P53 Codon 72 Gene Polymorphism in Patients with Hepatocellular Carcinoma on Top of Viral and Nonviral Etiologies

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ABSTRACT

Background and aim: Loss of p53 function has been suggested to be a critical step in multistage hepatocarcinogenesis. So, we aimed to investigate the frequency of P53 codon 72 gene polymorphism and its relation to plasma P53 levels in Egyptian patients with hepatocellular carcinoma (HCC) on top of viral and nonviral etiologies.

Methods: This is a hospital-based case-control study which included 159 HCC patients in addition to 83 healthy volunteers as controls. Patients were classified into: 63 patients with HCC complicating cirrhosis due to HCV; 55 patients with HCC complicating cirrhosis due to HBV and 41 patients with HCC complicating cirrhosis due to nonviral causes. Quantitative determination of plasma P53 levels was performed by ELISA. P53 Arg 72 Pro gene polymorphism was carried out by conventional PCR followed by restriction enzyme digestion (PCR-RFLP).

Results: There were significant increases in α-fetoprotein and plasma P53 levels in all studied groups in relation to the control group. AA genotype and A allele were more in the control group. PP genotype and P allele were more frequent in HCV related HCC group & HCC with non viral causes. AP genotype and P allele were more frequent in HBV related HCC group. P 53 plasma level showed significant increase in all groups in relation to the control group in AA genotype, AP genotype and PP genotype. There were significant increases in AP and PP genotype in all studied groups in comparison to AA genotype. Plasma P53 level showed significant increase in all groups in both allele A and allele P when compared with the control group. Also, it showed significant increase in their levels in P allele when compared with that of A allele in all studied groups. Conclusion: plasma p53 protein level could be considered as an additional tumor marker to AFP to increase the diagnostic potential of AFP in HCC patients. Therefore, P53codon 72 gene polymorphism could be used as an indicator of the genetic susceptibility for future development of HCC in Egyptian cirrhotic patients.

Key words: P53 codon 72 polymorphism –HCC- plasma P53.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common malignancy in the world. Its incidence is increasing worldwide ranging between 3-9%1. In Egypt, HCC was reported to account for about 4.7% of chronic liver diseases (CLD) patients2. Over a decade, there were nearly two-fold increases in
proportion of HCC among CLD patients in Egypt with a significant decline in HBV infection and a slight increase of HCV infection as a risk factor\(^{(3)}\). Development of HCC is generally preceded by chronic liver damage leading to cirrhosis. Screening liver cancer in patients at high risk by AFP and imaging diagnostics are conventional approaches for early detection. However, the cost effectiveness has long been debatable\(^{(4)}\). In addition, half the HCC patients are AFP negative\(^{(5)}\). Like most solid tumors, the development and progression of HCC are believed to be caused by the accumulation of genetic changes resulting in altered expression of cancer-related genes, such as oncogenes or tumor suppressor genes, as well as genes involved in different regulatory pathways, such as cell cycle control, apoptosis, adhesion and angiogenesis\(^{(6)}\).

P53 is encoded by the TP53 gene located on the short arm of chromosome 17 (17p13.1) and is of critical importance for the regulation of cell cycle and maintenance of genomic integrity\(^{(7)}\). Loss of p53 function has been suggested to be a critical step in multistage hepatocarcinogenesis\(^{(8)}\). A specific p53 mutation at codon 249 in exon 7 was associated with aflatoxin B1 (AFB1)-induced HCC in certain areas of high AFB1 contamination. The wild-type p53 gene exhibits a polymorphism at codon 72 in exon 4, with a single nucleotide change that causes a substitution of proline for arginine (Arg\(^{72}\)Pro). The two polymorphic variants of p53 are functionally distinct, and these differences may influence cancer risk. The polymorphism consists of a single base pair change of either arginine or proline which creates 3 distinct genotypes: homozygous for arginine (Arg/Arg), homozygous for proline (Pro/Pro) and a heterozygote (Pro/Arg). P53 codon 72 polymorphisms have been reported to be associated with cancers of the lung, esophagus, stomach, colorectal, breast, bladder, and cervix\(^{(9)}\).

Understanding the molecular events characterizing that carcinogenic pathway could be of importance in patient’s management, especially at the preneoplastic stage. So, the aim of the present study was to investigate the frequency of P53 codon 72 gene polymorphism and its relation to serum P53 levels in Egyptians patients with hepatocellular carcinoma on top of viral or nonviral etiologies.

**SUBJECTS & METHODS**

**Patients:**

This hospital-based case-control study included 159 HCC patients recruited prospectively from Out and Inpatient Clinics of Tropical Medicine Department, Mansoura University during the period from January 2010 to November 2012. Eighty three healthy volunteers were also included as controls. The study was approved by the Institutional Review Board of our university and an informed consent was obtained from all subjects.

Our patients were classified into: 63 patients with HCC complicating cirrhosis due to HCV (Group I); 55 patients with HCC complicating...
cirrhosis due to HBV (Group II) and 41 patients with HCC complicating cirrhosis due to nonviral causes (Group III) (seronegative and PCR negative for HCV and HBV).

HCC was diagnosed according to the diagnostic guidelines of the European Association for the Study of the Liver[10]. Patients with liver diseases other than HCC were excluded from the study. Patients diagnosed with HCC but without cirrhosis or with low AFP<200 ng/dl or patients with other liver tumors (liver metastasis) in addition to HIV infected patients were also, excluded.

**Samples:**
All subjects were instructed to fast for at least 12 hours. A 10 ml blood sample was withdrawn. Five ml were delivered to centrifuge tubes containing K2EDTA. One ml of that K2EDTA anti-coagulated blood sample was stored at -30°C for DNA extraction. The remaining 4.0 ml of that samples were prepared to obtain plasma for measurement of plasma P53 levels. Another 5 ml blood sample was allowed to clot for 15 minutes and centrifuged at 7000 rpm for 10 minutes for serum separation to determine: liver enzymes activities (AST, ALT and GGT), and serum levels of total proteins, albumin and α fetoprotein.

**DNA Extraction:**
Genomic DNA was extracted from K2EDTA-anticoagulated peripheral blood leucocytes using QIAamp DNA Blood Mini Kit supplied by Qiagen GmbH (Cat. No. 51104, Hiden, Germany)[13]. The average DNA concentration (0.127±0.005µg/µl) was determined from absorbance at 260 nm (Jenway, Genova Model, UK). All samples had a 260/280 nm absorbance ratio between 1.6 and 1.79. The integrity of the DNA was checked by electrophoresis on 0.8 % agarose gel stained with ethidium bromide.

**Genotyping of p53 Arg72Pro Polymorphism (12):**

**Polymerase Chain Reaction (PCR):**
The primers sequences used for DNA amplification are as follow: 5'-TTGCGTCCCAAGCAATGGATGA-3' (sense) and 5'-TCTGGGAAGGGACAGAAGATGAC- 3' (antisense). PCR was carried out in 50 µl final reaction volume using Ready Mix (RED. Taq-PCR Reaction Mix) (purchased from Sigma Aldrich, Saint Louis, USA). The following mixture was prepared for each sample: 25 µl RED-Taq PCR reaction Mix (1×), 1 µl (20 pmole) of forward primer, 1 µl (20 pmole) of reverse primer, 2 µl (200ng) of genomic DNA and 21 µl of double distilled deionizer water. This mix was put in a thin wall PCR microcentrifuge tube and gently centrifuged to collect all components to the bottom of the tube. Amplification was performed in a Thermal Cycler (TECHEN TC-312, Barloworld Scientific Ltd. Stone, Stafford Shire, st 150 SA,UK) using the following program: initial 5 minutes denaturation at 94°C followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds extension at 70°C for 30 seconds and a final extension for 7 minutes at 70°C. The resulting PCR product was 199 bp in length.

Amplified samples were digested with the specific restriction enzyme P (New England BioLabs) for 2 hours at
37 °C, electrophoresed on a 3% agarose gel for 60 minutes stained with ethidium bromide, visualized via light UV Transilluminator (Model TUV-20, OWI Scientific, Inc. 800 242-5560, France) and photographed and evaluated. Acc II digestion of the amplified fragment identified two alleles: the Arg allele produced 113 and 86 bp fragments and the Pro allele produced a 199 bp fragment (figure 1).

**Estimation of Plasma P53 Level:**
Quantitative determination of plasma P53 levels was performed by Human P53 ELISA Kits, Catalog number (IB39567), Immuno-Biological Laboratories, Inc. (IBL-America) 8201 Central Ave NE, Suite P, Minneapolis, MN 55432. This assay employs the quantitative sandwich ELISA technique which measures P53 in plasma. It was performed according to the manufacturer’s instructions. The absorbance of each sample was read on plate ELISA reader (Tecan, Sunrise Absorbance reader) at 450 nm wave length.

**Serum level of α-fetoprotein** was estimated using alpha Fetoprotein Human ELISA Kit (catalog number ab108838, Abcam, alpha Fetoprotein Human). This assay employs a quantitative sandwich enzyme immunoassay technique. It was performed according to the manufacturer’s instructions. The absorbance of each sample was read on plate ELISA reader (Tecan, Sunrise Absorbance reader) at 450 nm wavelength.

HCV antibody and HBsAg were estimated by an enzyme immunoassay (EIA) for the qualitative detection of IgG antibodies to Hepatitis C Virus (HCV)\(^{(13)}\) and HBsAg\(^{(14)}\) in human serum using RUO kits (catalog number; 6307125& 6307105 respectively)- LINEAR CHEMICALS S.L. Joaquim Costa 18 2ª Planta. 08390 Montgat,Barcelona, SPAIN. The confirming examination was genetically performed by the Cobas TaqMan HCV test real-time RT quantifiable PCR.

Serum levels of albumin\(^{(15)}\), total proteins\(^{(16)}\), total bilirubin\(^{(17)}\) and activities of alanine transaminase enzyme (ALT), aspartate transaminase enzyme (AST)\(^{(18)}\) as well as γ-glutamyl transpeptidase (GGT)\(^{(19)}\) were estimated by enzymatic methods.

**Statistical Analysis:**
The statistical analysis of data done using Excel program and SPSS program statistical package for social science version 10. The description of the data done in form of mean ± standard deviation (SD) for quantitative data and frequency & proportion for qualitative data. The analysis of the data was done to test statistical significant difference between groups. For quantitative data, student unpaired t-test was used to compare between 2 groups. One way ANOVA test was used to compare more than 2 groups. Chi square test was used to compare qualitative data. P is significant if \(< 0.05\) at confidence interval 95%.

**RESULTS**
There are significant increases in α-fetoprotein and serum P53 levels in all studied groups (HCC on top of virus and HCC with no virus...
infection) in relation to the control group. In addition, there is significant increase in the serum activity of liver enzymes and serum bilirubin level in HCC patients (group II & group III) in comparison with that of the control group as shown in table (1).

In the present study, figure (2) shows positive correlation between serum P53 levels and α fetoprotein as well as GGT levels, while, it shows negative correlation with serum albumin levels. Also, there is negative correlation between α fetoprotein and serum albumin but it shows positive correlation with serum GGT activities.

The genotype and allelic distribution of p53 gene and the frequency of its polymorphism is presented in table (2), where it is presented as follow: AA genotype and A allele is more in the control group (44.5% & 64% respectively), PP genotype is more frequent in group (41.2% & 58.5% respectively) and P allele is more frequent in the same group also(71.41% & 80.5% respectively. Lastly, AP genotype and P allele is more frequent in the group II.

In table (3), P 53 plasma level shows significant increase in the group I, group II and group III in relation to the control group in AA genotype, AP genotype and PP genotype, but it shows significant increase in AP and PP genotype in all studied groups in comparison to AA genotype in all of the studied groups.

Table (4) discriminates that plasma P53 level shows significant increase in group I, II & III in both allele A and allele P when compared with the control group. Also, It shows significant increase in their levels in P allele when compared with that of A allele in all studied groups.

**Table 1: Biochemical parameters in all studied groups:**

<table>
<thead>
<tr>
<th></th>
<th>Control (n=83)</th>
<th>Group I (n=63)</th>
<th>Group II (n=55)</th>
<th>Group III (n=41)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AFP</strong></td>
<td>4.79±1.53</td>
<td>645.7±38.2</td>
<td>526.4±34.8</td>
<td>698.45±44.3</td>
</tr>
<tr>
<td><strong>Bilirubin</strong></td>
<td>0.76±0.2</td>
<td>2.6±0.8</td>
<td>1.9±0.5</td>
<td>2.8±1.3</td>
</tr>
<tr>
<td><strong>GGT</strong></td>
<td>32.6±7.47</td>
<td>216.38±124.5</td>
<td>145.6±74.39</td>
<td>176.07±85.81</td>
</tr>
<tr>
<td><strong>ALKPH</strong></td>
<td>89.3±33.1</td>
<td>309.3±164.5</td>
<td>172.56±67.05</td>
<td>276.3±137.2</td>
</tr>
<tr>
<td><strong>AST</strong></td>
<td>23.2±6.2</td>
<td>64.7±11.5</td>
<td>70.8±10.9</td>
<td>69.57±12.19</td>
</tr>
<tr>
<td><strong>ALT</strong></td>
<td>23.1±10.8</td>
<td>44.5±8.82</td>
<td>60.35±13.3</td>
<td>48.6±7.6</td>
</tr>
<tr>
<td><strong>Albumen</strong></td>
<td>4.27±0.69</td>
<td>3.7±0.55</td>
<td>3.5±0.5</td>
<td>3.56±0.52</td>
</tr>
<tr>
<td><strong>Total protein</strong></td>
<td>5.94±0.97</td>
<td>5.89±0.6</td>
<td>5.56±0.39</td>
<td>5.7±0.35</td>
</tr>
<tr>
<td><strong>P53 level</strong></td>
<td>2.53±1.03</td>
<td>28.6±17.7</td>
<td>40.05±13.7</td>
<td>45.8±14</td>
</tr>
</tbody>
</table>

a: significance(P<0.05) between Control group and group I or group II or group III for each variable
b: significance(P<0.05) group I and group II or group III for each variable
c: significance(P<0.05) group II and group III for each variable
Table 2: Genotype distribution and allele frequency of P53 polymorphism in the studied groups

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Control No (%)</th>
<th>Group I No (%)</th>
<th>Group II No (%)</th>
<th>Group III No (%)</th>
<th>P value</th>
<th>Odd Ratio(OR)(95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype 1-1 (A / A)</td>
<td>37 (44.5%)</td>
<td>18 (28.5%)</td>
<td>9 (16.4%)</td>
<td>8 (19.5%)</td>
<td>0.37</td>
<td>0.42</td>
</tr>
<tr>
<td>Genotype 2-1 (A / P)</td>
<td>27 (32.5%)</td>
<td>19 (30.1%)</td>
<td>31 (70.4%)</td>
<td>9 (21.9%)</td>
<td>0.01</td>
<td>0.000</td>
</tr>
<tr>
<td>Genotype 2-2 (P / P)</td>
<td>19 (22.8%)</td>
<td>26 (41.2%)</td>
<td>15 (27.3%)</td>
<td>24 (58.5%)</td>
<td>0.11</td>
<td>0.005</td>
</tr>
<tr>
<td>Total No.</td>
<td>(n=83)</td>
<td>(n=63)</td>
<td>(n=55)</td>
<td>(n=41)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A allele</td>
<td>64 (77.1%)</td>
<td>37 (58.7%)</td>
<td>40 (72.7%)</td>
<td>17 (41.5%)</td>
<td>0.07</td>
<td>0.004</td>
</tr>
<tr>
<td>P allele</td>
<td>46 (55.4%)</td>
<td>45 (71.4%)</td>
<td>46 (83.6%)</td>
<td>33 (80.5%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P1: Significance of gpI relative to Control
P2: Significance of gpII relative to Control
P3: Significance of gpIII relative to Control
1: (OR)(95%CI) between Control and I
2: (OR)(95%CI) between Control and II
3: (OR)(95%CI) between Control and III
Table 3: A study of in AFP, Bilirubin and GGT in different Genotypes in the studied groups

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Control group</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Range</td>
<td>Mean</td>
<td>± SD</td>
</tr>
<tr>
<td>Genotype 1-1 (A/A)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFP</td>
<td>37</td>
<td>4</td>
<td>4.79</td>
<td>0.14</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>37</td>
<td>4</td>
<td>0.77</td>
<td>0.43</td>
</tr>
<tr>
<td>GGT</td>
<td>37</td>
<td>4</td>
<td>1.84</td>
<td>0.8</td>
</tr>
<tr>
<td>P53 level</td>
<td>27</td>
<td>4</td>
<td>4.32</td>
<td>0.47</td>
</tr>
<tr>
<td>Genotype 2-1 (A/P)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFP</td>
<td>27</td>
<td>4</td>
<td>0.8</td>
<td>0.22</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>27</td>
<td>4</td>
<td>83</td>
<td>1.3</td>
</tr>
<tr>
<td>GGT</td>
<td>27</td>
<td>4</td>
<td>144</td>
<td>2.4</td>
</tr>
<tr>
<td>P53 level</td>
<td>19</td>
<td>2.4</td>
<td>19.8</td>
<td>0.6</td>
</tr>
<tr>
<td>Genotype 2-2 (P/P)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFP</td>
<td>19</td>
<td>2.4</td>
<td>0.6</td>
<td>0.22</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>19</td>
<td>2.4</td>
<td>198.4</td>
<td>35.7</td>
</tr>
<tr>
<td>GGT</td>
<td>19</td>
<td>2.4</td>
<td>198.4</td>
<td>35.7</td>
</tr>
<tr>
<td>P53 level</td>
<td>19</td>
<td>2.4</td>
<td>19.8</td>
<td>0.6</td>
</tr>
</tbody>
</table>

P1: 0.04, P2: 0.17, P3: 0.027, P4: 0.000

a: significance (P<0.05) relative to the same parameter in same group between genotype (A/A) and genotype (A/P) or genotype (P/P)
b: significance (P<0.05) relative to the same parameter in same group between genotype (A/P) and genotype (P/P)
c: significance (P<0.05) relative to in the same parameter between control group and gp I or gp II or gp III either in genotype (A/A) or genotype (A/P) or genotype (P/P)
d: significance (P<0.05) relative to in the same parameter between gp I and gp II or gp III either in genotype (A/A) or genotype (A/P) or genotype (P/P)
e: significance (P<0.05) relative to in the same parameter between gp II and gp III either in genotype (A/A) or genotype (A/P) or genotype (P/P)
Figure (1): Agarose gel electrophoretic analysis of p53 Arg72Pro Polymorphism after Acc II digestion analysis represent as follow: A/A genotype is presented by 2 band at 113 & 86 bp. (lane 1), A/P genotype is presented by three bands 199, 113 & 86 bp. (lane 2). And P/P genotype is presented by one band 199 bp. (lane 3). Lane (M) represents the molecular marker (DNA molecular weight marker, purchased from Promega Technical Service, Catalog#G3161).

Figure (2): Correlations between the different biochemical parameters in all studied groups:
DISCUSSION

The aim of the present study was to investigate the frequency of p53 codon 72gene polymorphism and its relation to serum P53 levels in Egyptian patients with hepatocellular carcinoma on top of viral and nonviral etiologies healthy versus controls.

The present study demonstrated elevated plasma P53 levels in all studied groups (HCC on top of viral infection and HCC with non viral infection) in relation to the control group and that finding is in consistent with many other investigators (20-25).

On the other hand, Readle et al. showed no significant elevation of serum titers of anti-p53 in a large group of patients with HCV-related HCC and non-neoplastic lesions (23).

In the current study, there is a positive correlation between plasma P53 and serum AFP levels. This is in accordance with the results of Abdel Aziz, et al. (26).

The genotype and allelic distribution of p53 gene and the frequency of its polymorphism in the current study is presented as follow; AA genotype and A allele are more in the control group, PP genotype and P allele are more frequent in HCV-related HCC and HBV-related HCC. Lastly, AP genotype and P allele are more frequent in nonviral related HCC group. Teramoto et al. (27) showed that the incidence of p53 gene abnormality in HCC patients infected with either HBV or HCV is higher (45%) than those who are non infected (13%). A significant association between Pro allele and HCC in HBsAg positive males with chronic liver diseases or family history of HCC was reported in a Taiwanese case-control study conducted by Yu et al. (28). Zhu et al. (29) reported that p53 Arg72Pro is associated with a risk of HCC and homozygosly for the Pro allele is potentially one of the genetic risk factors for HCC in Chinese population.

There is inconsistency between these results and those of Anzola et al. (30) study that failed to observe any association between p53 Arg72Pro and HCC (30) which could be attributable to difference in genetic susceptibility between the study populations.

Yu et al. (28) reported that, no overall increase in HCC risk with the Pro variant allele of the p53 polymorphism was apparent. However, the combined effect of carrying the Pro allele and chronic liver disease is much higher than the effect of each alone on the risk of HCC. Because p53 is critical in cell-cycle arrest and apoptosis after DNA damage, alterations in its function may accelerate the progression from chronic liver disease to HCC.

The relation between P53 genotype and elevated level in HBV carcinogenesis could be explained by that HBx binds to p53 and inactivates p53-dependent activities including p53 sequence-specific DNA-binding activity in vitro and p53-mediated transcriptional activation in vivo, and represses p53 transcription (31). Moreover, HBx deregulates cell-cycle check point controls and blocks p53-mediated apoptosis. Interestingly, tumor-derived HBx mutants that lacked their transcriptional cotransactivation activity as well as proapoptotic activity (32) still retained
their p53-binding functions and blocked p53-mediated apoptosis. Furthermore, by losing the pro-apoptotic ability, the mutant HBx enhanced the transforming ability of ras and myc. The abrogation of p53-mediated apoptosis by HBx may provide a selective clonal advantage for preneoplastic or neoplastic hepatocytes and contribute to hepatocellular carcinogenesis. Recently, Iyer and Groopman explained this by that MutHBx binds to p53 and confers a different biological effect than WtHBx interaction with p53 provides a direction for understanding the elevated risk of HCC in people who have this mutation.

In the current study, there was a significant increase of AP and PP genotype in comparison to AA genotype also, in P allele when compared to A allele in all studied cancer groups. The same results were explained previously by Chen et al. as they stated that the Arg/Arg and Pro/Pro variants differ in binding activity, transcriptional activation, apoptosis induction and cell cycle arrest. The p53 Arg variant induces apoptosis faster and more efficiently than the p53 Pro variant. One explanation of such higher apoptotic potential is the greater ability of the Arg variant to localize to the mitochondria; that localization is accompanied by the proapoptotic release of cytochrome C into the cytosol. In addition, p53 Arg72 is more active than p53 Pro72 in the induction of apoptosis through a transcription-dependant pathway. In contrast, the Pro72 form appears to induce a higher level of G1 arrest than the Arg72 form. These data indicate that the two polymorphic variants of p53 are functionally distinct, and these differences may influence cancer risk.

In conclusion plasma p53 protein level could be considered as an additional tumor marker to AFP to increase the diagnostic potential of AFP in HCC patients. Also, this study suggests that the p53 codon 72 polymorphism may be associated with liver cancer regardless presence or absence of hepatitis virus infection. Finally, P53 gene polymorphism could be used as an indicator of the genetic susceptibility that might carry the risk of future development of HCC in Egyptian cirrhotic patients.

REFERENCES


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28. Yu MW, Yang SY, Chiu YH, Chiang YC, Liaw YF and Chen


الكبد

وDATES

البروتين المتعلق

وبعد مستوى P53 في بالزامو الدم في المرضى المصريين المصابين بسرطان الكبد لأسباب فيروسية أو غير فيروسية.

الكبد، وتكوين P53 والصرع في البول، وأيضًا، كما يمكن استخدام التعدد الجيني P53، كودون 72 كمؤشر مستقبل للقابضة الجينية، وللوفرة، الكبد، المتعاطين لسرطان الكبد في طريق المرضى الذين يعانون من اعتلال الكبد بعلاجات خارجية، ويعود إلى استخدام التعدد الجيني P53، كودون 72 كمؤشر مستقبل للقابضة الجينية، وللوفرة، الكبد، المتعاطين لسرطان الكبد في طريق المرضى الذين يعانون من اعتلال الكبد بعلاجات خارجية، ويعود إلى استخدام التعدد الجيني P53، كودون 72 كمؤشر مستقبل للقابضة الجينية، وللوفرة، الكبد، المتعاطين لسرطان الكبد في طريق المرضى الذين يعانون من اعتلال الكبد بعلاجات خارجية، ويعود إلى استخدام التعدد الجيني P53، كودون 72 كمؤشر مستقبل للقابضة الجينية، وللوفرة، الكبد، المتعاطين لسرطان الكبد في طريق المرضى الذين يعانون من اعتلال الكبد بعلاجات خارجية، ويعود إلى استخدام التعدد الجيني P53، كودون 72 كمؤشر مستقبل للقابضة الجينية، وللوفرة، الكبد، المتعاطين لسرطان الكبد في طريق المرضى الذين يعانون من اعتلال الكبد بعلاجات خارجية، ويعود إلى استخدام التعدد الجيني P53، كودون 72 كمؤشر مستقبل للقابضة الجينية، وللوفرة، الكبد، المتعاطين لسرطان الكبد في طريق المرضى الذين يعانون من اعتلال الكبد بعلاجات خارجية، ويعود إلى استخدام التعدد الجيني P53، كودون 72 كمؤشر مستقبل للقابضة الجينية، وللوفرة، الكبد، المتعاطين LTT