

A Panel of Molecular Markers in Hepatitis C Virus-Related Hepatocellular Carcinoma

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ABSTRACT

Background: Hepatocellular carcinoma is triggered by many factors including infection with hepatitis C virus. The molecular basis, however, of the development of HCV-related HCC remains unknown.

Objective: This work was designated to compare the circulating levels of some molecular markers between HCV-infected and HCV-free HCC patients.

Methods: We investigated 77 of HCC patients admitted to the National Cancer Institute, Cairo during the period 2002-2003. The plasma circulating levels of bcl-2, transforming growth factor beta 1 (TGF- β 1), vascular endothelial growth factor (VEGF) and beta2-microglobulin (β 2-MG) were investigated in HCV related HCC patients (n=40) compared to both HCV-free HCC patients (n=37) and a group of healthy subjects (n=20). Additionally, the LOH at the mannose 6-phosphate/insulin like growth factor-II receptor (M6P/IGFIIr) was investigated.

Results: The result did not predict a significant role of HCV infection on the circulating bcl-2 protein. In both HCC and HCC/HCV groups a limited number of patients had high levels of bcl-2. TGF- β 1 level increased particularly, but insignificantly in HCC associated with HCV infection. A similar pattern was obtained in the levels of β 2-MG, however the difference between HCC and HCC/HCV patients was significant ($p=0.001$). The infection with HCV was associated with a high incidence of LOH at M6P/IGFIIr site compared to HCV-free patients. Although the level of serum VEGF was significantly higher in all HCC patients than in healthy control, no significant difference, however was observed between HCV infected and HCV-free groups.

Conclusion: In HCC patients, HCV infection did not exclusively affect the levels of both bcl-2 and VEGF. TGF- β 1, β 2-MG and the LOH at M6P/IGFIIr, however were higher in presence of HCV infection.

Key Words: HCC – HCV – bcl-2 – TGF- β 1 – VEGF – M6P/IGFIIr – β 2-MG.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide [1]. Major etiologic factors associated with HCC include infection with hepatitis C (HCV) and hepatitis B (HBV) viruses, excess alcohol intake and aflatoxin B1 exposure [2,3]. HCC, with and without HCV infection, represents a major health problem in Egypt, where the two pathological conditions are integrated in many cases [4]. The mechanism of HCV-mediated oncogenic insults, required for initiation and/or progression of HCC, is not clear. HCV-RNA does not integrate into the host genome but likely induces HCC through the interaction of its proteins, such as the core, NS3 and NS5A, with the host cell proteins [5]. Over the past decade many reports have demonstrated that HCV proteins regulates growth and apoptosis related genes including c-myc, c-fos [6,7]. These findings and others suggest that HCV infection is, directly or indirectly, involved in HCV-mediated HCC.

The apoptosis-related gene bcl-2 gene, for example, provides a survival advantage to rapidly proliferating cells. It was suggested that bcl-2 expression inhibits diethylnitrosamine-induced HCC and counteracts the enhancing effect of transforming growth factor alpha (TGF α) [8]. Due to sequence homology, HCV mimics the effect of bcl-2 and maintains the growth of HCC, where HCV-NS5A region contains three bcl-2 homology domains (BH3, BH1 and BH2), interacts with Bax and inhibits apoptosis [9].

Transforming growth factor-beta 1(TGF- β 1), on the other hand, is a potent cytokine

involved in many functions such as epithelial mesenchymal transition, tissue morphogenesis, angiogenesis, and hence tumor progression, invasion, and metastasis [10]. In liver, TGF- β 1 has a profibrotic activity, and is involved in the pathogenesis of liver fibrosis [11], cirrhosis [12] and HCC [13]. Also, the angiogenic factors such as vascular endothelial growth factor (VEGF) are involved in neovascularization of malignant tumors. The effect of HCV on the expression of VEGF is controversial issue. Although HCV core protein activated expression of VEGF in HepG2 cells [14] no difference was detected among patients with HCC, patients with pre-malignant HCV and patients with steatohepatitis, as a non-malignant non-viral control. This predicted that VEGF mRNA expression is independent of the severity of HCV inflammation [15].

Additionally, mannose 6-phosphate/insulin like growth factor-II receptor (M6P/IGFIIr) is known to play a critical role in regulating cell growth by facilitating the activation of the growth inhibitor TGF- β 1 [16] and inactivating the growth stimulator insulin like growth factor-II (IGF2) [17]. Loss of this receptor may lead to both increase cell proliferation and reduction of apoptosis. Moreover, the development of HCCs from clonal expansions of phenotypically normal hepatocyte with mutated M6P/IGFIIr, supports the idea that M6P/IGFIIr functions as a liver tumor-suppressor gene [18].

A high serum level of β 2-microglobulin (β 2-MG) was detected in many infectious diseases including infection with HCV [19]. A significant correlation was found between β 2-MG and interleukin-6 (IL-6), α -fetoprotein and tumor size. This indicates that the elevation of β 2-MG seems to be a consequence of the stimulation of hepatocytes by humoral components such as IL-6. Weakening of the immune system, due to IL-6, may be responsible for a more severe progression of HCC and the overexpression of β 2-MG [20].

Such cross-talk between HCV proteins and host cell regulatory proteins was, extensively monitored, but still unable to clear the role of HCV in HCC initiation and/or progression. In this regard, the aim of this study is to reveal the interference of the HCV infection in HCC patients with a set of anti-apoptotic and growth factors inducing bcl-2, TGF- β 1, VEGF, M6P/IGFIIr and β 2-MG.

MATERIAL AND METHODS

Patients and Grouping:

This work included 97 subjects, 71 males and 26 females, with age ranged from 36 to 82 years, (mean \pm SD: 54.43 \pm 10.23). Subjects were categorized into 2 main groups including 77 HCC patients, admitted at the NCI, Cairo-Egypt, during 2002-2003, and a healthy group consisting of 20 subjects with no evidence of liver disease and serologically negative for both HCV and HBV. Non of the patients had a history of alcohol abuse or previous interferon treatment. Patients, however, had solid tumors with variable diameters ranging from 1.2cm to 12cm (mean \pm SD: 4.95 \pm 3.23cm). Out of 77 patients 24 (31.2%), 27 (35.1%) and 26 (33.8%) had tumors in grades I, II and III, respectively. According to HCV infection, confirmed by RT-PCR test on serum, HCC patients were further categorized into 2 subgroups including 40/77 (51.9%) patients infected with HCV and 37/77 (48.1%) HCV-free. In the later group HCC developed due to HBV and/or Schistosomal infection or to unknown etiology. All investigations were carried out on the same blood or tissue samples received for routine diagnosis and initial presentation of the patients. Lymph node metastasis was detected by CT scan and the clinically positive nodes were porta-hepatis. In some patients, lymph node metastasis was diagnosed by fine needle aspiration biopsies (FNAB). The histopathological grade of tumors was evaluated using standard staining procedures.

Detection of Anti-HCV and HCV-RNA:

Serum samples from all subjects were tested for anti-HCV using the commercially available EIA kit (Abbott HCV EIA-2; Abbott diagnostic division, USA) and RT-PCR was performed to confirm HCV infection.

The anti-apoptotic factor bcl-2 and Transforming growth factor β I (TGF β 1). The circulating level of bcl-2 protein was estimated by EIA using bcl-2 specific mouse monoclonal anti-human bcl-2 (Oncogene, Research Products, Boston, MA, USA) following the manufacturer's instructions. To determine the TGF- β 1, samples were diluted with assay buffer, acidified with HCl, neutralized with NaOH and then used to estimate TGF- β 1 protein using the commercially available EIA kit (DRG Interna-

tional Inc., USA) following the manufacturer's instructions.

Vascular endothelial growth factor (VEGF): The human VEGF protein ELISA kit (Oncogene Science, NY, USA) was used to measure VEGF in serum using mouse monoclonal antibody for capture and rabbit polyclonal serum for the detection of circulating VEGF protein, following the manufacture's instructions.

Mannose 6 phosphate/Insulin like growth factor II receptor (M6P/IGFIIr). The pattern of heterozygosity at the 3' untranslated region (3' UTR) of M6P/IGFIIr was investigated by PCR [21]. After extraction of DNA from liver tissue [22], about 20ng DNA were amplified by PCR using M6P1 and M6P2 primers. The forward primer was end-labeled with $\gamma^{32}\text{P}$ dATP using T4 polynucleotide kinase [23]. The labeling reaction was performed in a 100 μl mix containing 10 μl (100pmol) of each primer, 1.5 μl of 5XT4 polynucleotide kinase buffer, 2 μl $\gamma^{32}\text{P}$ dATP (10mCi/m) (Amersham, USA), and 1 μl (10U) T4 polynucleotide kinase (GIBCO-BRL, MD, USA). The reaction was incubated at 37° for 30min then heat-inactivated at 65° for 5min. The unincorporated $\gamma^{32}\text{P}$ ATP was removed by using Qiaquick nucleotide removal kit-50 (Qiagen Inc, CA, USA). The amplification was performed in a thermal cycling program consisting of initial denaturation at 94° C for 5min, and 35 cycles of denaturation at 94° C for 1min annealing at 60° for 1min, primer extension at 72° C for 1min followed by a single extension step at 72° C for 5min. The amplification products were resolved onto 8% denaturing polyacrylamide gel, which was dried and exposed to X-ray film from which LOH was scored according to the intensity of banding pattern.

β 2-Microglobulin: β 2-MG was estimated by the IMX β 2-MG assay using full-automated IMX system (Abbott Laboratory, USA). This method based on the microparticle immunoassay (MEIA).

Statistical analysis: Correlation between variables was evaluated using the nonparametric Spearman test. Comparisons between two groups with respect to numeric variables, Mann-Whitney test was performed. Kruskal-Wallis test was used for more than two groups. Comparisons between categorical measurements were done using the chi-square test. To measure

the HCV and lymph nodes in relation to VEGF levels, two-way analysis of variance (ANOVA) was performed [24].

RESULTS

HCV Infection and Tumor Characteristics:

Out of 77 HCC patients investigated, 40 (51.9%) of HCC patients were positive HCV-RNA by RT-PCR, whereas 37 (48.1%) were HCV-free (Table 1). In both HCV related HCC (HCC/HCV) and HCV-free groups, 67.5% and 59.5% of patients, respectively had tumor size bigger than 3cm. Patients with HCC/HCV, however, had insignificantly bigger mean tumor sized compared to HCV-free patients ($p=0.18$). The histopathological grades of tumors were as follows: Grade I: 31.2%; grade II: 35.1% and grade III: 33.8%. In presence of HCV the majority of patients (92.5%) had progressive HCCs (grades II and III) compared to 43.24% in absence of HCV. In the HCC/HCV group more patients had significantly positive lymph node metastasis compared with HCV-free patients ($p<0.001$) (Table 1). $\chi^2=<0.001$.

Relation between HCV and bcl-2 levels:

Compared to the average value of the healthy group, a limited number of patients (14/77, 18.18%) had 2-fold increase (or more) in the level of bcl-2 protein. In HCC/HCV and HCV-free groups 11/40 (27.5%) and 3/37 (8.1%), respectively had a high levels of bcl-2. The values of bcl-2 ranged from 100 to 1230U/ml and from 90.6 to 1924.1U/ml in HCC and HCV/HCV groups, respectively (Table 2). HCC/HCV patients were more heterogeneous compared to the HCC group. Although the changes of bcl-2 levels in both groups were insignificant compared with the control group, the increase of bcl-2 protein was significant ($p=0.013$, unpaired t -test) in presence of HCV compared to HCV-free group (Fig. 1B). There was no significant relation between the levels of bcl-2 and the tumor invasion represented by the VEGF level in both HCC and HCC/HCV groups ($r=0.085$, $p=0.6171$) and ($r=-0.199$, $p=0.22$), respectively (Spearman test). Similarly the relations between bcl-2 and TGF- β I were insignificant in both groups ($r=-0.113$, $p=0.504$ and $r=-0.276$, $p=0.085$, respectively). On the contrary β 2-MG level was significantly correlated with the bcl-2 level only in HCC/HCV patients ($r=0.43$, $p=0.006$) (Fig. 1C). Out of 11

HCC/HCV patients with abnormally high bcl-2 protein (2 fold or more): 1, 4 and 6 patients had tumors with grade I, II or III, respectively, whereas tumors of HCV-free HCC patients with high bcl-2 were limited to grades I or II.

Transforming Growth Factors β1:

The data showed that the average level of TGF-β1 expression was markedly increased in all patients investigated compared to the control group. Also, HCV-free HCC groups, had significantly higher levels of TGF-β1 compared to the control group. No significant difference was observed between HCC and HCC/HCV patients (Table 2).

Assessment of Angiogenesis and its Relation with HCV Infection:

Exclusively, all HCC patients we investigated had at least 10-fold increase in the circulating level of VEGF protein 1097±23.0pg/ml when compared with the reference value 167.4pg/ml. Serum VEGF levels were 1074.3±190.3 pg/ml and 1117.9±212.5pg/ml in HCC and HCC/HCV groups, respectively (Table 3). Although there was a significant increase of VEGF in HCC patients with and without HCV infection ($p < 0.001$ in both cases) compared with the reference value, no significant difference ($p = 0.204$) was

observed between the levels of VEGF in presence or absence of HCV. (Fig. 2A) illustrates the mean values of VEGF in patients with positive and negative lymph nodes metastasis. In presence of HCV an insignificant increase in VEGF was observed in patients with positive lymph nodes ($p = 0.201$). Similarly, in HCV-free HCC patients insignificant difference was observed between the patients with and without lymph node metastasis ($p = 0.60$). VEGF however did not have any correlation with tumor size (Fig. 2B).

Mannose 6-phosphate/insuline-like Growth Factor-II Receptor (M6P/IGFIIr):

The constitutional pattern of M6P/IGFIIr was detected in 31/77 (40.3%) of all patients investigated. LOH at this locus was observed in 13 (35.1%) and 33 (82.5%) of HCC and HCC/HCV patients, respectively ($p < 0.201$) (Fig. 3).

Beta2-microglobulin (β2-MG):

The average level of β2-MG in all HCC patients was significantly higher than the reference value (up to 3ug/ml) (Table 4). Beta 2 microglubulin was significantly higher in HCC/HCV patients compared to HCC patients ($p < 0.001$).

Table (1): Histopathological characteristics of HCC patients with and without HCV infection.

		Groups				
		Control	HCC/HCV	HCC group		
				HCC	All patients	p-value
Subject number (%)		20 (100%)	40 (51.9 %)	37 (48.1%)	77 (100%)	
Age (year):	Mean ±SD:	44.25±4.41a	57.88±9.0b	56.2±10.2b	57.08±9.60	0.22
	Range:	36-52	44 -75	37-82	37-82	
Sex	M:	11 (55.0%)	36 (90.0%)	24 (64.89%)	60 (77.9%)	
	F:	9 (45.0%)	4 (10.0%)	13 (35.1%)	17 (22.1%)	
Diameter of tumor size (cm):	Mean ±SD:	–	5.40±3.4 a	4.43±2.93 a	4.95±3.23	0.18
	Range	–	1.5-12	1.2-12	1.2-12	
	≤3:	–	13 (32.5%)	15 (40.5%)	28 (36.4%)	
	>3:	–	27 (67.5%)	22 (59.5%)	49 (63.6%)	
HCC grade	I	–	3 (7.5%)	21 (56.8%)	24 (31.2%)	<0.001
	II	–	14 (35.0%)	13 (35.1%)	27 (35.1%)	
	III	–	23 (57.5%)	3 (8.1%)	26 (33.8%)	
Lymph node metastasis	Positive	–	11 (27.5%)	6 (16.2%)	17 (22.1%)	0.233
	Negative	–	29 (72.5%)	31 (83.8%)	60 (77.9%)	

Legends: M: male, F: female, SD: standard deviation, HCC/HCV: patients with HCV-related HCC, HCC grading is hisptpathological grades I, II and III, Tumor size is bidirectional CT guided, SD: standard deviation. Percent values are calculated relative to the subgroup number. p-value less than 0.05 is considered significant. Similar small letters refer to statistically insignificant difference between means.

Table (2): Characteristics of HCC patients with and without HCV infection.

Parameters investigated	Groups					p-value
	Control	Diseased groups				
		HCC/HCV	HCC	All patients		
bcl-2 (U/ml)	<2 fold	20 (100%)	29 (72.5%)	34 (91.90%)	66 (85.71%)	0.001*
	>2 fold	0 (0.0%)	11 (27.5%)	3 (8.10%)	14 (18.18%)	
	Median	335.0 a	255.2 ab	177.0 b	200.0	
	Range	185- 450	90.6-1924.1	100-1230	90.6-1924.1	
TGF β 1 (pg/ml)	Median	390.0 b	485.0 ab	530.0 a	505.0	0.010*
	Range	220-410	105-1890	210-2350	105-2350	

* Comparisons between HCV-HCC and HCV-free HCC groups. p-value less than 0.05 is considered significant. Same small letters refer to statistically insignificant difference between medians.

Table (3): Vascular endothelial growth factor of HCC patients.

	Groups		
	HCC/HCV	HCC	All patients
VEGF mean \pm SD	1117.9 \pm 212.5 a	1074.3 \pm 190.3 a	1097 \pm 201.8
	p=0.204		

Table (4): Beta 2-microglobulin concentration of HCV infected and HCV-free HCC patients.

Parameter investigated	Groups		
	HCC/HCV	HCC	All patients
β 2-micro-globulin (mean \pm SD)	61.00 \pm 23.33 a	35.47 \pm 21.20 b	49.11
Range	10-150	8.75-81.25	8.7-250
Overall p-value	P=0.204		

p-value was estimated by ANOVA followed by Tukey-Kramer-multiple comparisons test. Group means sharing different small letter are significantly different than each other.

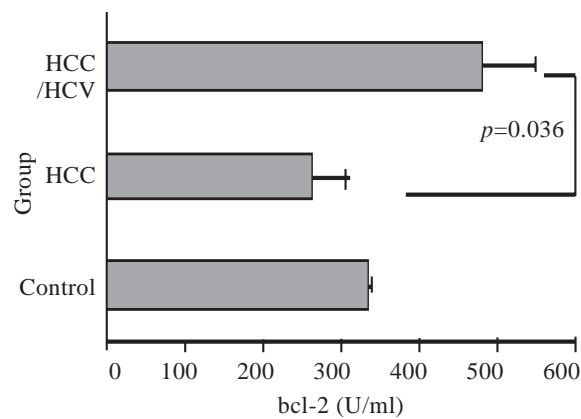


Fig. (1-A): Comparison of the mean values of bcl-2 in control subjects, HCC associated with HCV and HCC patients.

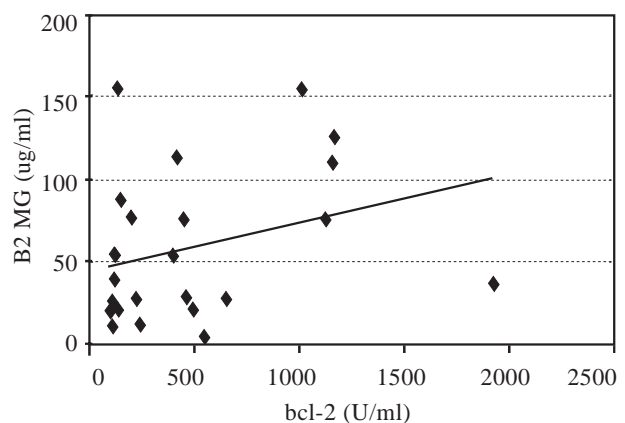


Fig. (1-B): The correlation between bcl-2 and β 2-MG levels in HCC/HCV patients.

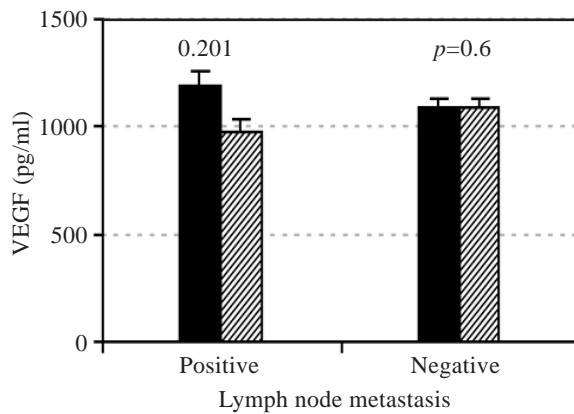


Fig. (2-A): Mean values of VEGF in HCC patients positive and negative lymph nodes. In presence of HCV (solid bar), patients with positive lymph nodes have a significantly higher levels of VEGF than HCV-free patients (patterned bar).

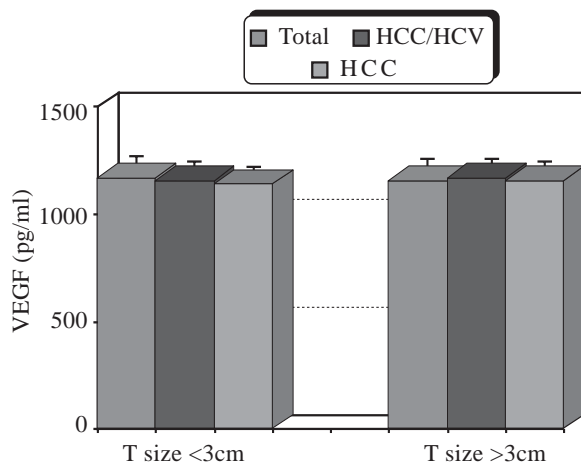


Fig. (2-B): Comparison of VEGF in all patients (dotted bar), HCV infected (solid bars) and HCV-free patients (patterned bar) with tumors with diameter less or equal to 3cm and greater than 3cm. No significant difference was observed between each subgroup.

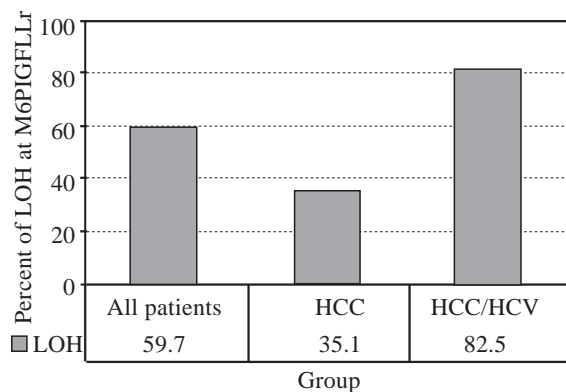


Fig. (3): Percent of patients with LOH at M6P/IGFIIr.

DISCUSSION

The RNA nature of HCV excludes the possibility of viral integration into the host cell genome. Thus, the oncogenic potential of HCV is limited to the effect of its proteins and/or the inflammatory response it causes. The literature have, extensively documented the effect of HCV infection on host cell genes expression, translation and a long list of cellular regulatory proteins. In this work the goal was to determine whether HCV infection in HCC patients affects the levels of bcl-2, TGF- β 1, VEGF, β 2-MG and the pattern of hetrozygosity at M6P/IGFIIr site. The data revealed that HCC was associated with HCV infection in about half (51.9%) of the investigated patients the majority were females. Histologically, tumors of a small number of cases (7.5%) belonged to grade I HCC because most of the cases were presented on top of post-hepatitis cirrhosis, whereas more than 2/3rd of the tumors belonged to higher grades (II, III) on top of cirrhosis with high tumor invasiveness both locally and matastatic. This may explain the unusual high percent of portahepatis lymph nodes due to advanced stage disease. Lymphatic spread, metastasis work up has been done to exclude the possibility of the underlying other types of malignancies rather than HCC using the tumor markers (carcininoembryonic antigen (CEA) and carbohydrate antigen CA199) and radiological investigation for chest, abdomen, and pelvic radiology (data not shown).

HCC patients with tumors expressing promoters of apoptosis (such as bax) versus inhibitors of apoptosis (such as bcl-2) may have increased survival [25]. Although there was an increase in the average level of bcl-2 protein in HCC/HCV patients compared to the healthy control subjects, the individual data did not predict that HCV was a direct cause for the elevation of bcl-2, where not all HCV infected patients had increased levels of bcl-2. Elevation of bcl-2 protein was observed in 18.2% of all patients and 11/40 (27.5%) of them were HCV infected. A similar ratio was reported using immunostaining [26]. Because we did not estimate the level of bcl-2 mRNA, it is not clear whether the low or normal bcl-2 levels detected, in most cases, were due to low initial expression or posttranslational degradation of bcl-2. Detection of highly expressed bcl-2 mRNA concomitant with low levels of its protein may

support the posttranslational degradation of bcl-2 [25]. It is not clear what factors led to stabilization of bcl-2 protein in 27.5% of HCV patients compared to 8.1% of HCV-free HCC patients. Interestingly, patients with higher bcl-2 showed low probability of metastasis, where 72.7% of these patients had negative lymph nodes metastasis. Although this does not confirm the inhibitory role of bcl-2 on tumor development, it may indicate, that bcl-2 may delay, but does not inhibit, the development of proliferating foci and clonal expansion of primary tumors [8]. Also, the moderate Spearman correlation ($r=0.4$) detected between bcl-2 levels and tumor size of all patients, partially excludes the inhibitory effect of bcl-2 on tumor growth. Subsequently, it seems that bcl-2 does not play a substantial role in the progression of HCC in patients with progressive tumors (grades II and III). Due to the domain homology between the non structural region (NS5A) of HCV and bcl-2 [9], it is speculated that HCV may exert an anti-apoptotic effect and enhances tumorigenesis. Both the increase in tumor size (by 8%) and presence of more patients with grade III tumors in HCC/HCV group may support this hypothesis. The overall picture does not predict a prognostic value of bcl-2 in HCC/HCV patients.

The increase in serum TGF- β 1 in HCC patients was repeatedly reported [13,28,29]. HCV may enhance the increase of this growth factor. TGF- β 1 levels, however were insignificantly increased in presence of HCV compared to HCV-free group. It was suggested that HCV core protein, rather than other viral proteins (E1/E2/p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) activated the TGF- β 1 promoter and upregulated TGF- β 1 [30]. Additionally, binding of M6P/IGFIIr with TGF- β 1 [31] may lead to its internalization and subsequent degradation in the lysosomes. Although the results support the trend of using serum (or urine) TGF- β 1 levels as a marker of HCC, however the implication of HCV-mediated increase may need more investigations.

Plasma VEGF is one of the positive prognostic markers secreted, abundantly in several human tumors including HCC [32]. The direct effect of HCV proteins on VEGF is not clear. The HCV core protein was able to activate the expression of VEGF in HepG2 cells [14]. On the contrary, the expression of VEGF was inde-

pendent of both HCV infection and HCV related liver inflammation [33]. In agreement with Makhoulouf and his coworkers [34], VEGF estimated in the present work was, dramatically elevated in all HCC patients investigated. In presence of HCV infection, the elevation of VEGF was significant compared to healthy subjects but insignificant compared to the corresponding HCV-free patients. This may, partially exclude the possible effect of HCV infection on the expression or posttranscriptional function modification of VEGF. HCV, however affects the VEGF expression, only, in early malignancy as indicated by the significant increase in HCV infected patients compared to the corresponding HCV-free HCC group. Also, the elevation of VEGF level was insignificantly higher in patients with grade III tumors compared with early HCC (grade I) in presence of HCV than in absence of HCV. In agreement with Li et al., [35], there was no significant difference in the level of VEGF between patients with bigger tumors (diameter >3cm) and smaller tumors (diameter \leq 3cm) ($p>0.05$ in presence or absence of HCV).

Few studies, if not, have reported the pattern of heterozygosity at M6P/IGFIIr locus in HCC patients in Egypt. Here, the data reported a high instability of M6P/IGFIIr gene in 54.05% of HCC patients and with a significantly higher percent (82.5%) in HCC associated with HCV infection. The high percent in HCV infected patients may be explained by the increased number of patients with invasive tumors. This observation supported previous reports indicating that the incidence of LOH may increase in HCC associated with the high risk factors such as hepatitis viruses [36]. The LOH was, uniformly distributed in HCC patients with tumors with different grades, where all patients with grade III tumors and the majority of patients with grades I and II showed LOH at M6P/IGFIIr site. This may support the idea that such genetic instability occurs early in liver carcinogenesis.

HCV infection is strictly associated with mixed cryoglobulinemia that may evolve to lymphoma. The vast majority of patients we investigated showed elevated levels of β 2-MG (equal to or greater than 3 fold). As previously reported [37], HCV infection enhanced the elevation of serum β 2-MG levels. This increase is due to stimulation of hepatocytes by humoral

components of immunological response, such as IL-6 [38]. In contrast to Dennis and Rifkin [39] the levels of β 2-MG did not reflect tumor size.

In summary, in spite of the heterogeneity of the sample population investigated, the data did not support the trend of using serum molecular markers in distinguishing HCC associated with or without HCV infection. HCC was associated with increased levels of TGF- β 1, VEGF and β 2-MG. HCV in HCC patients positively affects the circulating levels of β 2-MG, but not VEGF nor TGF- β 1. Also, HCV increases the incidence of LOH at M6p/IGFII receptor.

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