PROGNOSTIC SIGNIFICANCE OF THREE HEPATITIS MARKERS (p53 ANTIBODIES, VASCULAR ENDOTHELIAL GROWTH FACTOR AND ALPHA - FETO PROTEIN) IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

BY

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ABSTRACT

Hepatocellular carcinoma (HCC) is among the most common cancers in the world. It accounts for up to 85 % of primary liver cancers. The study included randomly selected 94 patients admitted to Gastroenterology Surgery Center during the period of December 2001 to February 2003. The patients were: 67 patients with hepatocellular carcinoma and 27 patients with cirrhosis. Ten healthy volunteers served as control group. Blood samples from all subjects were subjected to the following: 1) Determination of three hepatitis markers [a. p53 autoantibodies (p53 Abs), b- vascular endothelial growth factor (VEGF) and c-alpha-fetoprotein (AFP)]; 2) Liver profile tests and 3) Assay for antibodies of hepatitis C virus (HCV - Abs) and hepatitis B virus surface antigen (HBVsAg). The results reveal that the specificity of p53 Abs has the higher percentage in HCC patients (94.59 %). There is a significant correlation between VEGF and cirrhosis and HCC. There is no association between either p53 Abs or VEGF and AFP concentrations. It could be concluded that p53 Abs can be considered as an additional tumor marker to increase diagnosis potential of AFP in HCC patients and VEGF may offer a novel diagnostic value for HCC. However; it can not distinguish HCC from cirrhotic patients. p53 Abs positive patients have significant high serum level of VEGF; so both can be used in association for screening of patients with HCC.

INTRODUCTION

The hepatocellular carcinoma is linked to environmental, dietary and lifestyle factors, so that its incidence and distribution vary widely among ethnic groups, geographical regions and sex. The increased incidence of HCC in areas of the world where the prevalence of HBV infection is also high was a clue to the link between the virus and the tumor (Bosch et al., 2004).

HCV cirrhosis increases the risk of HCC dramatically (Di Bisceglie, 1997). It is estimated that between 2 and 5.7 % of all

patients with HCV cirrhosis will develop HCC over 10 years and the annual risk is 1 - 4 %. It is rare that HCC will develop in patients without cirrhosis or advanced fibrosis (Makris et al., 1993).

The p53 gene is a tumor suppressor gene. If a person inherits only one functional copy of the p53 gene from his parents, he is predisposed to cancer and usually develops several independent tumors in a variety of tissues in early adulthood. However, mutations in p53 are found in most tumor types, and so contribute to the complex network of molecular events leading to tumor formation (Kao et al., 2004). Investigations of the p53 tumor suppressor gene are an example of the recent progress in molecular aspects of cancer research (Ryan et al., 2001).

Furthermore, HCC is a highly vascular tumor, thus, angiogenesis is considered to be important for its progression. Vascular endothelial growth factor (VEGF) is a potent angiogenic factor which enhances vascular permeability, promoting the extravasations of protein to form a stromal matrix and tumor invasion (Jia et al., 2000).

Serum levels of VEGF may provide useful prognostic information in patients with various types of cancers. However, there has been a debate on whether serum VEGF level is a true reflection of tumor angiogenic activity in cancer patients (Niu et al., 1999).

Alpha fetoprotein (AFP) has been utilized as a circulating marker in the clinical management of HCC. AFP has been used as a prognostic marker and has been correlated with the clinical outcome following fulminant liver failure (Harn et al., 1999).The diagnostic capacity of AFP depends on the degree of its elevation in the serum. Concentrations of AFP greater than 500 ng / ml 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 (Khien et al., 2001).

The aim of the present work is to study the prognostic significance of three hepatitis markers (p53 antibodies, vascular endothelial growth factor and alpha fetoprotein) in patients with hepatocellular carcinoma.

SUBJECTS & METHODS

The study included randomly selected 94 patients admitted to Gastroenterology Surgery Center, Mansoura University, Egypt during the period of December 2001 to February 2003 and other 10 healthy volunteers served as control group. The patients were: 67 patients with hepatocellular carcinoma (HCC) (58 males & 9 females) and 27 patients with cirrhosis (23

males & 4 females). Ten healthy volunteers (7 males & 3 females) served as control group. A blood sample (10 ml) was collected from each subject (n = 104).

The sera of blood samples were used for testing the following:

I) Three hepatitis markers 1- p53 Abs of 52 HCC patients, 27 cirrhotic patients and 10 healthy volunteers according to Engvall and Perlman (1971); 2- VEGF of 60 patients with HCC, 23 patients with cirrhosis and 10 healthy volunteers according to Engvall and Perlman (1971) and 3- AFP of 65 patients with HCC and 10 healthy volunteers according to Fiore and Mitchell (1988).

II) Liver profile tests: albumin, bilirubin, total protein and liver enzymes [SGOT, SGPT and alkaline phosphatase (ALP)] of 67 HCC patients, 27 cirrhotic patients and 10 healthy volunteers.

III) Assay for antibodies of hepatitis C virus (HCV - Abs) and hepatitis B virus surface antigen (HBVsAg) were done to 58 HCC patients, 27 cirrhotic patients and 10 healthy volunteers.

Statistical Analysis:

The results were statically analyzed by the statistical analysis program package, GraphPad Instat, copyright © 1990-1993 GraphPad Software, Version 2.03, USA. Data were presented as mean ± SD and frequencies (%). Comparisons between two independent groups were performed by the Mann-Whitney U test for two non-parametric tests. A correlation test to investigate the relation between each two variables among each group was done using Ranked-Spearman correlation test (r). Associations between two variables were analyzed by Fisher's exact test. Values of $p < 0.05$ indicate significant differences.

RESULTS

Table (1): Serum AFP concentrations are determined in 65 patients with HCC and 10 healthy controls. The serum concentrations of AFP in HCC group ranged from 1.7 to >1210 ng/ml and averaged value equals to 291.4 ng/ml with standard deviation (SD) ± 476.3 ng/ml. These concentrations are significantly higher than those in healthy individuals who have serum concentrations of AFP ranged from 1.7 to 15.6 ng / ml and averaged value equals to 7.6 ng / ml with standard deviation (SD) ± 4.45 ng/ml ($p < 0.05$).

The cut - off value for the serum AFP concentration in the patients with HCC is arbitrarily defined as 20 ng/ml. Therefore, serum AFP concentrations above 20 ng/ml are considered as positive, and those below 20 ng/ml are negative. These results show that the positive serum AFP samples are found in 38 out of 65 (58.46 %) patients with HCC.

Table (2) shows the p53 antibodies (Abs) titers in sera of 52 HCC patients, 27 cirrhotic patients and 10 healthy volunteers: The p53 Abs titer [expressed as O.D. (optical density unit)] in HCC patients ranged from 0.12 to 1.43, and averaged value equals to 0.54 $\pm$ 0.23. These values are significantly higher than those in healthy individuals who have p53 Abs titers ranged from 0.14 to 0.31, and averaged value equals to 0.21 $\pm$ 0.068 ($p < 0.0001$). There is no significant difference in p53 Abs titers between healthy individuals group and cirrhotic patients ($p = 0.12$).

The cut - off value for the p53 Abs in the patients with HCC is found to be 0.418, which is 3SD above the mean of the healthy individuals. Therefore, serum p53 Abs titers above 0.418 are defined as positive, and those below 0.418 are negative. The present results show that the positive serum p53 Abs samples are found in 38 out of 52 (73.07%) patients with HCC. Only two out of 27 cirrhotic patients (7.4%) are found to be p53 Abs positive. The specificity of p53 Abs is calculated according to the non-malignant groups (including healthy individuals and cirrhotic patients) and found to be 94.59 %.

Table (3) represents the correlation between p53 Abs and serum AFP concentrations in 50 patients with HCC: there is a non significant positive correlation is observed ($r = 0.08$, $p = 0.25$).

Table (4) reveals the association between p53 Abs and tumor marker AFP in 50 patients with HCC: positive serum AFP concentrations (> 20 ng / ml) are noted in 30 (60 %) cases and positive p53 Abs are observed in 38 (76 %) cases. From 20 patients showing negative serum AFP concentrations, 7 (14 %) patients showing negative p53 Abs. On the other side, from 12 patients showing negative p53 Abs, 5 (10 %) patients have positive serum AFP concentrations. These results revealed that the mutations of p53 do not associate significantly with AFP concentrations.

Table (5) shows the assessment of serum levels of vascular endothelial growth factor (VEGF) in 23 cirrhotic, 60 HCC patients and 10 healthy volunteers: serum levels of VEGF (expressed as O.D.: optical density unit) in HCC patients ranged from 0.08 to 1.39 with a mean value of 0.52 $\pm$ 0.3. Serum levels of VEGF in cirrhotic patients ranged from 0.19 to 1.35 with a mean value equals to 0.55 $\pm$ 0.25. These values are significantly higher than those in healthy volunteers that have VEGF levels ranged from 0.12 to 0.21 with a mean value equals to 0.17 $\pm$ 0.034 ($p < 0.0001$). There is no significant difference in VEGF levels between HCC group and cirrhotic patients ($p > 0.05$ NS). The cut - off value for VEGF in the patients with HCC is arbitrarily defined as 0.277, which is 3SD above the mean of the healthy volunteers. Therefore, serum levels of VEGF above

0.277 are considered positive, and those below 0.277 are negative. These results showed that the positive serum VEGF samples are found in 50 out of 60 (83.33%) patients with HCC and 19 out of 23 (82.6%) patients with cirrhosis.

Table (6) shows the correlation between serum levels of VEGF and serum AFP concentrations in 56 patients with HCC: a non significant positive correlation is observed (Spearman correlation coefficient r = 0.124, one tailed p value = 0.178).

Table (7) reveals the association between serum levels of VEGF and tumor marker AFP in 56 patients with HCC: positive serum AFP concentrations (> 20 ng / ml) are noted in 34 (60.71 %) cases and positive serum VEGF are observed in 44 (79 %) cases. From 24 patients showing negative serum AFP concentrations, 7 (12.5 %) patients show negative VEGF. On the other side, from 12 patients showing negative VEGF, 5 (8.93 %) patients have positive serum AFP concentrations. These results reveal that the serum levels of VEGF do not associate significantly with AFP concentrations.

Table (8) shows the correlation between serum levels of VEGF and p53 Abs in 45 patients with HCC: It is found that not quit significant positive correlation is observed (Spearman correlation coefficient r = 0.215, one tailed p value = 0.078).

Table (9) shows the association between serum levels of VEGF and p53 Abs in 45 patients with HCC: positive serum p53 Abs are noted in 32 (71 %) cases and positive serum VEGF are observed in 38 (84 %) cases. From 13 patients showing negative serum p53 Abs, 2 (4 %) patients showing negative VEGF. On the other side, from 7 patients showing negative VEGF, 5 (11 %) patients have positive serum p53 Abs titers. These results reveal that the serum levels of VEGF do not associate significantly with p53 Abs titers.

DISCUSSION

Hepatocellular Carcinoma (HCC) is one of the most prevalent cancers in the world, particularly in Southeast Asia and Africa including Japan. Epidemiologic studies have established that chronic infection of the hepatitis B virus (HBV) and to a certain extent hepatitis C virus (HCV) and exposure to dietary aflatoxin B1 and intake of alcoholic beverages are important risk factors for the development of HCC. However, the precise molecular mechanisms of liver carcinogenesis still are not clearly understood (Katiyar et al., 2000).

p53 autoantibodies that have been detected in patients with different malignancies, including hepatocellular carcinoma are a serological parameter with unknown potential in patients with malignancies. The reason why and the mechanism

by which they develop in patients with cancer is still unclear (Tangkijvanich et al., 2000; Gottschlich et al., 2003).

In the present study, serum levels of p53 antibodies and serum vascular endothelial growth factor (VEGF) were investigated by enzyme linked immunosorbent assay (ELISA) in patients with HCC. To assess the clinical significance of p53 antibodies and VEGF in the sera of patients with HCC, we analyzed the association between these two markers and alpha-fetoprotein (AFP) level.

Many studies have documented the presence of p53 antibodies in patients with hepatocellular carcinoma by immunoblotting and by enzyme immunoassay, a study done by Volkmann et al., (1993) reported that, circulating antibodies against p53 were found in 25% (20 of 80) of the sera from patients with HCC but not in various non-malignant liver diseases, with specificity 100 %.

Bergquist et al. (2001) found detectable levels of p53 antibodies in 18.4% (26 of 114), of their HCC patients. Ryder et al., (1996) found p53 antibodies in 37% (14 of 38) of HCC patients. Tangkijvanich et al., (2000) found circulating antibodies against p53 in 13.2% (16 of 121) of patients with HCC.

Saffroy et al., (1999) reported that, 9 of 130 (7%) HCC patients had anti - p53 serum antibodies. Shiota et al., (1997) found that, anti - p53 antibody was positive in 32% (28 of 86) of the sera from patients with HCC, but in none of the 20 patients with chronic hepatitis and 20 patients with liver cirrhosis (sensitivity, 32 % and specificity, 100 %).

Raedle et al. (1997) found that, 3 of 7 of HCC patients had positive p53 antibodies, while zero of 140 patients without HCC, had sensitivity 42.85% and specificity 100%. While, Raedle et al., (1998) found detectable levels of p53 antibodies in 4% (15 of 377) of patients with chronic liver diseases, in 3.9 % (10 of 259) of patients with liver cirrhosis, and in 22.7% (17 of 75) of HCC patients, the sensitivity was 22.7%, and the specificity was 96.1%.

The sensitivity of serum p53 antibodies in the present study (73.07%) is higher than that (13 - 43%) in previous studies using ELISA technique in patients with hepatocellular carcinoma, while the specificity of our study (about 95%) is in agreement with most previous reports that ranged from 96 to 100%. Also, the sensitivity of p53 antibodies (73.07%) in our study, obtained by ELISA, is higher than the percentage of p53 mutation analysis (25 - 40%) that previously reported using an immunohistochemical technique (Volkmann et al., 1994). The discrepancy

between our results and others could be accounted for by number of factors. One of these may be the different number of subjects studied in different reports. In addition, the differences in the procedures of ELISA-type method used in these studies. Also, the population studied may have been different from ours (Egyptian's sample).

Most previous studies and the present study revealed that p53 antibodies are found predominantly in human cancer with a specificity ranged from 95 to 100%. Such antibodies are predominantly associated with p53 gene missense mutations and p53 accumulation in the tumor, but the sensitivity of such detection in previous studies did not exceed more than 45% resulting in a relatively high false negative rate (about 27% in our study, and more than 50% in previous studies). These results are not due to a lack of sensitivity of the current methods of detection but to a real absence of p53 antibodies (Soussi, 2000).

Alpha fetoprotein (AFP) has been utilized as a circulating marker in the clinical management of HCC. AFP has been used as a prognostic marker and has been correlated with the clinical outcome following fulminant liver failure (Harn et al., 1999).The diagnostic capacity of AFP depends on the degree of its elevation in the serum. Concentrations of AFP greater than 500 ng / ml 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 (Khien et al., 2001). Since the serum AFP concentration is increased not only in HCC but also in benign liver diseases including liver cirrhosis, so it is difficult to diagnose HCC only from increased AFP concentration (Soussi, 2000).

In the present study, serum AFP concentrations are determined in 65 patients with HCC. Our results show that the positive serum AFP samples (> 20 ng / ml) are found in 38 out of 65 (58.46%) patients with HCC. The sensitivity of serum AFP in this study (58.46%) is comparable to the study of Raedle et al., (1998) who found that the sensitivity of serum AFP was 69.3 % using the same cut off value (> 20 ng / ml).

The association between p53 antibodies and serum level of AFP in HCC patients is also studied in the present study (Table 3). The results reveal that, from 20 patients (40%) showing negative serum AFP concentrations, 7 (14%) patients showing negative p53 Abs. On the other hand, from 12 patients (24%) showing negative p53 Abs, 5 (10%) patients have positive serum AFP concentrations. These results prove that the presence of p53 antibodies do not necessarily

associated with positive serum AFP in patients with HCC..

The present results are in agreement with previous studies reported by several investigators. The findings of Tangkijvanich et al., (2000) revealed that positive p53 status (p53 antigen - positive and anti - p53 antibodies - positive) was not associated with serum AFP in their HCC patients. Volkmann et al., (1993) found that, from 21 HCC patients showing negative serum AFP concentrations, 5 (23.8%) patients had positive p53 antibodies using immunoblotting assay. So, p53 antibodies and serum AFP proved to be two independent variables. Thus the mutations of p53 protein represent a different biological process from AFP. We can conclude that, serological testing for p53 antibodies gives the opportunity to identify a sub - group of patients with HCC not detected by the conventional test for serum AFP. Also, Ryder et al., (1996) and Raedle et al., (1997) concluded that, the presence of p53 antibodies was independent of AFP status in their studies.

In conclusion, an antibody response against p53 developed in a significant proportion of patients with HCC but not in those with non malignant liver diseases as liver cirrhosis. Serological analysis of p53 antibodies, using ELISA, was relatively sensitive, specific for malignancy, and independent of AFP status, thus offering a new serological criterion in the fraction of tumor marker-negative patients. The serum p53 antibodies can be considered as an additional tumor marker to increase the diagnostic potential of AFP in HCC patients. Moreover, the ELISA for p53 antibodies is a convenient, rapid, easily performed, and specific test for the detection of humoral response to alterations in p53 gene expression and can be of value in the diagnosis and characterization of patients with HCC.

Vascular endothelial growth factor (VEGF) is the most potent angiogenic factor known so far, and it is secreted by a wide variety of human cancers (Marme, 1996). VEGF is a soluble glycoprotein that specifically stimulates endothelial cell proliferation and enhances vascular permeability. Several studies have demonstrated that a high serum VEGF level is associated with the disease progression and poor survival in patients with lung carcinoma (Salven et al., 1998), urinary bladder carcinoma (Miyake et al., 1999); gynacological cancers (Bachtiary et al., 2002); colorectal carcinoma (Chin et al., 2000); esophageal carcinoma (Vgurel et al., 2001and leukemia (Molika et al., 2002).

Poon et al., (2001a) suggested that serum VEGF is probably the best surrogate marker of tumor angiogenic activity among various circulating angiogenic factors investigated in cancer patients. The

expression of VEGF in patients with various malignancies has been made possible by measuring circulating VEGF concentrations with the enzyme linked immunosorbent assay (ELISA) (Kondo et al., 1994).

In the present study, we investigated serum vascular endothelial growth factor by enzyme linked immunosorbent assay (ELISA) in patients with HCC and its association with alpha-fetoprotein (AFP) level.

Serum levels of VEGF are determined in 60 patients with HCC, 23 cirrhotic patients. Sera and 10 healthy individuals used as controls. These VEGF values in both HCC & cirrhotic groups are significantly higher than those in control group ($p < 0.0001$). To our knowledge there are scarce data on the clinical significance of circulating VEGF in HCC patients. Jinno et al., (1998) reported that circulating VEGF levels were significantly elevated in patients with HCC compared with normal controls. Poon et al., (2001b) reported that serum VEGF level was significantly elevated in patients with HCC.

Also in the present study serum levels of VEGF in cirrhotic patients are significantly higher than healthy individuals. El - Assal et al., (1998) revealed that VEGF expression in non - tumor cirrhotic liver tissues was significantly higher that in the non - cirrhotic livers. Rosmorduc et al., (1996) and El - Assal et al., (1998) had observed an up-regulation of VEGF in the cirrhotic liver of patients with or without hepatocellular carcinoma, suggesting that this factor might be responsible for cirrhosis-associated angiogenesis.

In the present study there is no significant difference in VEGF levels between HCC group and cirrhotic group ($p > 0.05$). These findings are in contrast to those from another study done by Niu et al., (2000). They found that VEGF levels in HCC patients were significantly higher than in cirrhotic patients. It indicates that VEGF could play an important role in transforming liver cirrhosis into HCC.

Also, the correlation between serum levels of VEGF and serum AFP concentrations in patients with HCC is studied. It is found that, a non significant positive correlation is observed ($p = 0.178$). A significant difference in serum levels of VEGF is found between the patients with negative AFP levels ($\leq$ 20 ng / ml) and the patients with positive AFP levels (>20 ng / ml) ($p = 0.039$).

The association between serum levels of VEGF and AFP concentrations is studied in 56 patients with HCC. The data show that of 56 patients with HCC, positive serum AFP concentrations (> 20 ng / ml) are noted in 32 (57 %) cases and positive

serum VEGF are observed in 44 (79%) cases. From 24 patients showing negative serum AFP concentrations, 7 (13%) patients showing negative VEGF. On the other hand, from 12 patients showing negative VEGF, 5 (9%) patients have positive serum AFP concentrations. These results reveal that the serum levels of VEGF do not associate significantly with AFP concentrations ($p = 0.18$).

The correlation between serum levels of VEGF and p53 Abs in patients with HCC is studied. It is found that, a not quit significant positive correlation is elicited ($p = 0.078$).

The association between serum levels of VEGF and p53 Abs titers is studied in 45 patients with HCC. The data show that of 45 patients with HCC, positive serum p53 Abs are noted in 32 (71%) patients and positive serum VEGF are observed in 38 (84%) patients. From 13 patients showing negative serum p53 Abs, 2 (4%) patients showing negative VEGF. On the other hand, from 7 patients showing negative VEGF, 5 (11%) patients have positive serum p53 Abs titers. These results reveal that the serum levels of VEGF do not associate significantly with p53 Abs titers ($p = 0.67$).

In conclusion, VEGF level may offer a novel diagnostic value for HCC. However, it can not distinguish HCC from cirrhotic patients but p53 positive patients have significant high VEGF so both can be used in association with each other to confirm diagnosis of HCC..

CONCLUSION

It is concluded from results of the present study that: Alpha - Fetoprotein marker is not specific for diagnosis of HCC; p53 is highly specific for diagnosis of HCC (specificity is 94.59 %); there is a significant correlation between VEGF and cirrhosis and HCC; there is no association between either serum levels of VEGF or p53 Abs and AFP concentrations; no correlation between p53 Abs and VEGF serum levels; p53 Abs are a new serological parameter in patients with malignancies; p53 Abs are developed in patients with HCC but not in those with liver cirrhosis; serological analysis of p53 Abs using ELISA is sensitive for malignancy and independent of AFP status; thus offering a new serological criterion in patients with negative tumor marker; p53 Abs can be considered as an additional tumor marker to increase diagnosis potential of AFP in HCC patients and VEGF may offer a novel diagnostic value for HCC. However; it can not distinguish HCC from cirrhotic patients. But p53 Abs positive patients have significant high serum level of VEGF; so both can be used in association with each other.

It is recommended that every patient with a history of liver disease especially the cirrhotic patients must be submitted to periodic examination visits every 3 months. In every visit it is recommended to measure the titer of p53 Abs and serum level of VEGF to be able to diagnose HCC as early as possible, thus giving chance for such patients for proper management and better survival.

Table (1): Serum concentrations of AFP in HCC patients and control group.

Group	Serum AFP (ng / ml)	AFP status		Positivity	p value
	Range (Mean ± SD)	+ ve > 20 (ng/ml)	- ve ≤ 20 (ng/ml)		
Control group	1.7 - 15.6 7.6 ± 4.45	0	10	0	
Hepatocellular carcinoma group	1.7 - > 1210 291.4 ± 476.3	38	27	58.46	< 0.05

Cut - off value = 20 ng / ml.

Table (2): The sensitivity and specificity of p53 Abs in the studied groups.

Groups	n	Serum p53 Abs	p53 Abs status		Sensitivity %	Specificity %	p values
		OD Range (Mean ± SD)	+ ve > 0.418 (OD)	- ve ≤ 0.418 (O.D)			
Control group	10	0.14 - 0.31 0.21 ± 0.068	0	10	0	0	
Cirrhosis group	27	0.14 - 0.65 0.26 ± 0.1	2	25	7.4	0	0.12 (NS)
Hepatocellular carcinoma group	52	0.12 - 1.43 0.54 ± 0.23	38	14	73.07	94.59	<0.0001 *

OD = Optical Density, Cut - off value = Mean of HI + 3 SD = 0.214 + 3 (0.068) = 0.418, ≤ 0.418 is considered negative, > 0.418 is considered positive, Specificity was calculated according to the non-malignant group that includes healthy individuals and cirrhotic patients (10 + 27 = 37), NS: not significant, *p value < 0.05 is considered significant.

Table (3): Correlation between p53 Abs and AFP concentrations in HCC patients.

Variables	Correlation coefficient (r)	p Value
P53 Abs AFP	0.08	0.25 (NS)

NS: not significant.

Table (4): Association between p53 Abs status and AFP status in HCC patients.

Serum p 53 Abs Status (OD)	AFP		Total n (%)
	Positive > 20 (ng / ml) n (%)	Negative ≤ 20(ng / ml) n (%)	
Positive > 0.418	25 (50 %)	13 (26 %)	38 (76 %)
Negative ≤ 0.418	5 (10 %)	7 (14 %)	12 (24 %)
Total	30 (60 %)	20 (40 %)	50 (100 %)

OD = optical Density.

Table (5): Serum levels of vascular endothelial growth factor (VEGF) in the studied groups.

Groups	n	VEGF (O.D) Range (Mean ± SD)	VEGF status		Sensitivity (%)	p value
			+ ve > 0.277 (O.D)	- ve ≤ 0.277 (O.D)		
Control group	10	0.12 – 0.21 0.17 ± 0.034	0	10	0	
Cirrhosis group	23	0.19 – 1.35 0.55 ± 0.25	19	4	82.6	<0.0001*
Hepatocellular carcinoma group	60	0.08 – 1.39 0.52 ± 0.3	50	10	83.33	<0.0001*

OD = Optical Density, Cut - off value = Mean of HI + 3SD = 0.175 + 3(0.034) = 0.277, ≤ 0.277 is considered negative, > 0.277 is considered positive, *p value < 0.05 is considered significant.

Table (6): Correlation between serum levels of VEGF and AFP concentrations in HCC patients.

Variables	Correlation coefficient (r)	p Value
VEGF AFP	0.124	0.17 (NS)

NS: not significant.

Table (7): Association between VEGF status and AFP status in HCC patients.

Serum VEGF Status (OD)	AFP		Total n (%)
	Positive > 20 (ng / ml) n (%)	Negative ≤ 20(ng / ml) n (%)	
Positive > 0.277	29 (51.79 %)	15 (26.79 %)	44 (79 %)
Negative ≤ 0.277	5 (8.93 %)	7 (12.5 %)	12 (21 %)
Total	34 (60.71 %)	22 (39.29 %)	56 (100 %)

OD = optical density

Table (8): Correlation between serum levels of VEGF and p53 Abs titers in HCC patients.

Variables	Correlation coefficient (r)	p Value
VEGF P53	0.215	0.07 (NQS)

NQS: not quit significant

Table (9): Association between p53 Abs status and VEGF status in HCC patients.

Serum p53 Abs Status (OD)	VEGF (n = 45)		Total n (%)
	Positive > 0.277 (OD) n (%)	Negative ≤ 0.277 (OD) n (%)	
Positive > 0.418	27 (60 %)	5 (11 %)	32 (71 %)
Negative ≤ 0.418	11 (24 %)	2 (4 %)	13 (29 %)
Total	38 (84 %)	7 (16 %)	45 (100 %)

OD = Optical density.

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الأهمية التشخيصية لثلاثة دلالات كبدية (الأجسام المضادة لـ p ٥٣، معامل النمو الوعائى والألفافيتوبروتين) فى حالات أورام الكبد السرطانية

المشتركون فى البحث

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يعد سرطان الكبد من أكثر السرطانات إنتشاراً فى العالم فهو الخامس إنتشاراً بين أنواع السرطانات المختلفة والثانى فى سرطانات الجهاز الهضمى، وتليف الكبد من العوامل التى لها إرتباط وثيق بهذا المرض، وقد تم إجراء هذه الدراسة على ٩٤ مريض تم إختيارهم عشوائياً من الحالات التى تتردد على مركز جراحة الجهاز الهضمى فى الفترة من سبتمبر ٢٠٠١ إلى فبراير ٢٠٠٣، والحالات تتألف من : ٦٧ مريض بسرطان الكبد (٥٨ رجل ، ٩ إمرأة)، ٢٧ مريض بتليف الكبد تتراوح أعمارهم من ٣٠ - ٧٠ سنة، بالإضافة إلى مجموعة ضابطة تتكون من ١٠ متطوعين أصحاء (٧ رجال ، ٣ إناث)، وتهدف هذه الدراسة إلى تقييم دلالات الكبد الحديثة فى الكشف المبكر على حالات سرطان الكبد.

تم أخذ عينة دم ١٠ سم من كل شخص فى هذه الدراسة لإجراء التحاليل الآتية :

قياس مستوى ثلاث دلالات لإلتهاب الكبد فى الدم وهم : ١- الأجسام المضادة لـ p ٥٣ فى ٥٢ مريض بسرطان الكبد، ٢٧ مريض بتليف الكبد، و١٠ متطوعين أصحاء. ٢ - معامل النمو الوعائى فى ٦٠ مريض بسرطان الكبد، ٢٣ مريض بتليف الكبد، و١٠ متطوعين أصحاء. ٣- ألفا فيتوبروتين فى ٦٥ مريض بسرطان الكبد و ١٠ متطوعين أصحاء. بروفيل وظائف الكبد : (ألبيومين، بيليروبين، البروتين، إنزيمات الكبد (SGOT, SGPT & ALP).والكشف عن الأجسام المضادة لفيروس الكبد سى وأنتيجينات الفيروس الكبدى ب وتم إجرائها على ٥٨ مريض بسرطان الكبد، ٢٧ مريض بتليف الكبد، و١٠ متطوعين أصحاء.

وقد أظهرت الدراسة النتائج الآتية :

- نسبة الأجسام المضادة لـ p ٥٣ فى مرضى سرطان الكبد تراوحت بين ١٢ر٠ - ١٤٣ر١ والوسط الحسابى ٢٣ر٠ + ٥٤ر٠، وهذه المعدلات أعلى من نظيرتها فى المتطوعين الأصحاء الذين تراوحت النسبة فيهم بين ١٤ر٠ - ٣١ر٠ والوسط الحسابى ٠٦٨ر٠ ± ٢١ر٠، ولم تسجل الدراسة أى إختلاف ظاهر فى معدلات لـ p ٥٣ بين المتطوعين الأصحاء ومرضى تليف الكبد.

- وسجل معدل الأجسام المضادة لـ ٥٣ p أعلى خصوصية له فى مرضى سرطان الكبد (٥٩ر٩٤٪).

- قيمة الكت أوف لـ ٥٣ p فى مرضى سرطان الكبد كانت ٤١٨ر٠، بناء على هذا أى قيمة أعلى من هذه القيمة تعتبر موجبة وأى قيمة أقل من هذه القيمة تعتبر سالبة.

- أثبتت الدراسة عدم وجود علاقة بين نسبة الألفافيتوبروتين ووجود الأجسام المضادة فى ٥٠ مريض بسرطان الكبد.

- أثبتت الدراسة عدم وجود إرتباط بين وجود الأجسام المضادة لـ ٥٣ p والمرضى ذو نتيجة إيجابية للألفافيتوبروتين أو المرضى ذو نتيجة سلبية للألفافيتوبروتين.

- قد تم تعيين معدل عامل النمو الوعائى فى ٦٠ مريض بسرطان الكبد و ٢٣ مريض بتليف الكبد باستخدام الإيلايزا.

- تراوحت معدلات عامل النمو الوعائى فى مرضى سرطان الكبد من ١ر٣٩ O ٠٨ر٠ والوسط الحسابى ٣ر٠ ± ٥٢ر٠، بينما تراوحت المعدلات فى مرضى تليف الكبد من ١ر٣٥ O ١٩ر٠ والوسط الحسابى ± ٥٥ر٠ O ٢٥ر٠، وهذه المعدلات أعلى منها فى المتطوعين الأصحاء الذين تراوحت النسبة بينهم من ٢١ر٠ O ١٢ر٠ والوسط الحسابى ٠٣٤ر٠ ± ١٧ر٠٠.

- قيمة الكات أوف لعامل النمو الوعائى هى ٢٧٧ر٠ وأى قيمة أعلى من هذه القيمة تعتبر إيجابية وأى قيمة أقل من هذه القيمة تعتبر سلبية، وقد تم تعيين نتيجة إيجابية فى ٥٠ مريض من ٦٠ مريض بسرطان الكبد (٣٣ر٨٣٪)، وتم أيضاً تعيين نتيجة إيجابية فى ١٩ مريض من ٢٣ مريض بتليف الكبد (٦ر٨٢٪).

- أثبتت الدراسة عدم وجود علاقة بين نسبة الألفافيتوبروتين ومعدل عامل النمو الوعائى فى ٥٦ مريض بسرطان الكبد.

- وقد تم دراسة الارتباط بين عامل النمو الوعائى والألفافيتوبروتين فى ٥٦ مريض بسرطان الكبد، ومن هؤلاء المرضى تم تعيين نتيجة إيجابية للألفافيتوبروتين فى ٣٤ مريض (٧١ر٦٠٪) ونتيجة إيجابية لعامل النمو الوعائى فى ٤٤ مريض (٧٩٪)، ومن ٢٤ مريض ذو نتيجة سلبية للألفافيتوبروتين تم تعيين نتيجة سلبية أيضاً لعامل النمو الوعائى فى ٧ مرضى منهم (٥ر١٢٪).

- توجد علاقة موجبة غير هامة بين معدل عامل النمو الوعائى والأجسام المضادة لـ ٥٣ p فى ٤٥ مريض بسرطان الكبد (٠٧ر٠ = p، ٢١٤ر٠ = r).