus C has no significant role in induction of mutation but this study does not exclude the mutagenic role for the HCV.

Pontisso et al. (1998)[43] also found no correlation between p53 mutations and HCV infection in a small group of 15 Caucasian patients with HCC.They reported a mutation in exon 5 codon 176 for a patient infected with HCV genotype I.

Wong et al. (2000)[44] reported mutations at codon 242 (exon 7) for HCV related HCCs from Japan. They also noted a mutation in codon 232 (ATC → AGC) for another HCV-related HCC case.

El-Kafrawy et al. (2005)[45] collected tumor tissues from 41 subjects with HCC. HCV RNA was detected in the sera of 37 subjects (90%). A total of 17 of the 41 subjects (41%) had p53 mutations. Thirteen of these were in exon 7, of which 10 were in codon 249. The other three exon 7 mutations were found in codons 232, 242 and 248. A total of three mutations were detected in exon 5. One mutation was detected in exon 8 codon 275. These results indicate that exons 5 and 7 are the most frequent sites for p53 mutations in HCV associated HCC.

Infection of HBV is also a risk factor of chronic active hepatities, hepatic cirrhosis and hepatocellular carcinoma. It is well established that most HCCs related HBV infection contain HBV DNA sequence integrated into the host chromosomal DNA causing by itself or through proteins chromosomal rearrangements and fixed DNA mutations[46]. HBX, a protein of HBV, is an activator of transcription process involved in hepatocarcinogenesis. Some studies indicate the correlation between mutation, inactivation of p53 and HBX in hepatocarcinogenesis. In that process HBX will suppress p53 function, which lead to ineffective liver cell division and resulting in HCC[12].

The results of this study indicate that the incidence of HBV infection in HCC, cirrhotics and control group were 16.7% (7/42), 11.4% (4/35) and 0% (0/28) respectively. Out of the 7 HCC, 4 cirrhotics subjects positive for HBV we found five and three subjects respectively had mutation at codon 249 (exon 7). There was extrem significant correlation between HBV infection and p53 mutation (P<0.003) and so there was elevated

risks associated with HBsAg seropositivity and occurrence of p53 mutation OR 5.569 (95% CI: 1.576-19.678).

In a study in Taiwan of 110 cases of liver cancer and 37 controls, HBV status was assessed by assay for HBsAg, p53 status by immunohistochemistry. The main findings were elevated risks associated with HBsAg seropositivity (OR, 8.4) p53 mutation were found in 29% of cases and mutation at codon 249 in 13%. Mutation in codon 249 were found only in $HBsAg^+$ subjects, suggesting that HBV is involved in the selection of these mutation(47).

Another study on the relationship between HBV, and mutation at codon 249 of p53 showed that, seven of 21samples from patients with hepatocellular carcinoma in Tongan, China, had point mutations at codon 249 resulting in a G to T transversion. Only one of the patients was HBV-negative(48).

We can draw a conclusion from these data that p53 mutation frequency of these different groups provided the strong relationship between the detection of such mutation and the future development of HCC.This mutation may be regarded as an early detection marker or a prognostic molecular marker in HCC. The detection of 249^{ser} DNA may be useful as a marker of the neoplastic development, and the presence of the mutation in healthy subjects may reflect chronic exposure to high levels of AFB1.

The presence of the R249S mutation in exon 7, associated with AFB_1 exposure, may indicate these patients with HCC have been exposed to AFB_1. Further studies must be done to measure the AFB_1-albumin adducts in the sera of these subjects. Future studies of the mutational spectra of p53 and other important tumor suppressor genes in HCC should consider the possible roles of both viral and environmental factors.

REFERENCES

1- ***Parkin DM, Pisani P and Ferlay J. (1999):*** Global cancer statistics. CA Cancer J. Clin., 49: 33-64.

2. ***Pisani P. Parkin DM, Bray F and Ferlay J. (1999):*** Estimates of the worldwide mortality from 25 cancers in 19990. Int. J. Cancer; 83: 18-29.

3. ***Chen CJ, Yu, MW, Liaw UF (1997):*** Epidemiological characteristics and risk factors of hepatocellular carcinoma. J Gastroenterol. Hepatol; 12: 294-S308.

4- ***Jackson PE and Groopman JD (1999):*** Aflatoxin and liver cancer. Baillieres Best Pract Res Clin Gastroenterol; 13: 545-555.

5. ***Smela ME, Hamm ML, Henderson PT, Harris CM, Harris TM and Essigmann JM (2002):*** The aflatoxin B_1 formamidopyrimidine adduct plays a major role in causing the types of mutations observed in human hepatocellular carcinoma. Proc Natl Acad Sci USA; 99: 6655-6660.

6- ***Perz JF, Armstrong GL and Farrington LA (2006)***: The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. J. Hepatol.; 45: 529-538.

7. ***Johnson SM, Shaw JA and Walker RA (2002)***: Sprdic breast cancer in young women: prevalence of loss of heterozygosity at p53, BRCA1 and BRCA2. Int J Cancer; 98: 205-209.

8- ***Guan YS, La Z, Yang L, He Q and Li P (2007)***: p53 gene in treatment of hepatic carcinoma world. J. Gastroenterol; 13(7): 985-992.

9. ***Sorak M, Arsenljevic S, Lukie G and Baskic D (2007)***: Relationship of serum levels of tumor markers with tissue expression of gene products in ovarian carcinoma. J BUON; 12:99-104.

10- ***Huang XH, Sun LH, Lu DD, Sun Y, Zhang XR, Huang J and Yu L (2003)***: Codon 249 mutation in exon 7 of p53 gene in plasma DNA: may be a new early diagnostic marker of hepatocellular carcinoma in Qidong risk area China. World J Gastroenterol; 9:692-695.

11. ***Zhang YJ, Rossner PJr, Chen Y, Wang L, Lee PH and Santella RM (2006)***: Aflatoxin B_1 and polycyclic aromatic hydrocarbon adducts, p53 mutations and P^{16} methylation in liver tissue and plasma of hepatocellular carcinoma patients. Int J Cancer; 119: 985-991.

12. ***Dewantoro O, Garil RA and Akbar N (2006)***: Hepatocarcinogenesis in viral Hepatitis B infection: the role of HBX and p53. Acta Med Indones; 38: 154-159.

13. ***Oda T, Tsuda H, Scarpa A, Sakamoto M and Hirohashi S (1992)***: p53 gene mutation spectrum in hepatoceullar carcinoma. Cancer Res.; 52: 6358-6364.

14. ***Loffredo CA and Frank C (2002)***: Liver cancer and vinyl chloride. Clin. Occup. Environ. Med.; 2: 829-839.

15. ***Trivers GE, DeBenedetti VM, Cawley HL, Caron G, Harrington AM, Bennett WB, Jett JR, Colby TV, Tazelaar, H, Pairolero P, Miller R and Harris CC (1996)***: Anti-p53 antibodies in sera from patients with chronic obstructive pulmonary disease (COPD) can predate a diagnosis of cancer. Clin. Cancer Res.; 2: 1767-1775.

16. ***Wen WH, Bernstein L, Lescallett J, Beazer-Barclay Y, Sullivan-Halley J, White M and Press MF. (2000)***: Comparison of TP53 mutations identified by oliognucleotide microarray and conventional DNA sequence analysis. Cancer Res.; 60: 2716-2722.

17. ***Bressac B, Kew M, Wands J and Ozturk M (1991)***: Selective G to T mutation of p53 gene in hepatocellular carcinoma from Southern Africa. Nature; 350: 427-428.

18. ***Child CG and Turcotte JG (1964)***: Surgery and portal hypertension. In: The liver and portal hypertension. Child CG (ed). Philadelphia; 50-64.

19. ***Wong N, Lai P and Lee SW (1999)***: Assessment of genetic changes in hepatocellular carcinoma by comparative genomic hybridization analysis. Relationship to disease stage, tumor size and cirrhosis. Am J Pathol; 154: 37-43.

20. ***Lee CC, Liu JY, Lin JK, Chu JS, Shew JY (1998)***: p53 point mutation enhanced by hepatic regeneration in aflatoxin B_1-induced rat liver tumors and preneoplastic lesions. Cancer Letter; 125: 1-7.
21. ***Uzoaru I, Rubenstein M, Mirochnik Y, Slobodskoy L, Shaw M and Guinan P (1998):*** An evaluation of the markers p53 and Ki-67 for their predictive value in prostate cancer. J. Surg Oncol; 67: 33-37.
22. ***Martone T, Airoldi L, Magagnotti C and Vineis P (1998):*** Aminobiphenyl-DNA adducts and p53 mutation in bladder cancer. Int J. Cancer; 75: 512-516.
23. ***Wang JS, Huang T, Su J, Liang F and Grorpman JD (2001):*** Hepatocellular carcinoma and aflatoxin exposure in Zhuqing village, Fusui county, people's Republic of China. Cancer Epidemiol Biomarkers.; 10: 143-146.
24. ***Ezzikouri S, El-Feydi AE, Chatik A, Hassar M, Pineau P and Benjelloun S (2007)***: The pro variant of the p53 codon 72 polymorphism is associated with hepatocellular carcinoma in Moroccan population. Hepatol Res.; 37(9): 748-754.
25. ***Cox LS and Lan DP (1995):*** Tumor suppressors, kinases and clamps: how p53 regulates cell cycle in response to DNA damage, Bioassays, 17: 501-508.
26. ***Porter PL, Gown A and Kramp SG (1992)***: Widespread p53 overexpression in human malignant tumors: an immunohitochemical study using methacam fixed embedded tissue. Am J Pathol; 140: 145-153.
27. ***Livni N, Eid A and Ilan Y (1995)***: p53 expression in patients with cirrhosis and without hepatocellular carcinoma. Cancer; 75: 2420-2426.
28. ***Sarkis AS, Dalbagni G and Gordon C (1993):*** Nuclear overexpression of p53 protein in transitional cell bladder cancer carcinoma: a marker for disease progression. J Natl Cancer Inst; 85: 53-55.
29. ***Lane DP (1993):*** Cancer: a death in the life of p53. Nature; 362: 786-787.
30. ***Hollstein M, Sidransky D, Vogelstein B and Harris CC (1991):*** p53 mutation in the human cancers. Science, 253: 49-53.
31. ***Kirk GD, Camus-Randon AM, Mendy M, Goedert JJ, Merle P, Trepo C, Brechot C, Hainaut P, Montesano R (2000):*** 249^{ser} p53 mutations in plasma DNA of patients with hepatocellular carcinoma from the Gambia. J Natl Cancer Inst; 92: 148-153.
32. ***Goessl C, Heicappell R, Munker R, Anker P, Stroun M, Krause H, Muller M, Miller K. (1998):*** Microsatellite analysis of plasma DNA from patients with clear cell renal carcinoma. Cancer Res; 58: 4728-4732.
33. ***Xiang CQ, Shen CL. Wu ZR, Qin YQ and Zhang SN (2007)***: Detection of mutant p53 protein in workers occupationally exposed to Benzidine. J. Occup Health; 49: 279-284.
34. ***Park NH, Chung YH, Youn KH, Song BC, Yang SH, Kim JA, Lee HC, Yu E, Lee YS, Lee SG, Kim KW and Suh DJ (2001):*** Close correlation of p53 mutation to microvascular invasion in hepatocellular carcinoma. J Clin Gastroentero; 33: 397-401.
35. ***Edamoto Y, Hara A, Biernat W, Terracciano L, Cathomas G, Riehle HM, Matsuda M, Fujii H, Scoazec JY and Ohgkai H. (2003)***: Alterations of RB1, p53 and Wnt pathways in hepatocellular carcinomas associated with hepatitis C, hepatitis B and alcoholic liver cirrhosis. Int. J. Cancer; 106: 334-341.
36. ***Anzola M (2004)***: Hepatocellular carcinoma: Role of Hepatitis B and Hepatitis C viruses protein in Hepatocarcinogenesis. J Viral Hepato.; 11: 383-393.
37. ***El-Zayadi AR, Abaza H, Shawky S, Mohamed MK, Selim OE and Badran HM. (2001)***: Prevalence and epidemiological features of hepatocellular carcinoma in Egypt a single center experience. Hepatol Res.; 19: 170-179.
38. ***Loffredo CA, Ezzat S, Frank C, Hifnawy T, El-Din ARG, Enewold L, Abdel-Hamid M, Mokhtar N, Strickland GT and Eissa SAL (2003)***: Increased risk for hepatocellular carcinoma associated with agricultural pesticides and viral infections in Egypt. Genet Mol. Biol.; 26 (Suppl. 2): 103.
39. ***Deng L, Nagano-Fujii M, Ikeda M and Kato N (2006)***: N53 protein of hepatitis C virus associates with the tumor suppressor p53 and inhibit its function in an N53 sequence-dependent manner. J Gen. Virol; 87: 1703-1713.
40. ***Qadri I, Iwahashi M and Simon F. (2002)***: Hepatitis C virus NS5A protein binds TBP and p53, inhibiting their DNA binding and p53 interactions with TBP and ERCC3. Biochim. Biophys. Acta, 1592: 193-204.
41. ***Dharel N,Kato N, Shao PX and Kawab T. (2006)***: MDM2 promoter SNP309 is associated with risk of hepatocellular carcinoma in patients with chronic hepatitis C Clinic. Center Res.; 2: 4867-4877.
42. ***Dharel N, Kato N, Otsuka M and Wang Y (2008):*** Potential contribution of tumour suppressor gene p53 in the host defense against HCV. Hepatol; 47: 1136-1149.
43. ***Pontisso P, Belluco C, Bertorelle R, De Moliner L., Chieco-Bianchi L, Nitti D, Lise, M and Alberti A. (1998):*** Hepatitis C virus infection associated with human hepatocellular carcinoma lack of correlation with p53 abnormalities in Caucasian patients. Cancer; 83: 1489-1494.
44. ***Wong N, Lai P, Pang E, Fung LF, Sheng Z, Wong V, Wand W, Hayashi Y, Perlman E, Yuna S, Lau J, Johnson PJ (2000)***: Genomic aberration in human hepatocellular carcinoma of differentetiologies. Clin. Cancer Res.; 6: 4000-4009.
45. ***El-Kafrawy SA, Abdel-Hamid M, El-Daly M, Nada O, Ismail A, Ezzat S, Abdel-Latif C, Abdel-Hamid A, Shields PG and Loffredo S (2005):*** p53 Mutations in hepatocellular carcinoma patients in Egypt. Int. J. Hyg. Environ-Health, 208: 263-270.
46. ***Lee SG and Rho HM (2000)***: Transcriptional repression of the human p53 gene by hepatitis B viral X protein. Oncogene; 19: 468-471.
47. ***Lunn RM, ZhangYJ, Wang LY, Chen CJ, Lee, PH, Lee CS, Tsai WY, Santella RM (1997):*** p53 mutations, chronic hepatitis B virus infection and aflatoxin exposure in hepatocellular carcinoma in Taiwan. Cancer Res; 57:3471-3477.
48. ***Yang M, Shou H, Kong RY, Zhuang W and Yang S (1997):*** Mutations at codon 249 of p53 gene in human hepatocellular carcinomas from Tongan, China. Mutat Res; 381: 25-29.

أحد الطفرات المميزه فى جين p53 المثبط للأورام كدليل مبكر من دلالات الأورام بين مرضى سرطان الكبد

د / سناء محى الدين عبد العال، د / محمودالبندارى، د / مصطفى نعمة الله ، د / عماد حمدى الشحات، د / ممدوح الشربينى

من أقسام الميكروبيولوجيا والمناعة الطبية، طب المناطق الحاره، الكيمياء الحيويه وجراحة الجهاز الهضمى كلية الطب - جامعة المنصورة ، وقسم باطنة أورام - المعهد القومى للأورام – جامعة القاهرة

ملخص البحث

خلفية الموضوع: أحد الصفات المميزه لسرطان الكبد هو نوع من الطفرات التى تحدث فى الحمض الأمينى (السيرين) الذى تمثل شفرته الرقم ٢٤٩ فى جين p53 المثبط للأورام. وقد عرفت هذه الطفره على أنها نقطه ساخنه فى الطفرات الموجوده فى مرضى سرطان الكبد الذين يتعرضون لنوع فى سموم الفطريات "أفلاتوكسين" بالإضافه لإصابتهم بالتهاب الكبد الوبائى "ب ، سى".

هدف البحث

أجريت هذه الدراسه بغرض إمكانية إستخدام وجود هذه الطفره فى بلازما الدم لهؤلاء المرضى كدليل مبكر من دلالات الأورام.

خطة البحث

أجريت هذه الدراسه على ٧٧ مريضا من مستشفيات جامعة المنصورة والمعهد القومى للأورام منهم ٤٢ مريض بسرطان الكبد و٣٥ مريض بتليف الكبد بالإضافه الى ٢٨ أصحاء كمجموعه ضابطه. وتم استخلاص الحمض النووى فى بلازما الدم وقطعه بإنزيمات القص لتأكيد حدوث الطفره من عدمه حيث أن الجين فى الحاله الطبيعيه يعطى قطعه من الحمض النووى تحتوى على ٢٥٤ قاعده نيتروجينيه بعد تفاعل البلمره المتسلسل للجزء المحيط بالمنطقه التى تحدث فيها الطفره "القاعده الثالثه من الشفره رقم ٢٤٩" وبعد معاملة هذا الجزء بإنزيم القص "Hae III" ينتج قطعه من الحمض النووى طولها ١٥٨ قاعده نيتروجينيه فى حالة وجود الطفره.

نتيجة البحث

أثبتت الدراسه وجود هذه الطفره بين مرضى سرطان الكبد وتليف الكبد بفارق ذو دلاله احصائيه مقارنه بالمجموعه الضابطه، وعلى ذلك يمكن القول بأنه بالاضافه للطرق المستخدمه فى تشخيص سرطان الكبد يمكن استخدام وجود هذه الطفره فى بلازما الدم كدليل مبكر من دلالات الأورام.