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CASE REPORT

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Hepatocellular carcinoma in hepatitis-negative patients with thalassemia intermedia: a closer look at the role of siderosis

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ABSTRACT

Patients with thalassemia are often exposed to several risk factors for developing hepatocellular carcinoma (HCC) due to their repeated transfusions. However, even transfusion-independent patients with thalassemia intermedia (TI) can develop HCC, which is mainly attributed to a state of iron overload. We report here two cases and review the literature for the association between TI and HCC. Along with our cases, a total of 36 cases of HCC in thalassemic patients were reported in the literature. Of these, 22 (61%) were TI patients with 6 (27%) of them being hepatitis B and C negative. There was no consistency in their characteristics; therefore, we recommended screening thresholds for HCC in TI patients based on their total liver iron concentration (LIC).

Key words. Thalassemia intermedia. Hepatocellular carcinoma. Liver iron concentration. Iron overload. Risk factor.

INTRODUCTION

The thalassemias are a group of rare inherited disorders in the synthesis of hemoglobin where the alpha and beta chains are not expressed in stoichiometric proportions; alpha-thalassemia being a defect in the synthesis of the alpha chain, and beta-thalassemia in the beta chain. They can be divided according to clinical severity into thalassemia major, intermedia, and minor. Thalassemia major (TM) is characterized by severe anemia that usually manifests during the first year of life and requires lifelong transfusions and iron chelation therapy. Thalassemia minor is characterized by mild anemia that is asymptomatic. Thalassemia intermedia (TI) falls in between and permits survival without transfusion therapy.[1]

The pathophysiology behind the complications seen in TI is the result of three factors: ineffective erythropoiesis, iron overload, and hemolytic anemia.[1] The combination of ineffective erythropoiesis and chronic anemia lead to hepcidin suppression, increased iron absorption from the gut, and increased release of recycled iron from the reticuloendothelial system. This results in depletion of macrophage iron, relatively low levels of serum ferritin, and preferential portal and hepatocyte iron storage. This, in turn, leads to considerable hepatic iron overload and release of toxic iron species like non-transferrin-bound iron (NTBI) into the circulation.[2-5] With improved treatment techniques and iron chelation strategies, these patients are surviving longer, allowing the manifestations of iron overload to surface.

Iron overload is a known risk factor for developing hepatocellular carcinoma (HCC). The relationship between iron and HCC has been well studied in the case of hereditary hemochromatosis (HH) and African iron overload,[6] but few articles have dealt with HCC arising from iron overload in the thalassemias in general and TI in particular. We herein present two cases of HCC arising in hepatitis C (HCV) negative, non-cirrhotic, TI patients.

CASE 1

We report here the case of a 54-year old Middle Eastern Caucasian male diagnosed with TI at a young age [heterozygous IVS-I-6 (T → C)/β cod 44 (-C),

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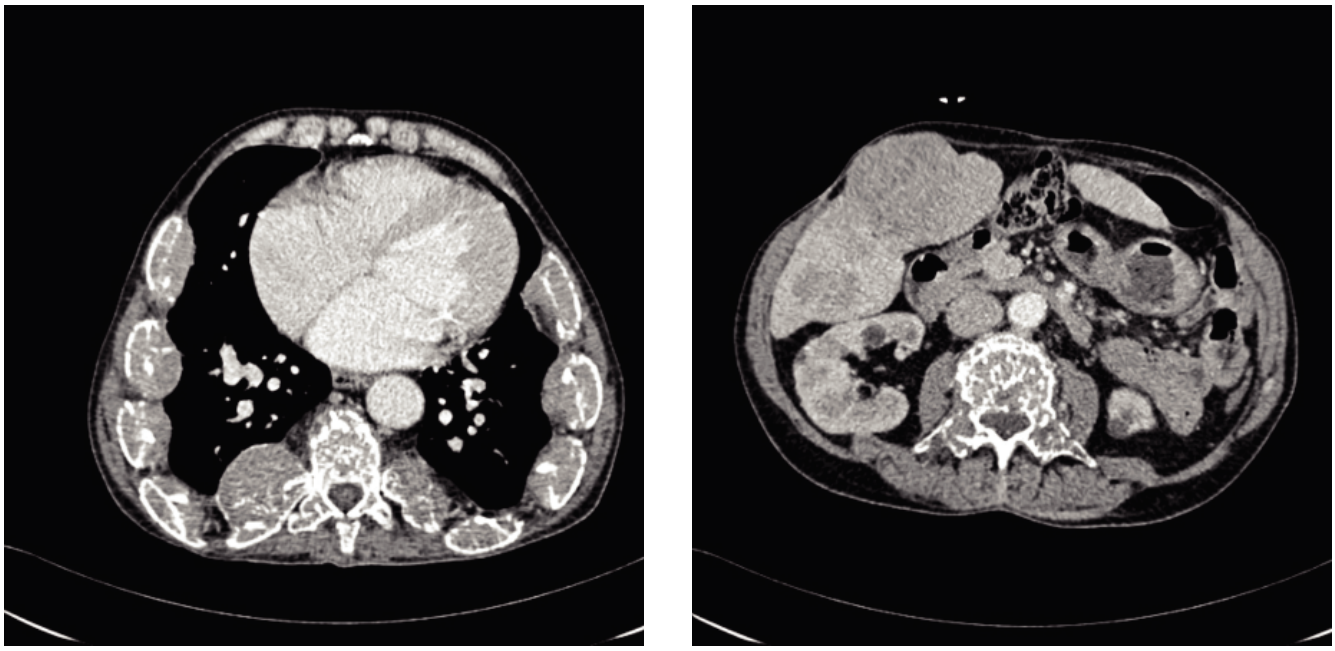

Figure 1. A. *Contrast enhanced CT of the abdomen shows severe bone marrow expansion with destruction of the overlying cortex. In addition there are paraspinal soft tissue densities compatible with extramedullary hematopoiesis.* ***B.*** *Contrast enhanced CT shows large pedunculated heterogeneous mass showing faint enhancement. Numerous other smaller lesions were seen in the liver consistent with EMH. The lesions were expanding in parallel with the bony deformities.*

no α deletion]. He underwent splenectomy for splenomegaly in childhood and received iron chelation therapy for a short period of time with desferrioxamine during his third decade. He has received sporadic transfusions, namely during the splenectomy surgery and once during an infectious episode. In 2001, he developed a massive pleural effusion on the right, and four months later, on the left, which proved to be the result of paraspinal extramedullary hematopoiesis (EMH). He was managed with sclerotherapy. The patient also had an episode of spontaneous superficial vein thrombosis. He had been maintained on hydroxyurea therapy with his hemoglobin stable between 6 and 7 g/dL. He presented to our clinic for regular follow-up in 2007 with a serum ferritin of 2,490 ng/mL. Two years later, he was started on oral iron chelation therapy with deferasirox and serum ferritin subsequently dropped to levels near 1,000 ng/mL. After one year of chelation, LIC by R2 magentic resonance imaging (MRI) was 12.3 mg Fe/g dry weight [normal is less than 7].

The patient presented to our attention with persistent right upper quadrant pain of 2 weeks duration. Previous imaging with ultrasound and computed tomography (CT) had shown three foci of EMH in his liver that were expanding in parallel with medullary tissue in the surrounding bones. Ultrasound and, later, a CT scan were done (Figure 1). Liver function tests were within normal range. His ferritin level was 1,100 ng/mL. A CT-guided biopsy showed the lesions to be compatible with multifocal HCC. There was no radiological evidence of cirrhosis. Hepatitis B and C testing were negative by antigen detection and RNA polymerase chain reaction (PCR), respectively. The patient received palliative treatment. He developed hepatorenal syndrome and rapidly succumbed to his illness.

CASE 2

A 55-year-old male diagnosed with TI (β° cod 6/β^{+} - 87) at a young age was maintained on regular transfusions until he underwent splenectomy at the age of 13 years and was able to maintain an adequate hemoglobin level. He was not compliant with iron chelation therapy; instead, he was treated with micro-phlebotomies. He was heterozygous for the hemochromatosis gene (Cys282 > Tyr: wt/wt; His63 > Asp: wt/MUT). In 2007, a routine screening ultrasound showed a suspicious lesion that was investigated with CT and then biopsied. It turned out to be a well differentiated, trabecular type HCC. The biopsy also showed widespread siderosis, but no evidence of cirrhosis. He was treated by percutaneous

radioablation and achieved complete response with absence of tumor areas. The patient tested negative for hepatitis B and C by antibody detection and RNA-PCR, respectively. His LIC at the time was 23.9 mg Fe/g dry weight by T2* liver MRI, so he was started on intravenous and subcutaneous desferrioxamine at a dose of 2.5 g. A CT scan one year later was suspicious for cancer recurrence, and so the patient underwent lobectomy. Later CT scans showed additional liver lesions and chest lymphadenopathy. One year later, his alpha-fetoprotein was 3.8 IU/mL. He was treated with palliative care. He slowly deteriorated until he passed away one year later because of hepatic failure.

DISCUSSION

HCC occurring in conjunction with thalassemia is not a rare complication. On the contrary, thalassemia and other transfusion-dependent anemias are frequently complicated by HCC secondary to infection with the hepatitis viruses and iron overload. HCC arising on a non-viral, non-cirrhotic liver is rare[6-8] and we report it here to attest to the severity of iron overload and its complications in TI patients specifically.

The role of iron in the pathogenesis of cancer has been well documented in HH, where some studies have shown a 200-fold increase in the risk for HCC.[9] The mechanism of iron overload in HH is similar to that in TI. Patients with HH have increased intestinal iron absorption[10] closely resembling TI patients. Hepcidin dysregulation plays a role in both. In HH, it is a genetic defect in one of the elements that governs the control of hepcidin;[11] while in TI, anemia, hypoxia, and ineffective erythropoiesis suppress the expression of hepcidin. In fact, the ineffective erythropoiesis is an unrelenting signal to downregulate hepcidin by increasing expression of growth differentiation factor 15 (GDF15) and hypoxia-inducible transcription factors (HIFs).[5] The end result in both is increased intestinal iron absorption and parenchymal iron overload. Other iron loading diseases, such as African iron overload, are also characterized by an increased risk for HCC, suggesting an independent carcinogenic role for iron overload.[6] The association of iron overload with HCC has also been reported in a transfusion-dependent patient with myelodysplastic syndrome.[12] The patient also had no evidence of cirrhosis or viral hepatitis.[12]

Iron has been epidemiologically related to carcinogenesis in several papers.[13,14] Moyo, *et al.* reported

Table 1. Characteristics of the 6 thalassemia intermedia patients who developed HCC in non-viral, non-cirrhotic livers.

Patient number	Sex	Age at diagnosis	Length of survival	HCV Ab	HCV RNA	HBV Ab	HBs Ag	AFP (kU/L)	Serum ferritin (µg/L)	Ferritin peak (µg/L)	LIC
1[18]	M	48	Alive at 26 months	-	-	-	-	2851	1520	5250	NA
2[18]	F	61	5 months	+	-	-	-	132	369	6000	NA
3[20]	F	59	25 months	+	-	NA	NA	NA	990	NA	NA
4[20]	M	73	7 months	-	-	NA	NA	NA	574	NA	NA
5	M	54	1 month	-	-	-	-	17.8	1291	2490	12.3
6	M	51	4 years	-	-	Vaccinated	-	3.8	5602	7138	23.9
	Average	57.67	19 months				751	1724	5220	18.1	
	SD	8.94	18 months				1401	1948	1978	8.2	

HCV: hepatitis C virus. HBV: hepatitis B virus. AFP: alpha-fetoprotein. LIC: liver iron concentration. NA: not available.

on 36 patients with HCC. After adjusting for age, sex, and the presence of cirrhosis or portal fibrosis, the risk of developing HCC was 3.1 (95% CI, 1.05-9.4) among patients with iron overload compared to those without.[15] Turlin, *et al*. assessed liver iron in patients with HCC in the absence of cirrhosis and found that patients with HCC were much more likely to have measurable and stainable iron in the liver compared to controls.[16] Animal and human *in vitro* studies have also pointed out the carcinogenic role of excess iron as a possible mechanism for hepatic carcinogenesis[17] with both direct and indirect effects. The direct effects include DNA damage by non-transferrin-bound iron resulting in inactivation of tumor-suppressor genes, such as p53, or their products. Indirect effects include the formation of reactive oxygen species, iron-induced lipid peroxidation, acceleration of fibrogenesis, and in addition, inducing immunological abnormalities that may be associated with decreased immune surveillance.[6]

HCC in thalassemia has been studied in a few papers, but never strictly in TI. We present a summary of the literature findings below. In a cross-sectional study by Borgna-Pignatti, *et al*. examining the prevalence of HCC in hemoglobinopathies, 12 out of 23 patients (52%) with HCC had TI, while only 8/23 (35%) had TM. Two of these patients were HCV negative.[18] In another prospective study by Mancuso, *et al.*, 2/2 (100%) patients who developed HCC during one year of follow up were affected with TI.[19] Restivo Pantalone, *et al*. looked at HCC in thalassemia with 6/9 (66%) HCC cases affected with TI. Altogether, with our two patients, 22 out of 36 (61%) thalassemia patients with HCC were affected with TI, 6 of which were HCV negative by RNA PCR.[20] Table 1 outlines the available characteristics of these 6 patients. These numbers, though scanty, instigated a thought that TI patients might be at a higher risk for developing HCC than TM patients. We believe that this might be due to the fact that TI patients accumulate iron, but unlike TM patients, this iron often goes unnoticed and unchelated. Chelation therapy and monitoring of iron overload is an overlooked issue in this patient population. Serum ferritin has been shown to underestimate the true iron burden in TI patients. In comparison with TM, for the same LIC value, serum ferritin was significantly lower in TI.[3] This suggests that we should have a lower threshold for chelating TI patients. In our patients, we were considering a ferritin of 1,000 ng/mL as being well chelated. It was not until we obtained a LIC by MRI that we realized our chelation therapy has been unsatisfactory. TI patients also survive longer than TM patients, so complications of cirrhosis and/or iron overload have enough time to develop. A third reason may be the fact that TI patients are diagnosed later than TM patients and consequently started on transfusion therapy later. This will increase their chance of developing chronic hepatitis leading to end-stage liver disease if infected with HCV, as the age of infection is related to the risk of developing chronic infection.[18] Further studies are needed to confirm the hypothesis that TI patients are truly at an increased risk of HCC and to dwell on the pathophysiology.

In addition to iron overload, the risk of developing HCC in TI depends on the presence or absence of cirrhosis and HCV status. There are no studies that address this issue specifically. One study looked at the annual risk of developing HCC in a thalassemic population of 105 patients. Out of these, 72 were found to have risk factors for the development of HCC, and 2 out of these 72 developed HCC in the one year follow-up giving an incidence rate of around 2%.[19] Interestingly, one of the patients was 39 years old.

In light of all these studies, we suggest thalassemia patients be screened for HCC and that screening be started early. The methods used for screening for HCC in the aforementioned studies were alpha-fetoprotein (aFP) and an abdominal ultrasound every six months. The American Association for the Study of Liver Diseases recommends using abdominal ultrasound for surveillance of patients with cirrhosis without aFP because aFP lacks adequate sensitivity and specificity.[21] Table 1 suggests that aFP might not be sensitive in our population too; moreover, we can see that none of the parameters was consistently elevated or depressed. We recommend patients undergo yearly LIC measures using non-invasive R2* MRI and surveying for HCC using an abdominal ultrasound every 6 months in at-risk patients. Since an LIC of 7 mg Fe/g dry weight has been associated with vascular complications and an LIC of 6 mg Fe/g dry weight was associated with endocrine morbidities,[22] we suggest a cut-off of 5 mg Fe/g dry weight to start screening patients for HCC. If LIC is not available, ferritin should be closely monitored so as to obtain a trend. With concomitant HCV infection, ferritin in TI should be titrated to a value of 300 ng/mL. Otherwise, a value of 500 ng/mL is acceptable. An attempt should be made to eradicate an existing HCV infection despite the fact that there is no clear-cut evidence for its benefit in preventing HCC in the setting of TI. We make this recommendation since it is reasonable to assume that the

benefit will be similar to the general population. The use of Ribavirin in TI is still controversial but we recommend close monitoring of blood counts during its use.

Treatment of HCC in TI should be a multidisciplinary approach. Successful treatment options have included percutaneous radio frequency thermoablation and ethanol injection, surgical resection, and chemoembolization.[8] TI should not be considered an absolute contraindication to liver transplant, especially with the marked improvement in medical care. Instead, patients should be selected and offered this treatment modality. In fact, there are two reports on two TM patients who had good outcomes after liver transplant for HCC.[8]

In conclusion, HCC is a serious yet overlooked complication in patients with TI. With proper screening and high index of suspicion, it can be detected early and treated with minimal complications. More studies are needed to properly assess the risk of HCC in TI and validate the cost-effectiveness of screening algorithms.

CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

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