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Mallory-Denk bodies: Correlation with steatosis, severity, zonal distribution, and identification with ubiquitin

LIVER

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Original Article

ABSTRACT

Background/Aims: Intermediate filament proteins contain few aggregates as their main component. Among those, Mallory–Denk bodies (MDBs) are by far the best recognized component. To identify the presence of MDBs in individuals having chronic liver disease and to evaluate the correlation among MDBs and steatosis as well as the severity and zonal distribution of hepatocyte balloon degeneration. Tertiary reference hospital.

Materials and Methods: Three hundred consecutive liver specimens derived from our patients with chronic liver disease were included in the current study.

Immunohistochemistry analysis was conducted on frozen liver biopsies fixed at room temperature with acetone anti-rabbit antibody to ubiquitin. In addition, histological activity was evaluated by the routine staining of liver biopsy sections with hematoxylin–eosin and periodic staining by acid-Schiff stain, reticulin, Masson trichrome, and iron. The presence of MDBs, steatosis, severity, and the zonal distribution of hepatocyte balloon degeneration were evaluated in every patient.

Results: Histopathologic diagnosis were chronic hepatitis B (n=219), alcoholic steatohepatitis (n=23), non-alcoholic steatohepatitis (n=20), chronic hepatitis C (n=20), overlap syndrome (n=10), and primary biliary cirrhosis (n=8). The distribution of MDBs stained positive for ubiquitin was 80% in the overlap syndrome, 86% in chronic hepatitis B, and 100% in alcoholic steatohepatitis, NASH, chronic hepatitis C, and primary biliary cirrhosis. There was a correlation between the severity of steatosis and ubiquitin positivity, particularly in zone 2. A conspicuous correlation existed between the severity of hepatocyte balloon degeneration and ubiquitin positivity.

Conclusion: These findings have demonstrated that the observation of MDB together with ubiquitin positivity will be helpful in the evaluation of the models of diagnosis, staging, and therapy in patients with chronic liver disease.

Keywords: Mallory–Denk bodies, ubiquitin, steatosis

INTRODUCTION

Intracellular misfolded protein aggregates are the morphological identity for the quality of neurodegenerative system, musculoskeletal system, and some other failures, for instance, neurofibrillary coils (Alzheimer's disease), Lewy bodies (Parkinson disease), Negri bodies (rabies), or Mallory–Denk bodies (MDBs; several chronic liver diseases) (1). The proteins within intracellular (perinuclear-intracytoplasmic) deposits are misfolded via accumulated β-plaque construction, which are phosphorylated, ubiquitinylated, and relatively degraded (2). Multiple intracellular deposits include some of these misfolded proteins as their chief components. Particularly, MDBs are optimally recognized. MDBs are intracellular protein aggregates comprising misfolded keratins that have wronged various post- translational alters, such as phosphorylation and transamidation, which may cause conformational alterations and crosslinking (3). MDBs reflect liver intracellular protein inclusions occurring in various chronic liver diseases such as alcoholic steatohepatitis (ASH) and non-alcoholic steatohepatitis (NASH), hepatocellular neoplasms, metabolic disorders, and chronic cholestasis as well as discriminate between the relatively benign simple steatosis. Hepatocellular ballooning degeneration identifies the morphological alteration that indicates the degeneration of hepatocytes and continued enlargement, inflation, rolling, and a characteristic retiform cytoplasm (4). These alterations

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are connected to the significant accumulation of fat droplets associated with the dilatation of both ER and cytoskeleton (5). Ballooning degeneration is characteristically related with MDB formation, yet this appearance is noninterchangeable. Not all ballooned hepatocytes contain MDBs, whereas hepatocytes associated with MDBs are mostly ballooned.

Nevertheless, it is yet uncertain whether ballooning degeneration and MDB are epiphenomena of the disruption of the cell preservative procedure or a cellular reaction that contributes to either the initiation of or the improvement of chronic liver disease. Surprisingly, both hepatocytes associated with ballooning degeneration and MDBs are mostly amphibiously and able to portion, and almost all of these alterations are reversible (6). Indeed, ballooned hepatocytes may generally be misidentified as MDB by autophagy (7). The objective of the present study was to detect the presence of MDBs in various chronic liver diseases and to evaluate the correlation of MDBs with steatosis, severity, and the zonal distribution of hepatocyte balloon degeneration.

MATERIALS AND METHODS

This study was approved by the local ethical committee in our hospital. (Number: 287-2011) Three hundred consecutive liver specimens derived from our patients with chronic hepatitis were included in this study.

Statistical analyses were conducted using the SPSS software, version 20 (SPSS. Inc., Chicago, IL, USA). The variables were surveyed using visual (histograms and probability plots) and analytical methods (Kolmogorov– Smirnov/Shapiro-Wilk test) to identify whether they are ordinarily distributed. Descriptive analyses were presented (using tables of frequencies for the ordinal variables) using medians and interquartile range (IQR) for the non-normally distributed and ordinal variables. While investigating the associations between non-normally disturbed and/or ordinal variables, the correlation matrix of the coefficients and their importance were calculated by using the Spearman's test.

Immunohistochemistry

Immunohistochemistry analysis was conducted on frozen liver sections fixed for 10 min with acetone at room temperature using anti-rabbit antibody to ubiquitin (Dako, Glostrup, Denmark; 1:200). Endogenous peroxidase action was blocked by incubation of 1% H2O2 in methanol for 10 min. The sections were incubated with the primary antibody for 1 h at room temperature; then, this was followed by washing and incubation with biotinylated multi-linked swine anti-goat, -mouse, and -rabbit Ig (Dako, 1:100) as secondary antibodies and ultimately with the ABC complex using 3-amino-9-ethylcarbazole (AEC) as chromogen (Dako).

Histology

Frozen liver biopsy sections routinely stained with hematoxylin–eosin and periodically stained with acid-Schiff stain, reticulin, Masson trichrome, and iron were evaluated by a pathologist who was uninformed of the patients' detailed clinical data.

The presence of MDBs, steatosis, severity, and zonal distribution of hepatocyte balloon degeneration was evaluated in every patient. The results of a total of 300 liver specimens obtained from six patient groups are as follows: chronic hepatitis B (CHB; n=219), ASH (n=23), NASH (n=20), chronic viral hepatitis C (CHC; n=20), overlap syndrome (autoimmune hepatitis with PBC; n=10), and primary biliary cirrhosis (PBC) (n=8). The characteristics of MDB in each disease are shown in Figures 1-3. The distribution of MDBs stained positive for ubiquitin according to the diagnosis were as follows: 189/219 (86%) patients with Hepatitis B showed mild ubiquitin positivity in 65% and 35% of the cases usually in zone 1 and 2, respectively; 23/23 (100%) patients with steatohepatitis showed mild (20%), moderate (35%), and diffuse (45%) ubiquitin positivity frequently seen in zone 2 and 3; 20/20 (100%) patients with NASH showed mild and moderate degrees of ubiquitin positivity detected in zone

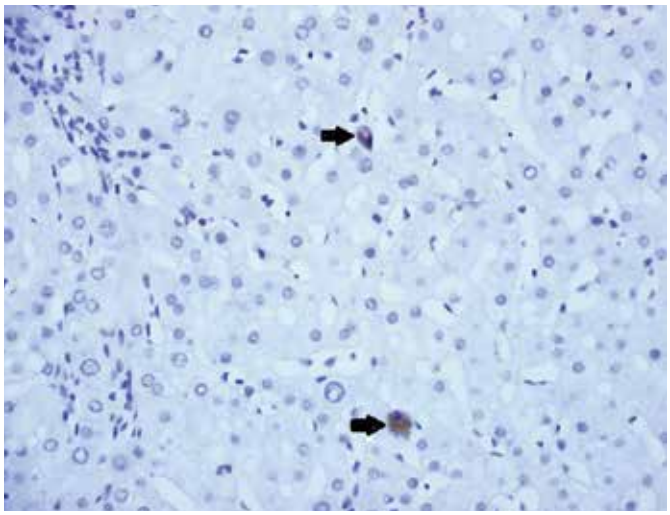

Figure 1. Ubiquitin painting perinuclear and intrastoplasmic positivity of MDBs keratin content as immunohistochemical (Ubiquitin × 400).

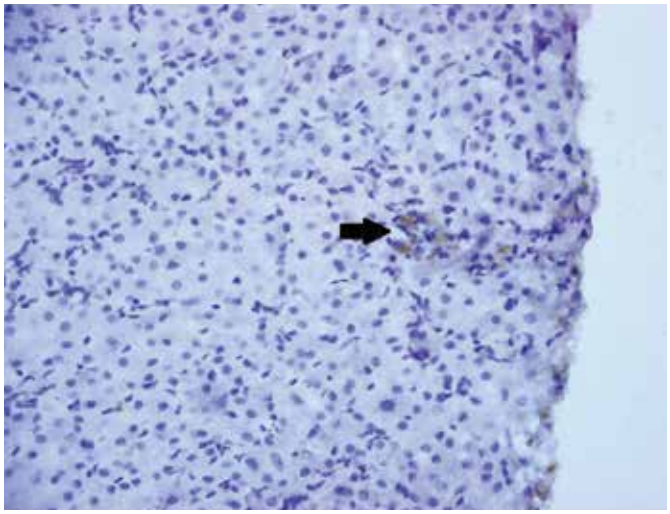

Figure 2. Ubiquitin painting perinuclear and intrastoplasmic positivity (Ubiquitin × 400).

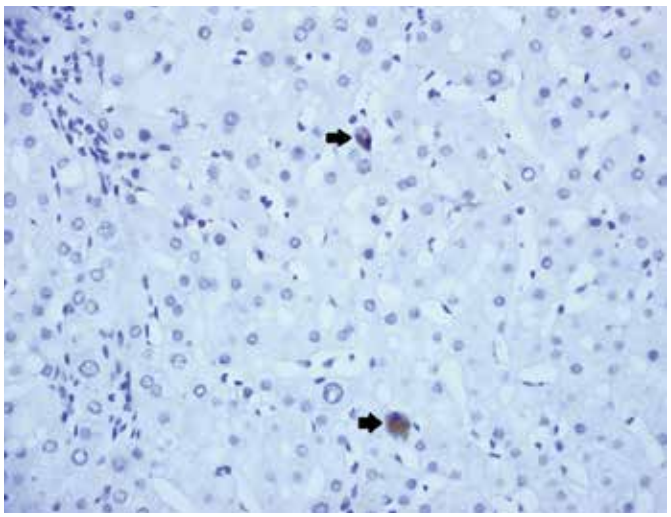

Figure 3. Ubiquitin painting of MDBs keratin content as immunohistochemical (Ubiquitin × 400).

2 and 3 in 60% and 40% of the cases, respectively; 20/20 (100%) patients with Hepatitis C showed mild and moderate ubiquitin positivity detected in 35% and 65% of the cases at every stage of the disease in zone 2 and 3; 8/10 (80%) patients with autoimmune hepatitis/overlap syndrome showed mild, moderate, and severe degrees of ubiquitin positivity noted in 10%, 80%, and 10% of the cases, respectively; 8/8 (100%) patients with PBS showed mild (10%), moderate (70%), and severe (10%) ubiquitin positivity frequently in zone 2 and 3. In biopsy specimens, macrovesicular steatosis was more prominent in zone 2 and 3. There was a correlation between the severity of steatosis and ubiquitin positivity, particularly in zone 2. In specimens with macrovesicular steatosis, balloon degeneration was more diffuse. A conspicuous correlation existed between the severity of hepatocyte balloon degeneration and ubiquitin positivity. Even in specimens without apparent steatosis, balloon degeneration was prominent with ubiquitin positivity.

DISCUSSION

The account of MDB generation in the identification and prognosis of chronic liver disorders, which is widely based on liver biopsy evaluation, has interesting caution. Morphologic properties frequently involve hepatocellular damage (fatty alters, ballooning hepatocyte degeneration, MDBs, giant mitochondria, apoptosis, and necrosis), inflammation, and fibrosis (both perisinusoidal and pericellular).

Hepatocyte ballooning degeneration is a characteristic of steatohepatitis and is frequently associated with MDB generation (8). Nevertheless, ballooning and MDB generation are noninterchangeable because not all ballooned hepatocytes include MDBs, although most MDB-including hepatocytes are ballooned.

As limitation; So, that, MDB generation is not only fixed feature in steatohepatitis. The density of MDBs based on the sensitivity of the determination method is examined.

As drawbacks, immunostaining not only demonstrates a mature form of MDB but also both its young forms and misfolded proteins such as CKs, ubiquitin, p62 protein, and HSPs in ballooned hepatocytes. In the present study, ubiquitin immunostaining was the preferred method. Hanada et al. (9) used ubiquitin immunostaining in 148 fine needle liver biopsies, which included 88 biopsies from patients with clinically diagnosed or suspected ASH and 60 selected biopsies from NASH. They showed the presence of MDB in ballooned hepatocytes by both hematoxylin-eosin and ubiquitin immunostaining in all of the 33 biopsies of patients with alcoholic hepatitis. Of the 55 biopsies from alcoholic patients, ubiquitin (+) cells were found in eight (14.5%) by immunostaining. Finally, they studied 60 selected biopsies from non- alcoholic patients and demonstrated a few ubiquitin (+) cells in 2 of the 10 patients with PBC. According to the study results, as ubiquitin immunostaining is a highly sensitive and specific tool in the detection of MDB in alcohol-consuming patients, its use in other forms of hepatitis is still limited. The importance of MDBs as an object in NASH is based on the histological scoring system utilized.

In an earlier classification system, Matteoni et al. (10) classified MDBs in patients with NASH as none, rare, and many. In that study on NASH, cirrhosis- and chronic liver disease-related mortality were connected to ballooning and either the presence of MDBs or degree of fibrosis. Mendler et al. (11) focused his study on the presence of MDBs, lobular inflammation degree and necrosis, hepatocyte ballooning degeneration, and perisinusoidal fibrosis rates. On the other hand, Kleiner et al. (12) indicated an emphasis in their scoring system of NASH on variable hepatocellular steatosis, lobular inflammation degree, hepatocellular ballooning degeneration, and fibrosis rates. In the latter case, MDBs were not regarded as a marker probably due to their association with hepatocyte ballooning. Frequency of MDB is reported to be variable in approximately 7%–90% in adult patients with NASH (13). Nevertheless, in the pediatric patients with NASH frequently deficiencies MDBs, which indicates that aging-related cumulative oxidative damages or genetic factors may take part a contributing effect (14,15). In addition, Haybaeck et al. (16) demonstrated that the structural features of Keratin 8 and Keratin 18 are genetic agents that are independent of the influence of genetic background as toxicity effects rely on the genetic background in a DDC-stimulated hepatotoxicity and MDB occurrence mouse form. At present, the in situ proximity ligation assay (isPLA) is an increasingly utilized technology for the in situ determination of protein interactive relations, post-translational modifications, and spatial connections of antigens in cells and tissues, in general. In another study, Zatloukal et al. (17) researched the colocalization of all three key MDB components, namely keratin 8, keratin 18, and p62 (sequestosome 1) by isPLA and immunofluorescence microscopy. As the function of proteins extreme bases on their interferences with other molecules and the localization of multiple constituent complexes in specific subcellular components, techniques for in situ invention of protein com-

plexes occur especial. On the other hand, in ASH with regard to the immunohistochemistry analysis with keratin antibodies appointed MDBs in 71% of cases, regardless of merely 40% in hematoxylin–eosin-stained tissue sections.

The histological examination revealed an increasing level of violence of hepatocellular damage, MDBs, neutrophil, mononuclear infiltration, and pericellular and periportal fibroses when non-alcoholic individuals, ambulatory patients with alcoholic liver disease, and hospitalized patients with ASH, respectively, were investigated (18). Men and women who drink more than 80 g and 40 g of ethanol/day, respectively, were considered to be at substantial risk of the development of liver disease in the previous studies. Recent studies showed that the risk of liver disease begins on consumption of 30 g of ethanol/day. The relationship between clinical alcohol-related liver disease expressions and morphological alterations analyzed in the liver biopsy sections is discussed in some literatures.

A few clinical-pathological investigations illustrate that MDB existence in combination with hepatocyte ballooning degeneration as well as necrosis and inflammation is a marker for disease activity, particularly in patients with alcohol-related liver disease such as ASH (19). This finding has led to a general recommendation that the maximum safe level of ethanol consumption is 20 g/day or two "drinks" per day for men and 10 g/day for women. However, it should be kept in mind that disease severity does not correspond to classic dose dependency. Although one of the major criteria for the diagnosis of NASH is no excessive alcohol consumption in all previous case series, as shortcoming; there has been no consensus on this issue and, in general, a wide range of alcohol consumption was allowed. Thus, these remarkable differences in the frequency of MDB might be explained with the generously allowed alcohol consumption in previous series. It must also be considered that patients sometimes under-report ethanol intake. In the present study, the distribution of MDBs stained positive for ubiquitin was 80% in overlap syndrome, 86% in chronic hepatitis B, and 100% in ASH, NASH, chronic viral hepatitis C, and primary biliary cirrhosis. In another study, Kucukoglu et al. (20) illustrated that the effects accountable for reproduced MDB creating can be observed in the high-fat diet fed animals contain remained CD73 degrees, aggregation Keratin 8 (K8)/Keratin 18 (K18), and p62, K8 cross bonding by means of TG2, conversely declined Hsp72 degrees which accommodated with researched misfolding intermediate filament protein.

In the high-fat diet fed animals, only did not change K8/K18 degrees also stimulated K8/K18 accretion both in K8tg and DDC- feeding animals.

As a matter of fact, this is not amazing that in as much as these keratins are stipulated stress-excitable genes raised in various liver disorders (20).

Cortez-Pinto et al. (21) classified persons with NASH. They were either asymptomatic ambulatory persons with ASH or hospitalized persons with severe ASH. Moreover, hepatocellular ballooning degeneration and MDBs were individually associated not only with perisinusoidal fibrosis but also with perivenular fibrosis in patients with NASH (22). Therefore, factors such as the existence of necroinflammation and MDBs identified a separate group of patients with cirrhosis without ASH and low mortality from another group with cirrhosis, ASH, and high mortality (23). In the present study, there was a correlation between the severity of steatosis and ubiquitin positivity, particularly in zone 2. A conspicuous correlation existed between the severity of hepatocyte balloon degeneration and ubiquitin positivity.

CONCLUSION

In this study, independent from the different forms of chronic hepatitis, we detected a positive correlation between the zonal distribution of steatosis, severity of macrovesicular steatosis, and ubiquitin positivity. However, even in hepatocytes without steatosis, ubiquitin positivity was observed. We have detected a positive correlation between macrovesicular steatosis, distribution of steatosis in zone 2, hepatocyte balloon degeneration, and ubiquitin positivity. These findings have demonstrated that the observation of MDB together with ubiquitin positivity will be helpful in the evaluation of the models of diagnosis, staging, and therapy in patients with chronic liver disease.

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