A 74-year-old woman with a past medical history of diabetes, hypertension, and alcohol abuse was brought to the emergency department and subsequently admitted to the intensive care unit with an altered mental status and weakness. Laboratories revealed acute renal failure (BUN 15 mg/dL, creatinine 2.5 mg/dL), elevated serum transaminase (AST of 83 IU/L), hyperammonemia (187 ug/dL), and marked normocytic anemia requiring transfusion of three units of packed red cells (hemoglobin 4.3 g/dL; hematocrit 13.1%). Blood ethanol level at the time of admission was <5 mg/dL, and full urine toxicology was negative. Alcohol abuse was reported to consist of, on average, “one pint of gin per day.” Her hospital course was nine days and included complete inotropic blood pressure support and intubation. On the ninth day, she was declared dead, and authorization for an unrestricted autopsy was granted by the coroner. At autopsy, two liters of serous ascitic fluid was drained from the peritoneal cavity, and non-ruptured, distended varices were identified at the gastroesophageal junction. Additional findings included changes compatible with hypertensive cardiovascular disease, including hypertrophy of the interventricular cardiac septum and glomerulosclerosis along with renal atrophy. The liver weighed 1,300 grams (normal 1,475 gm ± 362) and was markedly discolored yellow-tan. Its parenchyma was more firm than usual. Representative microscopic sections from the decedent’s liver are shown in the image below.

What are the pink inclusions seen within the hepatocytes’ cytoplasm, and with what liver diseases are they associated?

Figure 1: (A - left) Liver cells showing eosinophilic twisted rope appearing Mallory-Denk bodies in the hepatocyte cytoplasm, hovering around the cell’s nucleus (hematoxylin-eosin, original magnification x40). (B - right) Immunohistochemistry with an anti-pan cytokeratin antibody showing strong staining of Mallory-Denk bodies (original magnification x40).
**DISCUSSION**

Mallory-Denk bodies (MDBs) are eosinophilic cytoplasmic inclusions in liver cells that are characteristic of alcoholic liver disease. They are composed primarily of cytokeratin intermediate filaments (IF) complexed with other proteins such as ubiquitin and p62.1,2 Other widely recognized IF inclusion body diseases include Alzheimer’s (the neurofibrillary tangle) and Parkinson (Lewy bodies) disease. MDBs, however, are the most prevalent IF-related aggregates.3 IF cytokeratins function to maintain structural polarity and provide an intracellular scaffold that helps resist forces applied to the cell. In certain disease states, liver cytokeratin production can be altered and leads to cytokeratin misfolding. Such misfolding causes aberrant aggregation and accumulation of the proteins within the hepatocyte cytoplasm, such that they are rendered microscopically visible as MDBs.4,5

With routine hematoxylin and eosin (H&E) staining, MDBs take on an eosinophilic, twisted-rope-appearance. Though in the hepatocyte cytoplasm, they tend to hover around the cell’s nucleus. In the case presented here, they were widely distributed and diffusely present throughout all hepatic zones; a representative image from autopsy demonstrates several liver cells with prominent ropey inclusions in a perinuclear location (Figure 1a). By immunohistochemical staining with an anti-pan cytokeratin antibody, the MDBs are further highlighted (Figure 1b).

Intracytoplasmic, intrahepatic inclusions visible on H&E must also prompt the pathologist to consider the following: intracytoplasmic hyaline bodies (IHBs), ground glass inclusions, glycogen bodies, A1AT droplets, and megamitochondria. The size and morphology of the inclusion, the clinical context of the patient, and, on occasion, use of additional special staining modalities, usually makes the distinction between the various bodies a straightforward process.6 MDBs-like inclusions with similar morphology but different composite proteins have also rarely been seen in cells other than hepatocytes, such as type 2 pneumocytes and trophoblast cells. At the present time, it is currently unclear why hepatocytes, as contrasted with other cell types, are endowed with such a relative ability to accumulate inclusion bodies.6

The association of MDBs with alcoholic hepatitis was initially reported in 1911.7 Though most commonly attributed to an alcohol-related metabolic insult to the liver, MDBs can also be seen within the hepatocyte cytoplasm in Wilson disease, primary biliary cirrhosis, non-alcoholic steatohepatitis (NASH), alpha-1-antitrypsin (A1AT) deficiency, porphyria, and morbid obesity.8 By contrast, MDBs have not been seen in association with viral hepatitis, acute cholestasis, or with the majority of hepatotoxic injuries. As such, the prevailing thought is that MDBs signal chronic injury and are identifiable rather late in the course of disease after oxidative and other stresses have been incurred over some time.6

The usefulness for the pathologist of finding MDBs on liver biopsy related to discriminating between the diagnoses of steatosis (a.k.a. “fatty liver disease”) due to either alcohol or due to a cryptogenic cause, as would be the case in NASH, has attracted a good deal attention in the literature. The frequency of MDBs in steatosis is believed to be approximately 40% by routine H&E and up to 70% if immunohistochemical stains are utilized.9 Though the literature fails to reach a consensus on their frequency, specifically in NASH, with reports that range from 10-70% of cases, there does seem to be agreement that the MDBs in NASH are smaller in number, less well-developed in their appearance, and virtually absent in pediatric cases.10-12 As such, MDBs that are abundant and well-developed implicitly favor an alcohol-related etiology when seen in association with steatosis on histopathologic liver biopsy.

The clinical and prognostic significance of MDBs is yet to be clearly understood. Several animal models, including transgenic mice, and continued research efforts effectively continue to provide essential information about their pathogenesis and the cells that contain them. For example, such cells remain viable and capable of cellular division.13 Cells with MDBs are relatively more leukotactic and induce neutrophilic attraction to their surrounding tissue.14 And, finally, MDB accumulation appears reversible such that, in the mouse model, MDBs virtually disappear from injured hepatocytes during the recovery phases of experimentation.15

Alcohol-related diseases are the third most common preventable cause of death in the United States, accounting for approximately one death every seven minutes.16 The most important risk factor for the development of alcoholic liver disease is the total amount of alcohol consumed.5 According to the Dietary Guidelines for Americans, drinking in moderation is defined as no more than one drink per day for women and no more than two drinks per day for men where a standard drink contains 12 grams of alcohol.17 Comparisons of what constitutes a standard drink are shown in

<table>
<thead>
<tr>
<th>Type of drink</th>
<th>Amount in ounces</th>
<th>Alcohol content in grams</th>
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<tr>
<td>Wine</td>
<td>5</td>
<td>12</td>
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<tr>
<td>Beer</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Liquor (80 proof)</td>
<td>1.5</td>
<td>12</td>
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Table 1. And, for the risk of alcohol-related liver disease, recent studies have shown that the risk begins at 30 grams of ethanol per day. By report, the decedent in the current case ingested “a pint of gin” each day. With conversion, this translates into roughly 16 ounces of liquor of daily use or 10 times the amount that defines moderate drinking.

Diagnosing alcoholic liver disease can be a challenge for clinicians. Physical and laboratory findings are often nondiagnostic, especially in patients with early alcoholic liver disease. Patients commonly deny alcohol abuse and frequently underreport alcohol consumption. Therefore, clinicians must have a low threshold of suspicion for alcohol abuse and rely on indirect evidence such as information from family, questionnaires, or observation of physical, social, and psychological consequences of abuse. As clinicians may utilize liver biopsy information to help make clinicopathologic correlates about patients, it is important for them to consider whether or not MDBs were demonstrated along with steatosis, whether histologic staining was via the customary H&E or required use of immunohistochemical modalities, and whether there were an abundance of or a paucity of either vague or well-established forms of intracytoplasmic MDBs. By not only adding these descriptive features to the pathologist’s report, but also by increasing the clinician’s awareness of their meaning, the level of index of suspicion for whether chronic alcohol usage has served to play a role in the liver derangement being demonstrated may effectively be enhanced.

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REFERENCES


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