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Significance of Macrophage Migration Inhibitory Factor (MIF) and Anti P 53 Antibodies in Prognosis of Hepatocellular Carcinoma

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Abstract

Background and aim: Prognosis of hepatocellular carcinoma (HCC) is very poor and determining the prognosis rely many factors and we aim at defining the prognostic factor of macrophage migration inhibitory factor (MIF) , anti P53 and its correlation with other prognostic factors in HCC.

Patients: Serum macrophage migration inhibitory factor and anti-p53 antibodies were measured in139 patients diagnosed with HCC using a specific enzyme-linked immunosorbent assay (ELISA) kit. The clinicopathological characteristics of the patients were compared with respect to the presence of serum anti-p53 antibodies.

Results: In univariate analysis, the prognostic factors of overall survival with statistical significance were portal vein thrombosis, total serum bilirubin, serum albumin, serum AST, serum ALT, Prothrombin time, viral marker and anti p53 antibody and MIF and on multivariate analysis the prognostic factors were BCLC staging, presence of extrahepatic metastases, the patient received treatment or not, anti p53 antibody and MIF.

Conclusion: Both MIF and Anti p53 antibody are associated with poor prognosis in HCC and it increased the prognostic potential of alpha fetoprotein.

Keywords: MIF; p53; HCC; Prognostic; ELISA; Alpha fetoprotein

Abbreviations:

HCC: Hepatocellular Carcinoma; MIF: Macrophage Migration Inhibitory Factor; ELISA: Enzyme Linked Immunosorbent Assay; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; BCLC: Barcelona Clinical Liver Cancer; AFP: Alpha Fetoprotein; ALP: Alkaline Phosphatase; CLIP: Cancer Liver Italian Program; OS: Overall Survival; DFS: Disease Free Survival; PT: Prothrombin Time; TACE: Transarterial Chemoembolization

Introduction

Liver cancer is the tenth most common cancer and the fifth most common cause of cancer death among males, and the ninth most common cause of cancer death among females [1].

The death rate in HCC is almost equal to the incidence worldwide. Nearly 80% of deaths are due to underlying liver cirrhosis due viral hepatitis B and C [2].

There is no worldwide consensus on the use of any HCC staging system. Barcelona clinical liver cancer (BCLC) staging system uses prognostic factors including tumor stage, liver functional status, performance status, and cancer-related symptoms, the aim of BCLC classification to get link between prognosis and line of treatment (Figure 1) [3-5].

The estimation of prognosis of hepatocellular carcinoma is very complex process to determine the method of interference [6]. In addition to tumor stage at the time of diagnosis, the cirrhosis underlies HCC in most of the patients, [6,7] and the functional impairment of the underlying liver has a significant impact on prognosis irrespective of tumor stage. At the same time, liver function defines the choice of type of interference whether surgical, radiofrequency, transarterial chemoembolization, chemotherapy, target therapy or just best supportive care.

The p53 tumor-suppressor gene is involved in hepatocellular carcinoma (HCC). The patients with positive anti p53 antibodies are associated with shorter survival and bad prognosis [8-10].

Macrophage migration inhibitory factor (MIF) was originally identified as a lymphokine that concentrates macrophages at inflammatory loci. The roles of MIF in tumor genesis, proliferation of tumor cells and tumor angiogenesis were discovered. MIF expression may play a pivotal role in the dismal prognosis of patients with HCC that may be attributable to the modulation of angiogenesis [11-14].

Patients and Methods

Plasma MIF and Anti-p53 serum antibodies detection was performed on 139 consecutive outpatients with confirmed HCC (20 women, 119 men, and mean age 57.9 years, range 44-76) in period from January 2012 to January 2014. All the patients were assessed before treatment. Informed consent obtained from all patients included in this study. Diagnosis of HCC was made by

ultrasonography and triphasic computerized tomography and serum AFP. Size (maximal diameter of the tumor), number of nodules and total volume of the tumor were calculated using imaging techniques.

The number and size of nodules and the presence of portal vein thrombosis were evaluated. Bone scan was prescribed when there was bone pain.

In 87 out of 139 patients, HCC had developed on a cirrhotic liver, in 52 patients HCC had developed on a normal liver. HCC was of viral origin in 88 patients. All the patients were tested for presence of circulating anti-p53 antibodies at least once before beginning chemotherapy.

The diagnosis was done by triphasic CT abdomenoplevis and confirmed by histology and/or serum alpha-fetoprotein (AFP) levels above 400 IU/ml. The clinical and pathological data of the patients were recorded including sex, age, Iiver function tests (total bilirubin, SGOT, SGPT, alkaline phosphates, serum albumin, Prothrombin time,) severity of liver disease graded as Barcelona staging system, AFP level, and tumor characteristics, type of therapy and patients' survival time defined as the period from initial presentation to death.

There were 11 patients who had undergone surgical liver resection, 18 patients underwent radiofrequency, 34 patients with transarterial chemoembolization (TACE), 5 patients with systemic chemotherapy and 71 patients without any specific treatment due to the patients' advanced stages or refusal of therapy. This article does not contain any studies with human or animal subjects.

Detection of MIF levels in plasma samples

Peripheral blood samples were collected, anticoagulated by ethylene diaminetetraacetic acid (EDTA) and then centrifuged at 4°C for 15 min (3000 rpm). The plasma was removed, aliquoted, and snap frozen at −70°C until used. MIF levels in plasma were measured by quantitative sandwich enzyme-linked immunosorbent assay (ELISA) kits (Quantikine, R&D Systems, Minneapolis, MN) according to the manufacturer's protocols.

Detection of serum anti-p53 antibodies

The detection of anti-p53 antibodies in patient sera was performed with a commercially available enzyme-linked immunosorbent assay (Anti-p53 ELISA, Pharmacell Paris, distributed by Immunotech, Marseille, France). The assay was based on an indirect technique using microtitre plates coated with recombinant wild-type human p53 protein or with a control well coated with the neutral antigen.

This assay was performed according to the manufacturer's instructions with the following specifications: 1/100 diluted patient serum was added for 60 min at 20-25°C, with shaking, to microtitre wells coated with recombinant wild-type human p53 protein (to detect specific anti-p53 antibodies), or with a control protein (to detect nonspecific interactions). After washing, goat anti-human IgG antibody conjugated with peroxidase was added for 60 min at 20-25°C with shaking. Finally, the substrate 3.3¢, 5.5¢-tetramethylbenzidine (TMB) was added for 10 min. The enzymatic process was stopped by adding 2 N sulphuric acids. Light absorption was measured at 450 nm on a spectrophotometer (Dynatec, Paris, France). In this assay, nonspecific background of each sample corresponded to the absorbance measured on wells coated with control protein.

Anti-p53 antibodies were considered positive in a sample for an index value [specific signal of the sample (p53 net absorbance -control protein net absorbance) /specific signal of the lower positive /specific signal of the lower positive manufacturers control serum]1.1.

All sera were tested for HBsAg using a commercially available kit (Auszyme II; Abbott Laboratories, North Chicago, Ill, USA), and for anti-HCV by third generation enzyme-linked immunosorbent assay (ELISA) (Recombinant c22-3, c200, and NS5) obtained from Ortho Diagnostic Systems (Chiron, Emeryville, CA, USA).

Liver function test

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels were determined from each specimen by automated chemical analyzer (Hitachi 911).The normal levels obtained in healthy adults are within the range of 0-38 u/r for AST/ALT and 20-140 IU/I for ALP, respectively.

Statistical analysis

Data were presented as percentage, mean and standard deviation. The Chi-square test and unpaired t test were performed to compare clinical data according to the presence or absence of antip53 antibodies as appropriate. Survival curves were constructed using the Kaplan-Meier method and differences between curves were established using the log rank test. P values below 0.05 were considered statistically significant [15].

Results

Table 1 showed patient characteristics, mean age 44-76 with median 56 years with mean age 57.9 +7.8, male: female was 5.9: 1, 102 patients presented with high alpha fetoprotein more than 400. Cirrhosis was present in 87. Viral hepatitis was present in 88 cases. Portal vein thrombosis was presented 76 cases. BCLC staging was done; 28 stage A1,31 stage A2,24 stage A3 ,14 stage A4, 6 stage B, 20 stage C, 16 stage D. High serum bilirubin more than 1.1 in 80 cases. Serum albumin was low in 87 cases. Fifty seven had high Prothrombin activity. Eighty patients presented with multiple focal lesions. In forty two cases more than 50% of cases. No metastases were present in 119 cases. The anti p53 antibodies was positive in 17 cases of 139 cases. Eleven patients were subjected to surgical resection, eighteen patients were subjected to radiofrequency, transarterial chemoembolization (TACE) was done to thirty four patients, five patients received chemotherapy or target therapy, and seventy one patients received no treatment.

In table 2 analysis of different prognostic factors with anti p53 antibodies, it revealed the factor with statistical significance were age (P value 0.016), sex (p value=0.001), alpha fetoprotein (p value =0.03), viral hepatitis (p value=0.011) and Prothrombin time (p value =0.008).

Patients characteristics	**No.**	**%**
Age	44-76 with median 56 y- mean 57.9 ± 7.8	
< or equal 56 years	86	61.9
>56 years	53	38.1
Sex		
Male	119	85.6
Female	20	14.4

Alpha fetoprotein		
<20	10	7.2
20-400	27	19.4
>400	102	73.4
Cirrhosis		
Present	87	62.6
Absent	52	37.4
Viral hepatitis		
Present	88	63.3
Absent	51	36.7
BCLC		
A1	28	20.1
A2	31	22.3
A3	24	17.3
A4	14	10.1
B	6	4.3
C	20	14.4
D	16	11.5
Portal vein thrombosis		
Yes	76	54.7
No	63	45.3
Serum bilirubin		
<1.1	59	42.4
>1.1	80	57.6
ALP		
20-140IU/l	47	33.8
>140 IU/L	92	66.2
AST		
≤38	23	16.5
>38	116	83.5
ALT		
≤38	24	17.3
>38	115	82.7
Albumin level		
3.5-5.5g/L	52	37.4
<3.5gm/L	87	62.6
Prothrombin time(PT)		
9.5-13.5 sec	82	59
>13.5 sec	57	41
Number of tumors		
Single	53	38.1
Multiple	86	69.1
Size of tumor		
<5 cm	37	26.6
5 cm-less than 50%	60	43.2
More than 50%	42	30.2
Hepatic metastases		
No	119	86.3
Yes	20	17.3
Serum p53		
Negative	122	87.8
Positive	17	12.2
Treatment		
surgical resection	11	7.9
radiofrequency	18	12.9
TACE	34	24.5
Chemotherapy	5	3.6
No treatment	71	51.1

Table 1: characteristics of 139 cases with hepatocellular carcinoma

Patients characteristics	**Anti P53 positive antibodies**	**Anti P53 negative antibodies**	
	No. (17)	**No. (122)**	**P value**
Age			
< or equal 56 years	6	80	0.016*
>56 years	11	42	
Sex			
Male	10	109	0.001*
Female	7	13	
Alpha fetoprotein			
<20	-	10	0.03*
20-400	-	27	
>400	17	85	
MIF			
Low	10	7	0.495
high	61	61	

Cirrhosis			
Present	11	45	0.732
Absent	6	77	
Viral hepatitis			
Present	6	82	0.011*
Absent	11	40	
BCLC			
A1	5	23	0.431
A2	2	29	
A3	1	23	
A4	2	12	
B	2	4	
C	3	17	
D	2	14	
Portal vein thrombosis			
Yes	11	52	0.073
No	6	70	
Serum bilirubin			
≤1.1	4	55	0.092
>1.1	13	67	
ALP			
20-140IU/l	3	44	0.133
>140 IU/L	14	78	
AST			
≤40	3	20	0.896
>40	14	102	
ALT			
≤40	3	21	0.965
>40	14	101	
Albumin level			
3.5-5.5 g/L	4	48	0.207
<3.5 mg/L	13	74	
Prothrombin time (PT)			
9.5-13.5 sec	5	77	0.008*
>13.5 sec	12	45	
Number of tumors			
Single	6	47	0.797
Multiple	11	75	
Size of tumor			
<5 cm	5	32	0.799
5 cm-less than 50%	6	54	
More than 50%	6	36	
Hepatic metastases			
No	16	104	0.318
Yes	1	18	
Treatment			
surgical resection	3	8	0.154
radiofrequency	2	16	
TACE	3	2	
Chemotherapy	5	31	
No treatment	7 2	3 64	

Table 2: Correlation of different prognostic factors with anti p53 antibody

One year survival was 73.3% with median survival 13 months (Figure 1). Analysis of different prognostic factors with survival were done table 3. In univariate analysis the prognostic factors with statistical significance were portal vein thrombosis, total serum bilirubin, serum albumin, serum AST, serum ALT, Prothrombin time, viral marker and anti p53 antibody. While in multivariate analysis the MIF and anti p53 antibodies were the factors with statistical significance (p value =0,028; p value =0.001 respectively). One year survival in relation anti p53 and MIF was shown in (Figures 2-4) with the negative anti P 53 had better one year survival (76.4) than positive (45.1%) which was statistically significant (p value 0.001) and low MIF had better one year survival (81.9%) than high MIF (64%) which was statistically significant (0.001).

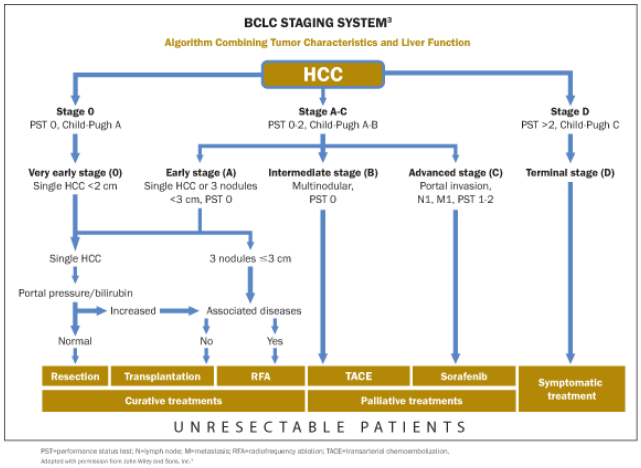

Figure 1: Barcelona clinical liver cancer staging system

Prognostic factors	Univariate analysis	Multivariate analysis
Age	0.052	0.879
Sex	0.811	0.584
Alpha fetoprotein	0.676	0.079
Liver cirrhosis	0.494	0.426
Portal vein thrombosis	0.001*	0.599
Total serum bilirubin	0.001*	0.168
Serum albumin	0.001*	0.789
Alkaline phosphatase	0.479	0.366
Serum AST	0.002*	0.975
Serum ALT	0.001*	0.965
Prothrombin time	0.001*	0.168
Viral marker	0.001*	0.879
Tumor size	0.296	0.407
Extra hepatic metastases	0.595	0.002*
BCLC	0.352	0.051*
Anti p53 antibody	0.001`*	0.001*
MIF	0.001*	0.022*
Treatment (yes/no)	0.767	0.033*
Type of treatment	0.247	0.876

Table 3: Prognostic factors with overall survival

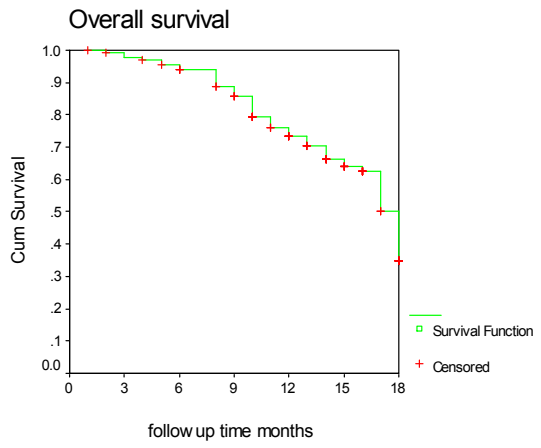

Figure 2: overall survival of 139 patients with HCC

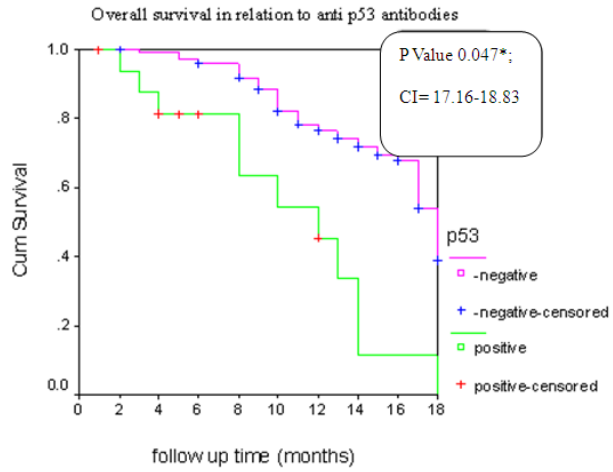

Figure 3: overall survival in relation to anti p53 antibodies

Discussion

Hepatocellular has very bad prognosis and high relapse rate despite of treatment technique. Tumor suppressor gene p53, its wild-type protein is responsible for cell-cycle regulation and apoptosis after DNA damage. In case of mutated p53, the cancer escape from apoptosis and turn into malignant cells [16,17].

The anti-p53 serum antibodies have been reported in 2-25% and 32% among HCC patients [17].

The roles of MIF in tumor genesis, proliferation of tumor cells and tumor angiogenesis were discovered. MIF expression may play a pivotal role in the dismal prognosis of patients with HCC that may be attributable to the modulation of angiogenesis. The cutpoint of plasma MIF level in HCC was 35.3 ng/ml. High MIF expression levels had a significantly worse (=0.025) disease-free survival, and this finding remained significant as an independent prognostic factor in the multivariate analysis. Plasma macrophage migration inhibitory factor (MIF) levels have prognostic value in HCC patients. Plasma MIF levels have a significant association with overall survival (OS) and disease-free survival (DFS) of HCC patients, even in patients with normal serum AFP levels and Tumor Node Metastasis (TNM) stage I HCC [18-24].

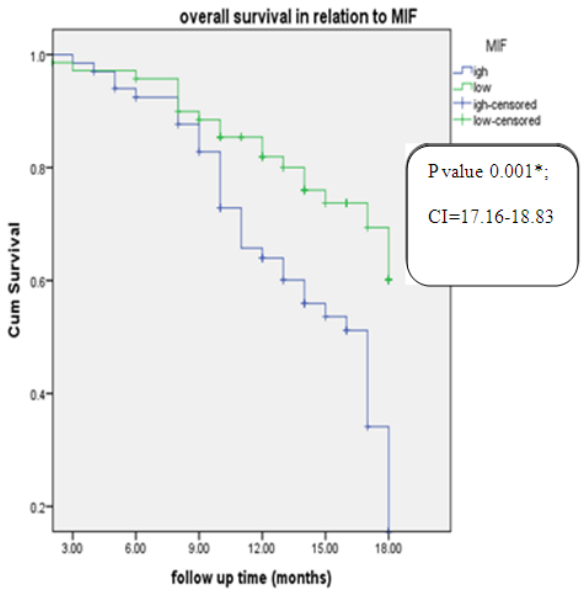

Figure 4: overall survival in relation to MIF

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