

Hypercoagulable States in Patients with Hepatocellular Carcinoma

DEMETRIOS N. SAMONAKIS, MD,* IOANNIS E. KOUTROUBAKIS, MD,*
AEKATERINI SFIRIDAKI, MD,† NIKI MALLIARAKI, MD,‡ PAVLOS ANTONIOU,*
JOHN ROMANOS, MD,§ and ELIAS A. KOUROUMALIS, MD, PhD*

Hepatocellular carcinoma (HCC) patients have an increased risk for venous thromboembolism, mainly portal venous thrombosis (PVT). The aim of this study was to assess the role of acquired and hereditary thrombotic risk factors in HCC patients. Thirty-one patients with HCC, 30 patients with cirrhosis but without HCC or PVT, and 48 matched healthy controls were studied. Mean levels of plasma protein C, protein S, antithrombin, and serum lipoprotein (a) were significantly lower in patients with HCC and in the cirrhotic group compared to the healthy controls. Mean serum homocysteine levels were significantly higher in patients with HCC compared to cirrhotics and healthy controls. The prevalence of activated protein C resistance, factor V Leiden mutation, prothrombin gene mutation G20210GA, and C677T methylenetetrahydrofolate reductase polymorphism was not significantly different among the three groups. In conclusion, thrombophilic defects are common in HCC patients and they might contribute to the observed thrombotic complications in this malignancy.

KEY WORDS: cirrhosis; hepatocellular carcinoma; homocysteine; factor V Leiden; portal vein thrombosis; thromboembolism.

Prothrombotic state of malignant diseases is a well-known cause of morbidity and mortality in cancer patients. Moreover, venous thromboembolism is often the presenting feature of an occult cancer case (1). The underlying mechanisms for the hypercoagulability in malignancy are not entirely understood (2). Portal vein thrombosis is a frequent complication of hepatocellular carcinoma (HCC) (3, 4), whereas it is also increasingly recognized in non-malignant chronic liver disease (5–7). Although in some cases thromboses in the portal vein's trunk or branches are neoplastic in origin (8), in a significant percentage there is no satisfying explanation for the increased incidence in patients with HCC.

Factors like hyperhomocysteinemia (9) and resistance to activated protein C (APC) have been proposed as important factors for venous thromboses (10, 11). Moreover, APC resistance is considered to play a major contributory role in the thrombotic tendency of cancer patients (12, 13). The effect of factor V Leiden (FVL) mutation is also considered significant (14). On the other hand, in portal vein thrombosis (PVT) prothrombotic disorders, according to recent data, seem to play an important role in disease pathogenesis (15–17). Genetic defects such as deficiencies in protein C, protein S, and antithrombin (18, 19), FVL mutation, and prothrombin (PT) 20210GA mutation (16, 17, 20, 21) as well as acquired factors such as increased antiphospholipid antibodies have been reported in patients with PVT (22). The presence of similar risk factors in patients with HCC with or without portal vein thrombosis has not been adequately studied, and there is just one case report in the literature (23).

The aim of our study was to estimate the presence of thrombophilic defects in patients with HCC and to correlate them with thrombotic complications.

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From the Departments of *Gastroenterology and §Surgical Oncology and †Laboratory of Clinical Chemistry, University Hospital Heraklion, and ‡Regional Blood Bank Center, Venizelion Hospital Heraklion, Crete, Greece.

Address for reprint requests: Ioannis E. Koutroubakis, MD, Department of Gastroenterology, University Hospital Heraklion, P.O. Box 1352, 71110 Heraklion, Crete, Greece; ktjohn@her.forthnet.gr.

THROMBOSIS AND HEPATOCELLULAR CARCINOMA

TABLE 1. CLINICAL CHARACTERISTICS OF PATIENTS INCLUDED IN THE STUDY

Parameter	HCC patients	Cirrhotic patients	Healthy controls
Number	31	30	48
Median age (range), years	64 (46–85)	57 (35–81)	59 (36–77)
Sex (M/F)	24/6	23/7	29/19
Etiology			
Viral	18	15	—
Alcohol	7	9	
Other	6	6	
Okuda stage			
I	13 (43.3%)	—	—
II	14 (46.7%)		
III	3 (10%)		

PATIENTS AND METHODS

We studied 31 newly consecutively diagnosed patients with HCC between January 2000 and December 2002. The diagnosis was made with either liver biopsy to a suspected mass or a pathognomonic increase in α -fetoprotein (>400 ng/ml) in a patient with a compatible liver imaging on contrast-enhanced spiral CT or MRI. For the above period monitoring of every thrombotic event was registered. The spectrum was variable, from deep vein thrombosis of lower extremities to portal vein thrombosis or pulmonary embolism. Diagnosis was through clinical examination and radiological confirmation. Cases with apparent portal vein invasion by the tumor (two cases) were excluded. The clinical characteristics of HCC patients are presented in Table 1. PVT was confirmed by either Doppler ultrasound, CT, or MRI (with MR angiography). The HCC group was matched as to gender, age, and origin with two control groups. The first one consisted of 30 consecutive cirrhotic patients, not different from the HCC group in etiology of liver disease or Child–Pugh's score. A Doppler ultrasound was also performed in all these patients and no case with PVT was found. In the second group 48 healthy volunteers recruited from personnel of the clinic and visitors of the hospital wards were studied. All patients gave informed consent for their participation in the study and the protocol was approved by the Ethics Committee of the Medical Faculty of Crete.

Laboratory Studies. Fasting venous blood was collected from all patients and controls, and plasma, serum, and EDTA-blood from each patient were stored at -80°C until assay.

Protein C was determined by ELISA according to the manufacturer's instructions (Biochem Diagnostics, France; normal range, 65–140%). Protein S (total and free) was also assayed by ELISA (Biochem Diagnostics; normal range of free protein

S, 70–140%). Antithrombin (AT) activity was measured using a chromogenic substrate kit (Organon Technica, USA; normal range, 85–111%). APCR was measured by determining the activated partial thromboplastin time (APTT) in the absence and presence of APC (Coatest activated protein C resistance kit; Chromogenix, Sweden). Normal values of APCR were 1.8–5 sec.

The serum concentrations of lipoprotein (a) were measured by rate nephelometry (LPA test; Beckman Instruments Inc., Galway, Ireland). The measurement of serum total homocysteine (tHcy) was done by the IMX homocysteine assay (Abbot Laboratories).

Genomic DNA isolation from EDTA blood, polymerase chain reaction, and detection of FVL mutation, prothrombin 20210A mutation, and C677T methylenetetrahydrofolate reductase (MTHFR) polymorphism were done using Factor V Gene Mutation Assay (VIENNA LAB; Austria), Prothrombin Gene Mutation Assay (VIENNA LAB), and MTHFR Mutation Assay (VIENNA LAB). FVL mutation was assayed in patients and controls with abnormal APCR, whereas the other two mutations were assayed in all cases.

Statistical Analysis. Comparisons among the three diagnostic groups in terms of continuous measurements were made by analysis of variance (one-way ANOVA). Post hoc multiple-comparison tests were made by least squares difference (LSD) and Newman–Keuls test. The difference in prevalence of acquired and genetic thrombophilic defects between HCC patients and controls was studied by means of 2×2 table analysis using Fisher's exact test. A 5% significance threshold was adopted. To show the strength of the associations, we have included *P* values, odds ratios (ORs), and 95% confidence intervals in the text.

RESULTS

Table 2 shows the serum and plasma levels of the examined thrombophilic parameters in the three groups. The plasma levels of protein C, protein S, and antithrombin were significantly lower in both HCC and cirrhotic patients compared to healthy controls ($P < 0.0001$). The serum levels of lipoprotein (a) were also significantly lower in HCC and cirrhotic patients compared to healthy controls ($P = 0.0001$). Mean serum homocysteine levels were significantly higher in patients with HCC compared to cirrhotic patients and healthy controls ($P = 0.0001$). The above-mentioned statistical differences were confirmed by multiple-comparison post hoc test (LSD). The

TABLE 2. PLASMA AND SERUM THROMBOPHILIC PARAMETERS IN HCC PATIENTS COMPARED TO CIRRHOTICS AND HEALTHY CONTROLS

Parameter	HCC	Cirrhosis	Healthy controls
Protein C (%; mean \pm SD)	63.5 \pm 25.2	41.9 \pm 21.6	108.5 \pm 36.1
Protein S (%; mean \pm SD)	69.5 \pm 28.7	72.6 \pm 19.2	95.7 \pm 29.3
Antithrombin (%; mean \pm SD)	62.2 \pm 18.6	40.8 \pm 19.0	98.0 \pm 18.4
Lipoprotein (a) (mg/dl; mean \pm SD)	12.8 \pm 12.2	8.1 \pm 7.1	22.7 \pm 21.3
Homocysteine (μM ; mean \pm SD)	19.5 \pm 10.5	13.1 \pm 7.8	9.7 \pm 3.5
APCR (N; %)	3 (10.3)	2 (7.1)	1 (2.1)

TABLE 3. GENETIC THROMBOPHILIC PARAMETERS IN HCC PATIENTS COMPARED TO CIRRHOTICS AND HEALTHY CONTROLS

Parameter	HCC	Cirrhosis	Healthy controls
FVL mutation (N; %)	1 (3.4)	1 (3.6)	1 (2.1)
Prothrombin 20210A mutation (N; %)	3 (10.3)	1 (3.6)	0 (0)
C677T MTHFR (N; %)	6 (19.4)	4 (13.3)	6 (12.5)

prevalence of APCR was not significantly different among the three groups.

Table 3 shows the prevalence of prothrombotic genetic risk factors in patients with HCC, patients with cirrhosis, and healthy controls. The prevalence of FVL, PT G20210GA, and C677T MTHFR was not significantly different among the three groups. However, it is notable that the prothrombin 20210A mutation was present (heterozygous) in three HCC cases compared to one cirrhotic and none of the healthy controls.

Concerning the C677T MTHFR polymorphism and its association with homocysteine levels, carriage of the C677T MTHFR polymorphism was associated with hyperhomocysteinemia. Homocysteine levels in HCC patients with the C677T MTHFR polymorphism were higher, although just failing to reach statistical significance, compared with those in patients without this polymorphism ($P = 0.09$).

Among the six cases with thromboembolic manifestations, in four patients (66%) at least one thrombotic risk factor was found. Table 4 shows the characteristics and the identified thrombotic risk factors in patients with thromboembolic manifestations. Three of four cases with thrombosis had a combination of thrombotic risk factors.

DISCUSSION

Our study showed that thrombophilic defects are common in patients with HCC and they could interfere in the observed thromboembolic complications. HCC patients with thrombotic manifestations usually present with a combination of thrombotic risk factors.

Prothrombotic tendency in malignancies has been suggested to be related to the ability of tumor cells to produce

and secrete procoagulant/fibrinolytic substances and inflammatory cytokines, as well as the physical interaction between tumor and vascular cells. Moreover, other factors, like inflammation, abnormal protein metabolism, and anticancer therapy, may significantly increase the risk of thrombosis (2). Apart from these factors, activation of pathways of coagulation/fibrinolysis in malignancies has been suggested (24).

Although thromboembolism, mainly PVT, is a frequent complication of HCC, the mechanisms of thrombosis have not been investigated in depth in HCC patients. A case of a child with HCC and PVT in association with FVL mutation has been reported (23), but as far we know no study on the prevalence of FVL in HCC has been published. Increased levels of plasma thrombomodulin in HCC have been reported (25). On the other hand, two studies on lipoprotein (a) in HCC have shown conflicting results (26,27).

Recent developments in the definition of genetic and acquired risk factors of thrombosis have revolutionized the way in which the etiology of venous thrombosis is viewed. Studies in patients with PVT have shown that single or combined deficiencies of natural anticoagulant proteins are a common finding, suggesting that the majority of deficiencies are acquired, presumably as a consequence of PVT and not because of a hereditary genetic defect (17–22). A minority of cases of PVT may have a true underlying hereditary anticoagulant protein deficiency, and this can only be confirmed by careful investigation of family members (18).

We found decreased levels of protein C, protein S, antithrombin, and lipoprotein (a) in HCC and cirrhotic patients compared with healthy controls. However, these proteins are produced by the liver and it is expected that their levels may decrease in patients with chronic liver disease. On the other hand, we found significantly higher levels of homocysteine in HCC patients compared to cirrhotic and healthy controls. Hyperhomocysteinemia, as shown by several studies, is an established thrombotic risk factor in the general population (28). This relationship, however, has not been consistently corroborated by studies of patients with genetic polymorphisms that alter homocysteine metabolism.

TABLE 4. CHARACTERISTICS AND RISK FACTORS FOR THROMBOSIS IN HCC PATIENTS WITH THROMBOEMBOLIC MANIFESTATIONS

No.	Sex	Age	Thromboembolic manifestation	Thrombotic risk factors
1	F	63	Pulmonary embolism	Increased lipoprotein (a), hyperhomocysteinemia
2	M	76	Portal vein thrombosis	—
3	M	68	Portal vein thrombosis	Heterozygous prothrombin 20210A
4	M	57	Portal vein thrombosis	Hyperhomocysteinemia heterozygous FVL, homozygous C677T MTHFR
5	M	81	Deep vein thrombosis	—
6	M	72	Portal vein thrombosis	Heterozygous prothrombin 20210A

Concerning genetic thrombotic risk factors, in our study no significant differences among the three groups were established. A trend, however, of an association between HCC and prothrombin 20210A mutation was noted. This mutation has recently been recognized as a risk factor for a selected group of patients with idiopathic PVT (21). On the other hand, carriage of the C677T MTHFR polymorphism, although higher in HCC patients, was not significantly different compared to the two groups of controls. We could therefore suggest that the etiology of hyperhomocysteinemia in HCC patients is multifactorial, attributable not only to genetic factors but also to nutritional or other factors.

It should also be stressed that apart from HCC, thrombotic risk factors are frequently detected in patients with chronic viral hepatitis, while their presence has recently been associated with more advanced fibrosis (28).

In conclusion, thrombophilic defects are common in patients with HCC and they could interfere in the observed thrombotic complications. The most important thrombotic risk factor in our HCC patients was hyperhomocysteinemia. Questions raised by these findings should address the possible benefits of extensive thrombophilic screening for HCC patients.

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