International Journal of Cancer Research 1 (2): 94-100, 2005
ISSN 1811-9727


# Clinical Significance of Serum p53 Antigen in Patients with Hepatocellular Carcinoma

Mohamed Mohamed Abdel-Aziz, Nabil Ali Gad El-hak and Ayman Tallatt Abbas
Department of Biotechnology Research, Gastroenterology and Surgery Center,
Mansoura University, Mansoura, Egypt

**Abstract:** The aim of this study was to analyse the serum p53 antigen concentrations in 98 patients with hepatocellular carcinoma (HCC) using ELISA based on mouse anti-p53 monoclonal antibody (Bp53-12 mAb). A single band of 53 kDa was detected in sera of selected HCC patients using western blot assay. The ELISA technique revealed that the mean serum concentrations of p53 antigen in HCC patients was significantly higher than in controls ($p<0.0001$). The positive serum of p53 protein (>0.212 ng $mL^{-1}$) samples were found in 60 out of 98 (61.22%) patients with HCC. There were no significant differences in serum p53 protein levels according to age, sex, or serum AFP, but serum levels of p53 protein in patients with smaller tumor size (<5 cm) were significantly higher than those in patients with larger tumor size (>5 cm) ($p = 0.03$) . There were significant differences in p53 protein level according to Child Pugh classification (Child A vs. combined Child B and C) ($p = 0.011$). There was no significant association between serum p53 protein concentrations and serum AFP levels. Positive status of serum p53 protein and/or AFP were found in 57 out of 69 (82.6%) patients with HCC. These results suggest that the quantification of soluble p53 in serum may be an additional useful biochemical marker for immuno-diagnosis of patients with HCC using ELISA as a non-invasive technique.

**Key words:** p53, serum, hepatocellular carcinoma, Enzyme Linked Immunosorbent Assay (ELISA)

## INTRODUCTION

p53 tumor suppressor gene plays an important role in preventing cancer development, by arresting or killing potential tumor cells. Mutations with the p53 gene leading to the loss of p53 activity, are found in about half of all human cancers[1] and can result in the production of abnormal protein with novel oncogenic properties and a prolonged half life thereby leading to its accumulation in tumor cells[2]. It has been suggested that the intracellular accumulation of mutant p53 protein in vivo in some cases may result in correspondingly high levels of p53 protein in extracellular fluids, such as serum, which could be quantified using antibodies that are specific for these mutant p53 proteins[3].

Extensive investigation of p53 alterations has been proposed for a decade using different methodologies: Molecular analysis of the p53 gene remain the gold standard method but it is difficult to perform in routine clinical settings[4] because the direct sequencing of the p53 gene is time consuming and difficult to obtain fresh surgical tissue specimens[5]. The strong correlation between p53 gene mutation and p53 protein accumulation[6] allows the use of simple immunologic methods of p53 protein detection instead of the complex and labor-intensive procedures of determining p53 alterations at the nucleic acid level[7]. Immunohistochemistry is a commonly used immunologic method for assessing p53 alterations in human cancers. This method can be performed in protein analysis but some mutations may not lead to p53 protein accumulation, or p53 over-expression may be detected in the absence of mutation of the p53 gene. Also, this method requires a tissue specimens[4,5]. Because the accumulation of mutant p53 protein in tumor cells can be released into the extracellular environment such as the serum and the presence of strong correlation between p53 expression in the tumor tissue determined by immunohistochemistry and the levels of mutant p53 protein in serum[3], the p53 protein could be quantified in serum using an ELISA. The ELISA does not require a tissue specimen, is a non invasive technique and easy to perform, so can be widely used in different laboratories[7].

HCC is one of the most prevalent cancers in the world, particularly in Southeast Asia and Africa including Japan[8]. HCC, that its prognosis is extremely poor, often develops from chronic liver diseases such as chronic hepatitis and liver cirrhosis[9]. p53 is mutated in about 30%

**Corresponding Author:** Dr. Mohamed Mohamed Abdel-Aziz, Department of Biotechnology Research, Gastroenterology and Surgery Center, Mansoura University, Gehan Street, Mansoura, Egypt
Tel: +2 050 2243320 2243319 2349248 Fax: +2 050 2243220 E-mail: azizhkh@yahoo.com

of HCC low incidence areas, e.g. Western Europe and the USA. However in areas of high incidence, p53 is mutated in over 50% of cases[10]. The most frequent mutations of the p53 gene in HCC is an AGG $^{Arg}$ to AGT$^{Ser}$ missense mutation at codon 249 of exon 7[8].

In the present study, serum p53 antigen was detected by western blot analysis and investigated its concentration in serum of patients with HCC by ELISA using specific anti-p53 monoclonal antibody. The association between serum p53 status and serum level of α-Fetoprotein (AFP) was also studied.

## MATERIALS AND METHODS

**Human subjects:** This study was approved by the ethical committee of the Gastroenterology and Surgery Center, Mansoura University, Mansoura, Egypt. Serum samples were obtained from 98 patients with HCC admitted to Gastroenterology and Surgery Center. Ninety eight cases consisted of 80 male and 18 female patients with age range 41-72 years. Patients subjected to surgical treatment (n = 51) were diagnosed according to the pathological investigation of biopsy tissues. Other patients (n = 47) subjected to non surgical treatment (radio-frequency or alcohol injection) were diagnosed clinically based on liver tumor characteristics detected by ultrasound and helical Computed Tomography (CT) scan and confirmed by history and/or serum AFP level. Sera of 30 healthy individuals consisted of 19 male and 11 female with age range 28-53 years were used as negative controls. All sera were stored at -20°C until used.

**Western blot analysis:** The SDS-PAGE resolved serum samples was transferred to nitrocellulose paper according to Towbin *et al.*[11]. The paper was incubated with 1:50 dilution of a mouse monoclonal antibody Bp53-12 (Sigma). After washing, alkaline phosphatase conjugated goat anti-mouse antibody (Sigma) diluted 1:500 was added for 3 h, washed three times and then exposed to alkaline phosphatase substrate [5-bromo-4-chloro-3-indolyl phosphate (BCIP)/nitroblue tetrazoluim (NBT)] for 10 min. The reaction was stopped using distilled water.

**Determination of serum p53 antigen concentrations by ELISA:** After optimization of reaction conditions, polystyrene microtiter plates were coated with 50 μL/well of each serum sample diluted 1:1000 in carbonate/bicarbonate buffer (pH 9.6). The plates were incubated over night at room temperature and washed three times using 0.05% (v/v) PBS-T20 (pH 7.2) and then incubated for 1 h at room temperature with 200 μL/well of 0.2% (w/v) non-fat milk in carbonate/bicarbonate buffer (pH 9.6). After washing, 50 μL/well of mouse monoclonal antibody Bp53-12 (Sigma), diluted 1:100 in PBS-T20, were added and incubated at 37°C for 2 h. After washing, 50 μL/well of anti-mouse IgG alkaline phosphatase conjugate (Sigma), diluted 1:250 in PBS-T20, was added and incubated at 37°C for 1 h. Excess conjugate was removed by extensive washing and the amount of coupled conjugate was determined by incubation with 50 μL/well p-nitrophenyl phosphate (Sigma) for 30 min at 37°C. The reaction stopped using 25 μL/well of 3M NaOH and absorbance was read at 405 nm using microplate autoreader (Bio-Tek Instruments. WI, USA). Standard concentrations of a commercial recombinant human mutant p53 (Oncogene Science, Cambridge, UK) or of the purified p53 antigen were used to establish a standard curve for quantification of p53 antigen in serum. Cutoff level of ELISA above or below which the tested samples is considered positive or negative was calculated as the mean concentrations of 30 serum samples from healthy individuals + 2SD The cutoff level is equivalent to p53 concentration of 0.212 ng $mL^{-1}$.

**Determination of serum AFP level:** Serum AFP level was determined using the Electrochemiluminescence Immunoassy (ECLIA) on the Roch Elecsys 1010/2010 and modulator analytics E170 (Elecsys module) immunoassy analysers. Ten microliters of sample, a biotinylated monoclonal AFP-specific antibody and a monoclonal AFP-specific antibody labeled with a ruthenium comlex react to form a sandwich complex. After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with procell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are determined via a calibrator curve which is instrument: specifically generated by 2-point calibration and a master curve provided via the reagent barcode. The analytical sensitivity was 0.5 IU $mL^{-1}$ or 0.605 ng $mL^{-1}$ and the maximum of the master curve was 1000 IU $mL^{-1}$ or 1210 ng $mL^{-1}$. Values above the measuring range are reported as >1000 IU $mL^{-1}$ or 1210 ng $mL^{-1}$.

**Statistical analysis:** The results were computed on IBM PC microprocessor by the statistical analysis program package, GraphPad Instat, copyright © 1990-1993 GraphPad Software (Version 2.03, USA). Data were presented as mean±SD. Comparisons between two independent groups were performed by the Mann-

Whitney U test for two non- parametric tests. Associations between serum p53 protein concentrations and serum AFP level was analysed by Fisher's exact test. Values of $p<0.05$ were considered to indicate significant differences.

## RESULTS

**Identification of serum p53 protein in patients with HCC using western blot analysis:** A mouse monoclonal antibody Bp53-12, that recognizes both wild type and mutant forms of the p53 protein, was used as an immunochemical probe for identification of the p53 antigen in serum of patients with HCC using western blot analysis. An intense single immunoreactive band was observed at apparent molecular weight of 53 kDa in sera from patients with HCC. No reaction was observed in sera from healthy individuals (Fig. 1).

**Determination of p53 protein concentrations in serum of patients with HCC using ELISA:** Serum p53 protein concentrations were determined in 98 patients with HCC using ELISA. Sera from 30 healthy individuals were used as controls. The serum concentrations of p53 protein in HCC patients ranged from 0.105 to 0.552 ng $mL^{-1}$ with a mean value of 0.237 ng $mL^{-1}$ and a Standard Deviation (SD) equals to 0.073 ng $mL^{-1}$. These concentrations were significantly higher than those in healthy individuals that had serum concentrations of p53 protein ranged from 0.073 to 0.198 ng $mL^{-1}$ with a mean value of 0.14 ng $mL^{-1}$ and SD equals to 0.032 ng $mL^{-1}$ ($p<0.0001$). The cutoff value for the serum p53 protein concentration in HCC cases was arbitrarily defined as 0.212 ng $mL^{-1}$, which was 2SD above the mean of the healthy individuals. Therefore, serum p53 protein concentrations above 0.212 ng $mL^{-1}$ were defined as positive and those below 0.212 ng $mL^{-1}$ were designated as negative. Present results showed that the positive serum of p53 protein samples were found in 60 out of 98 (61.22%) patients with HCC (Table 1). There were no significant differences in serum levels of p53 protein between younger patients (<60 years) and older patients (≥60 years) and also between male and female patients. Mean serum levels of p53 protein in patients with negative AFP levels (≤17 ng $mL^{-1}$) were similar to those in patients with positive AFP levels (>17 ng $mL^{-1}$). On the other hand, the serum levels of p53 protein in patients with smaller tumor size (<5 cm) were significantly higher than those in patients with larger tumor size (>5 cm) ($p=0.03$). Also, there were significant differences in p53 protein level according to Child Pugh classification (Child A vs. combined Child B and C) ($p = 0.011$) (Table 2).

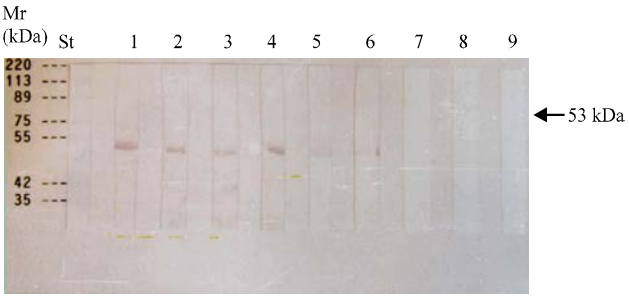


Fig. 1: Detection of p53 antigen in selected serum samples from patients with hepatocellular carcinomas and from healthy individuals using western blot based on mouse anti-human p53 monoclonal antibody, Bp53-12mAb. Lane St: Molecular weight standard; lane 1-5: HCC patients; lane 6-9: healthy individuals

**Correlation between concentrations of p53 protein and serum AFP in HCC patients:** The association between serum p53 protein concentrations and AFP concentrations was studied in 69 patients with HCC using Fishers exact test. In Table 3, from 69 patients with HCC, positive serum AFP concentrations (>17 ng $mL^{-1}$) were noted in 44 (64%) cases and positive serum p53 protein concentrations (>0.212 ng $mL^{-1}$) were observed in 37 (54%) cases. Of the 25 patients showing negative serum AFP concentrations, 13 (19%) patients had positive serum p53 protein concentrations. Of the 32 patients showing negative serum p53 protein concentrations, 20 (29%) patients had positive serum AFP concentrations. Twenty four cases out of 69 (35%) patients showed positivity for both serum p53 protein and AFP concentrations. These results revealed that the mutations of p53 protein did not associate significantly with AFP concentrations (the two-sided p value was 1.000, Odds ratio was 1.108 and 95% Confidence Interval CI = 0.4141-2.963).

**Combination between p53 and AFP positivity status in patients with HCC:** By combination between serum p53 protein and/or AFP positivity status in patients with HCC, it was found that of the 69 patients, 57 (82.6%) patients had positive serum p53 protein or AFP concentrations (Fig. 2).

## DISCUSSION

Currently, the number of types of p53 mutations described in the world literature increases by two or three thousand every year. It is very likely that this trend will continue in the coming years, confirming the status of p53 as a central piece of the puzzle in the molecular biology of

Table 1: Serum p53 protein concentrations in patients with HCC compared to healthy individuals as controls

| Group | Sex (M/F) | Age (years) | Serum p53 level (ng mL$^{-1}$) range Mean±SD | p53 status[a] Positive (No) | Negative (No) | Positive (%) | p-value |
|---|---|---|---|---|---|---|---|
| Controls | 19M/ 11F | 28-53 | 0.073-0.198 0.14 ± 0.032 | 0 | 30 | 0 | |
| HCC Patients | 80M/ 18F | 41-72 | 0.105-0.552 0.237± 0.073 | 60 | 38 | 61.22 | <0.0001 |

[a]Cutoff = Mean of Controls+2SD = 0.212 ng mL$^{-1}$, Positive status means serum p53 protein concentration >0.212 ng mL$^{-1}$
Negative status means serum p53 protein concentration ≤0.212 ng mL$^{-1}$, p-value<0.0001 is considered extremely significant, M: Male, F: Female

Table 2: Mean values of serum p53 levels according to variables of HCC patients

| Variables | Serum p53 level ng mL$^{-1}$ Mean±SD | Positive (%) | p-value |
|---|---|---|---|
| Age (years) | | | |
| <60 (n=60) | 0.23±0.057 | 60 | NS |
| ≥60 (n=38) | 0.22±0.063 | 61.53 | |
| Sex | | | |
| Male (n=80) | 0.23±0.073 | 65 | NS |
| Female (n=18) | 0.23±0.074 | 44.44 | |
| Child Pugh classification | | | |
| A (n=38) | 0.20±0.059 | 39.47 | 0.011* |
| B,C (n=31) | 0.25±0.055 | 77.4 | |
| AFP (ng mL$^{-1}$) | | | |
| ≤17 (n=25) | 0.22±0.068 | 52 | NS |
| >17 (n=44) | 0.22±0.054 | 54.54 | |
| Tumor size (cm) | | | |
| <5 (n=41) | 0.25±0.068 | 72.72 | 0.03* |
| >5 (n=28) | 0.22±0.041 | 53.33 | |

*p-value<0.05 is considered significant, NS: Not Significant

Table 3: Association between serum p53 protein concentrations and AFP concentrations in 69 patients with HCC

| Serum p53 protein | Serum AFP (No) (%) Negative (≤17 ng mL$^{-1}$) | Positive (>17 ng mL$^{-1}$) | Total |
|---|---|---|---|
| Negative (≤0.212 ng mL$^{-1}$) | 12 (17) | 20 (29) | 32 (46) |
| Positive (>0.212 ng mL$^{-1}$) | 13 (19) | 24 (35) | 37 (54) |
| Total | 25 (36) | 44 (64) | 69 (100) |

Fisher's exact test: the two - sided p-value was 1.000 considered not significant. Odds Ratio = 1.108, 95% CI: 0.441-2.963

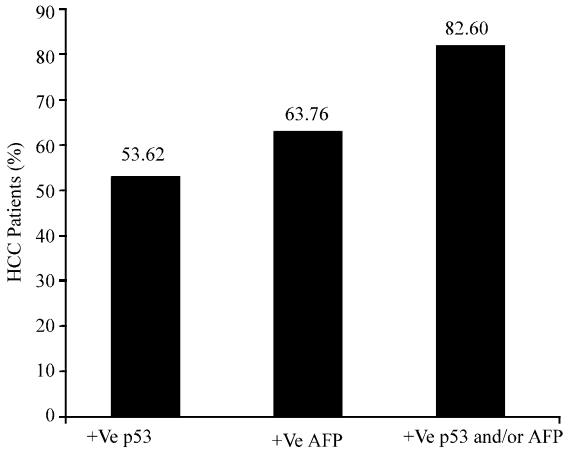


Fig. 2: Combination between p53 and AFP positivity in 69 patients with HCC

human cancer[10]. p53, sometimes, produces immunogenic protein, usually concomitant with either missense alterations in the highly conserved regions of the DNA, nuclear accumulation of p53 protein, or both[12]. The accumulation of mutant p53 protein in tumor cells can be released into the extracellular environment, such as into serum and can thus be examined by ELISA[5].

Several studies have been used an ELISA technique based on monoclonal and polyclonal antibodies for serum p53 quantification in different human cancer such as lung cancer[13,14], pancreatic carcinomas[5], colorectal cancer[3,15], esophageal cancer[16], gynecological cancer[17], bladder cancer[18], head and neck squamous cell carcinoma[19] and hepatocellular carcinoma (HCC)[7]. Most of these studies use a commercial ELISA kit that was developed for the detection of p53 protein in cell or tumor extracts but has not been fully verified on serum samples. Sera have always been tested undiluted, which can lead to high background and false positive[20]. Also, all current ELISA-type methods for p53 quantification include rabbit polyclonal anti-p53 detection antibodies and consequently cross reactivity with human IgG present in serum specimens (heterophilic antibodies) will occur. So, the immunological assays for p53 protein based only on monoclonal antibodies will probably prove more successful for the assay of serum specimens[21]. In present study, previous technical problems are avoided by optimization the reaction conditions of ELISA method using diluted serum samples before analysis and only monoclonal antibody (Bp53-12mAb) was used to avoid the heterophilic antibodies interference. In this study, the serum concentrations of p53 antigen was examined in 98 patients with HCC, by ELISA, and analysed the association between serum p53 antigen concentrations and serum AFP level. Results showed that, using western blot assay, a single immunoreactive band at 53 kDa was identified in selected samples from HCC patients using Bp53-12mAb. The serum p53 antigen concentrations in patients with HCC, assayed by ELISA, were significantly higher than in healthy individuals ($p<0.0001$) and patients with positive serum p53 antigen concentrations

(>0.212 ng $mL^{-1}$) were found in 60 out of 98 (61.22%) patients with HCC.

The serum p53 antigen concentrations in this study was in range of $10^2$ pg $mL^{-1}$. This level was comparable to that previously reported using ELISA techniques for serum p53 quantification in different human cancers[3,5,13-15,17,19]. Unlike all of the above investigators and us, were studies reporting levels of p53 protein in colon cancer patients sera up to $10^9$ pg $mL^{-1}$ determined by High Performance Liquid Chromatography (HPLC) after partial isolation on Gel Fiber Glass (GFG) affinity chromatography columns to extract tumor associated antigens from sera[22-24]. This findings deviates tremendously from data obtained by ELISA and should be interpreted with caution.

The serum p53 antigen positivity of this study (61.22%) was higher than (13-34%) in previous studies using ELISA technique in different human cancers[3,5,13,17,19] while, present percentage was in agreement with the previously reported by Sobti *et al.*[25] who found 61.5% of the patients with invasive cervical cancer had higher levels of serum p53 protein than did healthy controls. In contrast with all previous reports, the presence of p53 protein in the sera of patients with lung cancer is not supported by the results of Levesque *et al.*[21].

Mutations of the p53 gene have been extensively investigated in HCC in different populations world wide and a high prevalence of p53 alterations (30-50% of HCC patients) was reported in previous studies[10,26,27]. The quantification of serum p53 protein in HCC patients was studied by Charuruks *et al.*[7] only who used a commercial one-step immunoassay, a p53 pan ELISA kit. They found detectable levels of serum p53 protein in 22.7% of their HCC patients. It is clear data from this study the results was higher than that (22.7%) in the study of Charuruks *et al.*[7] while, was lower than that in previous report in which serum p53 antigen were detected in 75% (15 of 20 HCC patients)[28]. This difference could be accounted for by two factors. One of these may be the smaller number of subjects studied (only 20 patients) in that report. In addition, the previous report was based on different anti-p53 monoclonal antibody (DO-7 mAb). Also, present percentage was higher than that (25-40%) previously reported in HCC patients using an immunohistochemical technique[29]. The reason for false negative cases in serological analysis may be the fact that not all tumors with p53 gene over-expression necessarily release p53 protein into the blood stream, or that the presence of p53 protein antibodies can impair the detection of p53 protein in the serum[5].

Results revealed that, there were no significant differences in serum levels of p53 protein according to age or sex. Also, patients with negative AFP concentrations (≤17 ng $mL^{-1}$) and those with positive AFP concentrations (>17 ng $mL^{-1}$) have the same mean value of serum p53 protein concentration. On the other hand, the serum levels of p53 protein in patients with smaller tumor size (<5 cm) were significantly higher than those in patients with larger tumor size (>5 cm) ($p = 0.03$). Also, serum levels of p53 protein in patients with Child A were significantly lower than those in the patients with Child B combined with Child C ($p = 0.011$) So, the quantification of serum p53 protein may be useful in the clinical evaluation and characterization of patients with HCC.

AFP have been utilised as a circulating marker in the clinical management of HCC[30]. The serum concentrations of AFP greater than 500 ng $mL^{-1}$ almost always indicate the presence of HCC, but values below this level are less useful because they may also occur in chronic liver disease and yolk sac tumors[9]. Also, the serum AFP concentrations is increased not only in HCC but also in benign liver diseases including liver cirrhosis, so it is difficult to diagnose HCC only from an increased AFP concentration[31]. In this study, we noticed that the positive serum AFP (>17 ng $mL^{-1}$) cases were found in 44 out of 69 (63.76%) patients. Of the 25 patients showing negative serum AFP concentrations (≤17 ng $mL^{-1}$), 13 patients had positive serum p53 protein concentrations. Results showed that the serum concentrations of p53 protein did not correlate significantly with serum AFP level. Also, the findings of Charuruks *et al.*[7] revealed that positive p53 status (p53 antigen-positive and anti-p53 antibodies-positive) was not associated with serum AFP in their HCC patients. Thus the presence of serum p53 protein may be represents a different biological process from AFP. When both of p53 antigen-positive group and/or AFP-positive group were combined together as a single group, this combined group accounted for 57 of the 69 (82.6%) patients. So, the serum p53 mutant protein can be regarded as an additional tumor marker to improve the serological sensitivity of AFP in patients with HCC. In conclusion, the serological analysis, using ELISA, of p53 antigen may be useful in the clinical assessment of patients with HCC and the serum p53 antigen can be considered as an additional tumor marker to increase the diagnostic potential of AFP in HCC patients.

## ACKNOWLEDGEMENTS

The authors would like to thank the Surgery Stuff of Gastroenterology and Surgery Center, Mansoura University for providing the samples.

## REFERENCES


1. Vousden, K.H., 2002. Activation of the p53 tumor suppressor protein. Biochim. Biophys. Acta, 1602: 47-59.
2. Vojtěšek, B., C.J. Fisher, D.M. Barnes and D.P. Lane, 1993. Comparison between p53 staining in tissue sections and p53 proteins levels measured by an ELISA technique. J. Cancer, 67: 1254-1258.
3. Luo, J.C., A.I. Neugut, G. Garbowski, K.A. Forde, M. Treat, S. Smith, W.P. Carney and P.W. Brandt-Rauf, 1995. Levels of p53 antigen in the plasma of patients with adenomas and carcinomas of the colon. Cancer Lett., 91: 235-240.
4. Tominaga, O., P. Hammel, R. Hamelin, H. Nagawa, T. Muto and Y. Remvikos, 1998. Quantification and characterization of total cellular p53 protein in colorectal cancer. Cytometry, 31: 60-66.
5. Suwa, H., G. Ohshio, N. Okada, Z. Wang, M. Fukumoto, T. Imamura and M. Imamura, 1997. Clinical significance of serum p53 antigen in patients with pancreatic carcinomas. Gut, 40: 647-653.
6. Collier, J.D., M. Carpenter, A.D. Burt and M.F. Bassendine, 1994. Expression of mutant p53 protein in hepatocellular carcinoma. Gut, 35: 98-100.
7. Charuruks, N., P. Tangkijvanich, N. Voravud, R. Chatsantikul, A. Theamboonlers and Y. Poovorawan, 2001. Clinical significance of p53 antigen and anti-p53 antibodies in the sera of hepatocellular carcinoma patients. J. Gastroenterol., 36: 830-836.
8. Katiyar, S., B.C. Dash, V. Thakur, R.C. Guptan, S.K. Sarin and B.C. Das, 2000. p53 tumor suppressor gene mutations in hepatocellular carcinoma patients in India. Cancer, 89: 2322-2323.
9. Khien, V.V., H.V. Mao, T.T. Chinh, P.T. Ha, M.H. Bang, B.V. Lac, P.T.V. Ho, N.A. Tuan, L.V. Don, Taketak and S. Satomura, 2001. Clinical evaluation of lentil lectin-reactive α-fetoprotein-L3 in histology-proven heptocellular carcinoma. Intl. J. Biol. Markers, 16: 105-111.
10. Szymanska, K and P. Hainaut, 2003. TP53 and mutations in human cancer. Acta. Biochimica. Polonica., 50: 231-238.
11. Towbin, H., T. Stachlin and J. Gordou, 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. Procedure and some applications. Proc. Natl. Acad. Sci. USA., 76: 4350-4354.
12. Cawely, H.M., S.J. Meltzer, V.M.G. DeBenedetti, M.C. Hollstein, K-R. Muehlbauer, L. Liang, W.P. Bennett, R.F. Souza, B.D. Greenwald, J. Cottrell, A. Salabes, H. Bartsch and G.E. Trivers, 1998. Anti-p53 antibodies in patients with Barrett's esophagus or esophageal carcinoma can predate cancer diagnosis. J. Gastroenterol., 115: 19-27.
13. Luo, J-C., R. Zehab, S. Anttila, M. Ridanpaa, H. Husgafvel-Pursiaine, W. Carney, I. DeVivo, C. Milling and P.W. Brandt-Rauf, 1994. Detection of serum p53 protein in lung cancer patients. J. Occup. Med., 36: 155-160.
14. Segawa, Y., N. Takigawa, K. Mandai, Y. Maeda, I. Takata, N. Fujimoto and K. Jinno, 1997. Measurement of serum p53 protein in patients with small cell lung cancer and results of its clinicopathological evaluation. Lung Cancer, 16: 229-238.
15. Shim, K.S., K.H. Kim, B.W. Park, S.Y. Lee, J.H. Choi, W.S. Han and E.B. Park, 1998. Increased serum levels of mutant p53 proteins in patients with colorectal cancer. J. Korean Med. Sci., 13: 44-48.
16. Sobti, R.C and K. Parashar, 1998. A study on p53 protein and anti-p53 antibodies in the sera of patients with esophageal cancer. Mutat. Res., 422: 271-277.
17. Barbati, A., L. Mariani, M.G. Porpora, M. Anceschi, P. Collini, V. Lauro and E.V. Cosmi, 2000. Serum evaluation of p53 protein in patients with gynaecological cancer. Anticancer Res., 20: 1033-1035.
18. Morita, T., N. Tachikawa, T. Kumamaru, A. Nukui, H. Ikeda, K. Suzuki and A. Tokue, 2000. Serum anti-p53 antibodies and p53 protein status in the sera and tumors from bladder cancer patients. Eur. Urol., 37: 79-84.
19. Chow, V., A.P. Yuen, K.Y. Lam, W.K. Ho and W.I. Wei, 2001. Prognostic significance of serum p53 protein and p53 antibody in patients with surgical treatment for head and neck squamous cell carcinoma. Head Neck, 23: 286-291.
20. Soussi, T., 2000. p53 antibodies in the sera of patients with various types of cancer: A review. Cancer Res., 60: 1777-1788.
21. Levesque, M.A., M. D'Costa and E.P. Diamandis, 1996. p53 protein is absent from the serum of patients with lung cancer. Br. J. Cancer, 74: 1434-1440.
22. Zusman, I., B. Sandler, P. Gurevich, R. Zusman, P. Smirnoff, Y. Tendler, D. Bass, A. Shani, E. Idelevich, R. Pfefferman, B. Davidovich, M. Huszar and J. Glick, 1996. Comparative study of the role of serum levels of p53 antigen and its tumor cell concentration in colon cancer detection. Hum. Antibodies Hybridomas, 7: 123-128.
23. Zusman, I., 1998. Gel fiberglass membranes for affinity chromatography columns and their application to cancer detection. J. Chromatogr. B. Biomed. Sci. Applied, 715: 297-306.
24. Sandler, B., P. Smirnoff, A. Shani, E. Idelevich, V. Kaganski, R. Pfefferman, B. Davidovich and I. Zusman, 1999. The role of blood vessels of soluble 53 kDa protein and CEA in monitoring colon cancer patients. Anticancer Res., 19: 4229-4233.

25. Sobti, R.C., K. Parashar, R. Kaur and N. Capalash, 2002. Detection of human papillomavirus DNA, serum p53 and p53 antibodies in patients with cervical cancer. J. Environ. Pathol. Toxicol. Oncol., 21: 79-85.
26. Hsu, I.C., R.A. Metcalf, T. Sun, J.A. Welsh, N.Y. Wang and C.C. Harris, 1991. Mutational hot spot in the p53 gene in human heptocellular carcinoma. Nature, 350: 427-428.
27. Bressac, B., M. Kew, I. Wands and M. Ozturk, 1991. Selective G-to T mutations of p53 gene in hepatocellular carcinoma from Southern Africa. Nature, 350: 429-431.
28. Attallah, A.M., M.M. Abdel-Aziz, A.M. El-Sayed and A. Tabll, 2003. Detection of serum p53 protein in patients with different gastrointestinal cancers. Cancer Det. Prev., 27: 127-131.
29. Volkmann, M., W.J. Hofmann, M. Muller, Rathu, G. Otto, H. Zentgraf and P.R. Galle, 1994. p53 over-expression is frequent in European hepatocellular carcinoma and largely independent of the codon 249 hot spot mutation. Oncogene, 9: 195-204.
30. Harn, K.D., P. Wax, S.M. Schneider, T.G. Marhin, J.S. Nine, M.A. Moraca, M.A. Virji, P.A. Aronica and K.N. Rao, 1999. Biomarkers of liver regeneration allow early predication of hepatic recovery after acute necrosis. Am. J. Clin. Pathol., 112: 351-357.
31. Shimauchi, Y., M. Tanaka, R. Kuromatsu, R. Ogata, Y. Tateishi, S. Itano, N. Ono, S. Yutani, H. Nagamatsu, S. Matsugaki, S. Yamasaki, K. Tanikawa and M. Sata, 2000. A Simultaneous monitoring of lens culinaris agglutinin. A-reactive α-fetoprotein and des-γ-carboxyprothrombin as an early diagnosis of hepatocellular carcinoma in the follow-up of cirrhotic patients. Oncol. Rep., 7: 249-256.