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Case study

Sequential development of hepatocellular carcinoma and liver angiosarcoma in a vinyl chloride–exposed worker☆

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Summary Strong experimental and clinical evidences have definitely linked occupational vinyl chloride exposure to development of angiosarcoma of the liver. In contrast, despite the International Agency for Research on Cancer having included vinyl chloride among the causes of hepatocellular carcinoma, the association between vinyl chloride exposure and hepatocellular carcinoma remains debated. This issue is relevant, because occupational exposure to high levels of vinyl chloride may still occur. We report a unique case of sequential occurrences of hepatocellular carcinoma and angiosarcoma of the liver, in a vinyl chloride–exposed worker without cirrhosis and any known risk factor for chronic liver disease. Both the hepatocellular carcinoma and the surrounding normal liver showed micronucleus formation, which reflects genotoxic effect of vinyl chloride. Angiosarcoma showed a *KRAS* G12D point mutation, which is considered to be characteristic of vinyl chloride–induced angiosarcoma. This case supports the pathogenic role of vinyl chloride in both hepatocellular carcinoma and angiosarcoma development.

1. Introduction

Liver angiosarcoma (ASL) is a rare neoplasia arising from the malignant transformation of endothelial cells, whose most relevant known risk factor is occupational exposure to vinyl chloride (VC). Hepatocellular carcinoma (HCC) ranks among the 5 most common cancers worldwide, and it is associated with defined risk factors, including hepatitis virus infections, alcohol consumption, aflatoxin exposure, and nonalcoholic steatohepatitis. HCC evolves from cirrhosis in most cases, particularly in Western countries. Despite the International Agency for Research on Cancer having included VC among the causes of HCC [1], the association between VC exposure and HCC remains controversial [2,3]. The issue is relevant because occupational exposure to high levels of VC may still occur. Furthermore, VC has been identified as a degradation product of chlorinated solvents, being present in landfill leachate and thus constituting a potential environmental carcinogen

Abbreviations ASL, liver angiosarcoma; VC, vinyl chloride; HCC, hepatocellular carcinoma; MN, micronucleus; TASH, toxicant-associated steatohepatitis.

☆ Disclosures: None.

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[4,5]. Mechanisms of VC-related tumorigenesis are not fully elucidated. VC has been shown to be mutagenic and genotoxic, and indeed, VC exposure induces DNA strand breaks, sister chromatid exchanges, micronucleus (MN) formation, and other chromosomal aberrations [6]. Characteristic VC-induced *KRAS* point mutations have been demonstrated at codon 13 and, less frequently, at codon 12: both of them cause the substitution of glycine by aspartic acid in the resulting p21 protein [2,7].

We report a case of HCC and ASL sequentially occurring in a noncirrhotic liver, in a VC-exposed worker, without any known risk factor for HCC.

2. Case report

The patient was a 78-year-old man, who had been exposed to VC for 6 years, from 1961 to 1966, while he was an autoclave worker (cumulative exposure: 4100 ppm × years, and peak exposure: 750 ppm/year). He had never been under surveillance for VC occupational exposure. In 2009, he presented a 4-cm nodular lesion in the hepatic segment VII, diagnosed as HCC by fine-needle aspiration biopsy. At the time of the diagnosis, liver function tests, serum cholesterol, and triglyceride levels were within the normal ranges. Serologic markers of hepatitis B virus infection (including HBsAg/anti-HBsAb, HBeAg/anti-HBeAb, anti-HBcAb) were absent. Serologic tests for hepatitis C virus as well as for human immunodeficiency virus were also negative. Laboratory data did not show any evidence of autoimmune or metabolic diseases, Wilson's disease, or hemochromatosis. There was no history of alcohol abuse, and the patient was not taking any drug. He had a body mass index of 25.8, and he was not diabetic. α-Fetoprotein level was within the normal limit. In the absence of associated hepatic lesions, the patient underwent curative segmentectomy. Pathological examination showed an Edmondson's grade 3 HCC (Fig. 1A), without microvascular invasion, and with tumor-free resection margins. The background liver was not cirrhotic and did not display portal or lobular inflammation, or fibrous septa. In the central lobular zones, there were mild macrovescicular steatosis and sinusoidal fibrosis (Fig. 1B-C), a picture resembling toxicant-associated steatohepatitis (TASH) occurring in non-obese, non-drinking workers with high VC exposure [5]. To test genotoxic damage, MN formation was investigated in tumor and nontumor liver tissue, by using Feulgen stain for DNA, as previously described [8]. A rate of 5 and 9 micronucleated cells × 1000 hepatocytes was found in normal and tumor tissue, respectively (Fig. 1D). In 2012, a new liver nodule developed. It was interpreted as relapsing HCC and treated with multiple transarterial chemoembolization.

The patient died in 2014, in a general condition of neoplastic cachexia, for hepatic and renal failure during hospitalization. To evaluate the casual relationship between the neoplasia and the occupational exposure to VC, autopsy was required by the public prosecutor, who has authorized the report of this case. At autopsy, a 4-cm nodular area of hemorrhage and necrosis was detected. The surrounding liver was not cirrhotic and showed congestion. Histologic examination revealed that the hemorrhagic nodular lesion was an ASL (Fig. 2A). Histologic diagnosis was confirmed by immunostains, which demonstrated the absence of cytokeratin expression (MNF116 clone; Dako, Glostrup, Denmark; working dilution 1:200) and a strong positivity for CD31 (JC70A clone; Dako; working dilution 1:20) and CD34 (QBEnd/10 clone; Thermo Scientific, Waltham, MA; working dilution 1:100) (Fig. 2B). The surrounding liver tissue was diffusely congested and showed minimal macrovescicular steatosis.

DNA was extracted from both HCC and ASL, as well as from surrounding non-neoplastic liver tissue, after enrichment of neoplastic cellularity using manual microdissection. Exons 2, 3, and 4 of the *KRAS* gene were investigated by Sanger sequencing (primers upon request). A characteristic VC-related G12D point mutation was detected in ASL, but not in HCC nor in the surrounding normal liver tissue.

For the aim of prosecution, patient's charts were carefully reviewed, confirming the absence of any previous or concomitant hepatic or extrahepatic disease. The public prosecutor acknowledged the occupational origin of the liver neoplasms, as suggested by the forensic pathologist.

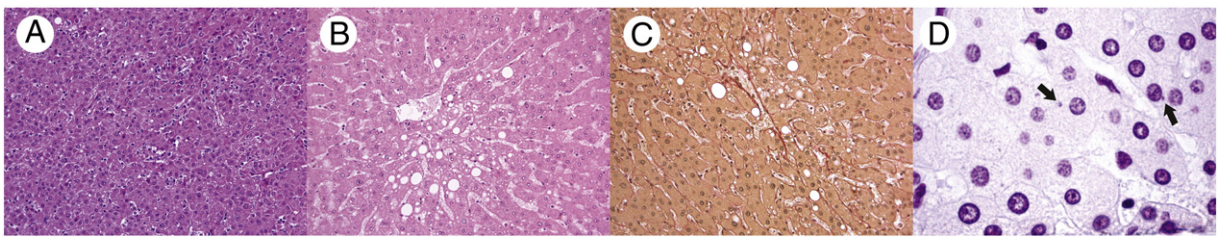

Fig. 1 A, Hepatocellular carcinoma (hematoxylin-eosin stain, original magnification ×40). B, Surrounding liver tissue showing mild steatosis in central lobular zones (hematoxylin-eosin stain, original magnification ×40). C, Mild sinusoidal fibrosis was evident in central lobular zones of surrounding liver tissue (Van Gieson stain, original magnification ×40). D, Micronuclei (arrows) in surrounding non-neoplastic liver tissue (Feulgen stain, original magnification ×60).