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HEPATOCELLULAR CARCINOMA AND ANGIOGENIC GROWTH FACTORS IN CASES WITH HCV AND/OR HBsAG COINFECTION

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Abstract

Angiogenic growth factors are involved in several pathological conditions including solid growth, wound healing and organ regeneration. An interaction between HCC and viral hepatitis conferred by HCV and HbsAg remain debatable. The aim of the present work is to assess the role of certain angiogenic growth factors in HCC respective to the influence of chronic HCV and HBsAg. To achieve this objective, thirty male with HCC (mean age 52+ 6.8) were subclassified into three groups (GIa, b,c). Cases in GIa (n = 9) had chronic HCV and HbsAg, cases in GIb n=11 had HCV; cases in GIc n=10 had HBSAg. Twenty cases were equally classified into those with chronic HCV (GII) and those with HbsAg GIII) and compared to ten normal healthy states representing the control group (GIV) .The results revealed increased levels in GIa>GIb>GIc>gII>GIII relative to the control group (GIV) for the assessed angiogenic growth factors: platelet derived endothelial cell growth factor (PD-ECGF), basic fibrobalst growth factor (bFGF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and transforming growth factor alpha (TGF-a). In parallel, a similar pattern of change was presented for the tumor marker alpha fetoprotein (AFP) and liver function tests. In conclusion, the increments in assesses angiogenic growth factors and the tumor marker versus alterations in liver function verified the viremic impact influenced by chronic HCV and HbsAg in HCC cases.

Introduction

HEPATOCELLULAR carcinoma (HCC) has been identified as the most common lethal solid human malignancy in the world with a mortality index of 0.94[1]. The annual incidence of HCC has been estimated to be at least one million new patients worldwide[2-5]. The pronounced geographic variation of HCC appeared to be the most common cancer in men globely[6,7].

Furthermore, both geogrphic variation and the prevalence of HCC provided convincing evidence that the development of human cancer is associated with chronic HBV as well as HCV infection[8-11].

Angiogenic growth factors are involved in several pathological conditions including solid tumor growth, wound healing, and organ regeneration[12]. Platelet derived endothelial cell growth factor (PD-ECGF), acidic and basic fibroblast growth factors (FGFs) and transforming growth factor alpha (TGF-α) are among the angiogenic factors that have been shown to stimulate endothelial cells both in vitro and in vivo. However , the target cell specificities of these factors differ. Thus, FGFs and TGF-α stimulate proliferation of a wide variety of cells including Fibroblasts, whereas, PD-ECGF has not been found to stimulate any cell type other than endothelial cells[13].

Evidently, new vessel development, which is required for extracellular matrix invasion, involves the angiogenic cytokines basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF). They represent two potent heparin-binding mediators of angiogenesis with a synergistic effect in vitro[14].

Another important angiogenic factor that stimulates endothelial cell motility and growth is hepatocyte growth factor (HGF) which is expressed in non-parenchymal liver cells such as Kupffer, endothelia and Ito cells[15,16]. HGF represents an endocrine or growth hepatotrophic factor that may reflect liver necrosis and dysfunction followed by active regeneration[16-18].

On the other hand, besides the potential importance of TGF as an autocrine growth regulator, it may play a role as an angiogenic mediator in malignancy associated neovascularization. Thus, TGF-(may contribute to the generation of a local micro-environment that is favorable for solid tumor growth[19].

As the mechanism of interaction between HCC and viral hepatitis conferred by HCV and HbsAg remains debatable, the present work aims to assess the role of angiogenic growth factors in such cases.

Subjects And Methods

Thirty male with HCC (mean age 52 + 6.8) were subclassified into three groups (GIa, b, c) .Cases in GIa (n = 9) had chronic HCV and HbsAg, those in Gb (n = 11) had chronic HCV, and those in GIc (n = 10) had HbsAg. Twenty cases were equally classified into those with chronic HCV (GII) and those with HbsAg (GIII) and compared to ten normal healthy states representing the control group (GIV). All cases were subjected to the following:

- Thorough history taking with special emphasis on history of hepatitis, blood transfusion, drug abuse, dialysis, surgery and any symptoms of hepatocellular decompensation.
- Clinical examination with special emphasis on liver, spleen presence or absence of ascites, and signs of hepatocellular decompansation.
- Serological markers for HCV and HbsAg .
- Diagnosis of HCC was established by clinical assesssment combined with findings on imaging studies (ultrasonography, spiral CT scanning, isotope liver scanning,magnetic resonance imaging) and confirmed by liver biopsy.
- Sera of all patients and controls were examined for the levels of the following angiogenic growth factors: platelet derived endothelial cell growth factor (PD-ECGF), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), hepatocyte growt factor (HGF) and transforming growth factor alpha (TGF-A). All these growth factors were determined by ELISA technique using commercial available kits from "Oncogene Research Products, Cambridge, MA. These parameters were monitored relative to alterations in tumor marker alpha fetoprotein (AFP), which was determined by radioimmunoassay according to the method of Silver, 1973[20].
- Liver function tests included serum enzyme activities of alanine and aspartate transaminases[21], alkaline phosphatase[22], and total serum bilirubin[23].

Results

Table (1) represents the angiogenic growth factors in groups under study . The b-FGF, VEGF, HGF and TGF were significantly increased in GIa> GIb>GIc>GII>GIII compared to normal control (GIV), P<0.001, while the PD-ECGF was insignificantly increased in GIa>GIb>GIc>GII>GIII compared to normal control (GIV) P>0.05.

Table (2) represents the tumor markers and liver function tests (ALT,AST,ALP and serum albumin). AFP was significantly increased in GIa>GIb>GIc>GII>GIII compared to normal control P<0.001. The AST, ALP, S.Bilirubin were statistically increased in GIa>GIb>GIc>GII>GIII compared to normal control P<0.001 while ALT level was insignificantly increased in GIa>GIb>GIc>GII.GIII compared to normal control P>0.05.

Table (I): Angiogenic Growth Factors in Groups Under Study.
Data are Mean ± SD.

Biochemical Parameter	Cases with HCC (n=30)			Cases with HCV	Cases with HBsAg	Control Group	F-value
	HCV+HBsAg (GIa, n=9)	HCV (GIb, n=11)	HBsAg (GIc, n=10)	(GII, n=10)	(GIII, n=10)	(GIV, n=10)	(P)
PD-ECGF (U/mL)	7.1±2.8 (II,III,IV)*	6.4±2.4 (II,III,IV)*	6.0±2.1 (III,IV)*	4.9±1.7	4.6±1.5	4.2±1.1	3.13 (0.0143)
bFGF♦ (pg/mL)	93.7±25.4	81.5±21.6	64.3±17.9	23.2±9.1	16.1±4.6	11.2±3.4	50.01 (<0.0001)
VEGF♦ (pg/mL)	647±189	529±155	485±123	285±84.6	170±54.9	93.8±28.6	32.20 (<0.0001)
HGF◊ (pg/mL)	1.25±0.38	0.99±0.29	0.78±0.22	0.55±0.16	0.46±0.14	0.31±0.10	(22.17 (<0.0001)
TGF- a◊ (pg/mL)	362±89.4	249±65.2	126±32.1	45.3±10.6	36.2±9.3	6.1±2.1	87.44 (<0.0001)

*Comparison between individual groups using LSD (least significant difference) at 5% level of significance.

PD- ECGF = platelet derived endothelial cell growth factor
bFGF = basic fibroblast growth factor
VEGF = vascular endothelial growth factor
HGF = hepatocyte growth factor
TGF-a = transforming growth factor alpha
♦ = All groups are statistically significant with each other
◊ = All groups are statistically significant with each other except G II with GIII

Table (II): Tumor Marker and Liver Function Tests in Groups Under Study.
Data are Mean ± SD.

Biochemical Parameter	Cases with HCC (n=30)			Cases with HCV	Cases with HbsAg	Control Group	F- value (P)
	HCV+HBsAg (GIa, n=9)	HCV (GIb, n=11)	HBsAg (GIc, n=10)	(GII, n=10)	(GIII, n=10)	(GIV, n=10)	
AFP (µg/mL)	489±126 (II,III,IV)*	453±99.4 (II,III,IV)*	425±87.5 (II,III,IV)*	202±58.9 (IV)*	175±43.7 (IV)*	10.3±3.3	57.35 (<0.0001)
ALT (U/mL)	47.8±11.6 (Ic,II,III,IV)*	41.5±10.2 (II,III,IV)*	36.4±9.3 (IV)*	28.9±8.7 (IV)*	35.9±10.1 (IV)*	21.7±6.8	4.01 (0.00343)
AST (U/mL)	53.6±14.3 (Ic,II,III,IV)*	48.3±12.1 (Ic,II,III,IV)*	41.2±10.0 (II,III,IV)*	34.7±9.8 (III,IV)*	27.5±8.5 (IV)*	20.6±6.3	14.14 (<0.0001)
ALP ♦ (U/dL)	17.2±4.7	15.1±4.3	13.8±3.2	9.1±2.9	6.4±2.2	4.14±1.3	24.37 (<0.0001)
Bilirubin ◊ (mg/dL)	1.74±0.52	1.58±0.47	1.43±0.42	1.31±0.39	1.06±0.27	0.81±0.25	7.24 (<0.0001)

*Comparison between individual groups using LSD (least significant difference) at 5% level of significance.
AFP = alphafetoprotein
ALT = alanine transaminas
AST = aspartate transaminase
ALP = alkaline phosphatase
♦ = All groups are statistically significant with each other
◊ = All groups are statistically significant with each other except GIa with GIb and GIa with GIc.

Discussion

The role of HBV or HCV as direct carcinogens is unclear because the HCV genome is not integrated into the human host, while the HBV genome is integrated in a seemingly random fashion[24]. Howevere, it is thought that hepatic destruction and replicative repair leads to the accumulation of mutations associated with cancer development. In accordance, angiogenesis represents a complex process that involves several steps inccluding migration and proliferation of endothelial cells which is associated with the release of certain growth factors[13]. It is evident from the present data that, coinciding with the magnitude of hepatotoxic insult, variable alterations were assessed in the levels of angiogenic growth factors.

The present data conforms with the restricted target cell specificity of PD-ECGF and the fact that platelets are a main source of it . This suggests that PD-ECGF has a role in maintaining the integrity of blood vessels by promoting repair of endothelial cell layer[13]. Evidently, angiogenesis represents a net balance between positive and negative regulators of neovascularization whereby endothelial cell matrix remodeling, migration and proliferation are central to the angiogenic process[25,26]. Consistently, the assessed alterations in VEGF herein appears to follow a similar pattern to PD-ECGF. This agrees with postulations referring to the angiogenic potency of VEGF being a potent endothelial mitogen[27].

The parallel increase in bFGF to VEGF incremental levels reflects new vessel development which is required for extracellular matrix invasion. This manifests a graded response to hepatic insult with great variation in HCC cases with dual impact of HCV and HbsAg(14). The mechanism by which tumors induce trauma which provides the vascular supply that tumors require for obtaining nutrients, gas exchange and waste disposal have focused on angiogenic cytokines bFGF and VEGF[28-30].

In alignment, the assessed increase of HGF in groups under study verifies its association with the magnitude of hepatic affection. It occurs on the basis of the activity of HGF to stimulate mitogenesis of mature hepatocytes.[31] Increasing evidence suggests that HGF not merely acts as a humoral mediator of liver regeneration but has more diverse activities on a variety of epithelial cells other than hepatocytes[32-34]. This agrees with the potential role reported for HGF in tumor progression presenting a paracrine mediator[35] Hence, HGF mRNA level in the injured liver was reported to increase 10-20 times the normal values with a marked compensatory hepatocyte DNA synthesis 48-72 hours later.

The major source of HGF increase in liver damage may be from extrahepatic organs such as lung and spleen. The increased levels of HGF assessed herewith may represent a decrease in

hepatic clearance of HGF which may be caused by reduction of intact hepatocytes as reported in fulminant hepatitis or liver cirrhosis . Moreover , the increase in HGF levels in hepatocellular necrosis should be considered in relation to hepatic macrophage inactivation[36].

On the other hand, the assessed increments in TGF-a herein would reflect a role in neoplastic pathogenesis through an autocrine growth regulated mechanism[19]. This, TGF-α in a variety of solid tumors which depend on neovascularization for their development, reflects the ability of TGF-a to promote angiogenesis by targeting endothelial cells[19]. Evidently, as observed herewith and reported elsewhere, clinical differences between HCC patients with HBV in various geographic areas and between HCC patients with HBV and with HCV occurs[37,38]. These differences are attributable to additional exposure to environmental factors, life style, and diet [24].

Compatible with such data, alteration in levels of AFP coincided with disturbances of liver function that identified persistence of hepatic pathological changes and hepatocellular injury. The monitored increase in AFP and ALT levels aligns with the significant relationship between them and HCV PCR positivity as well as the grade of viremia representing their significance as markers in liver disease.

In conclusion, the higher levels of angiogenic growth factors in HCC cases (GI a > GI b > GIc). Reflects the magnitude of hepatocyte insult by viral hepatitis (chronic HCV and HbsAg). These relative changes coincided with alterations in the tumor marker (AFP) and liver function. It verifies the viremic impact influenced by chronic HCV and HbsAg that initiated HCC rather than the impact of individual viral infection.

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