

非酒精性脂肪肝病肝组织超微结构特点 *

王 爽¹ 黄晓峰^{1△} 尹 文¹ 韩 英² 王瑞安³ 李增山³ 李永强¹ 亢君君¹ 鲁亚成¹

(1 第四军医大学基础医学院中心实验室 2 第四军医大学西京消化病医院;

3 第四军医大学基础医学院病理学与病理生理学教研室 陕西 西安 710032)

摘要 目的:阐明非酒精性脂肪肝病(NAFLD)的超微结构特点。方法:收集我校和其他单位送检的3例单纯性非酒精性脂肪肝,16例NASH患者和4例NAFLD肝硬化患者的肝穿刺组织。用2.5%戊二醛、1%锇酸双固定、Epon 812包埋,超薄切片70nm,醋酸铀和柠檬酸铅染色后,JEM-2000EX透射电镜观察。结果:单纯性脂肪肝患者主要表现为大小不等的脂滴沉积、以小脂滴为主,可互相融合。NASH患者的肝细胞都可出现大量脂滴积聚,为大小脂滴混合型、内容物主要为中等电子密度、比较均一的甘油三酯,部分脂滴周围可见磷脂成分,NASH患者肝细胞内脂滴也互相融合。肝细胞线粒体的超微结构改变包括多形性线粒体、基质颗粒增多、线粒体增大和嵴的丧失是主要的电镜异常发现,线粒体内还可见副晶格样包涵体。部分NASH患者肝细胞内可见Mallory小体。NASH患者肝细胞周围可见淋巴细胞浸润。肝血窦Kupffer细胞增生不明显,NAFLD肝硬化患者Disse间隙和肝细胞间可见胶原纤维增生。结论:NAFLD具有较为明确的超微结构改变,电镜检查有助于诊断。

关键词 非酒精性脂肪肝病;超微结构;诊断;电镜检查

中图分类号 R-575.1 R-365 文献标识码 A 文章编号:1673-6273(2012)14-2703-03

Ultrastructural Features of Liver Tissues in Non-alcoholic Fatty Liver Disease*

WANG Shuang¹, HUANG Xiao-feng^{1△}, YIN Wen¹, HAN Ying², WANG Rui-an³, LI Zeng-shan³,
LI Yong-qiang¹, KANG Jun-jun¹, LU Ya-cheng¹

(1 Central Laboratory, School of Basic Medicine, Fourth Military Medical University, Xi'an 710032, China;

2 Department of Xi'jing Digestive Disease Hospital, Fourth Military Medical University, Xi'an 710032, China;

3 Department of Pathology and pathophysiology, Fourth Military Medical University, Xi'an 710032, China)

ABSTRACT Objective: To investigate the ultrastructural features of liver tissues in non-alcoholic fatty liver disease (NAFLD).

Methods: 23 cases of NAFLD were collected, included 3 cases of steatosis, 16 cases of nonalcoholic steatohepatitis (NASH) and 4 cases of liver cirrhosis. The percutaneous liver aspiration biopsy was performed, the biopsy tissues were fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, embedded with Epon 812, 70 nm ultrathin sections were prepared. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a JEM-2000EX transmission electron microscope at 80 kV. **Results:** The number and size of lipid droplets vary in steatosis, small lipid droplets was seen frequently and they can fuse each other. The numerous lipid droplets was found in NASH. The macrovesicular and microvesicular or mixed types of steatosis were existed in the same cell. The content of lipid droplets was mainly medium electron dense triglyceride, and phospholipid was found in the periphery of some lipid droplets. The fused lipid droplets were also found in NASH. Ultrastructural changes in mitochondria of hepatocytes included polymorphic and enlarged mitochondria, increased metrical granules, fragmented cristae. The paracrystalline inclusions of mitochondria and Mallory body were also found in NASH. Lymphocytic infiltration is sometimes present in the vicinity of hepatocytes in NASH. The proliferation of Kupffer cells was not obvious. Increased collagen fibers were found in Disse spaces and intercellular spaces of hepatocytes in liver cirrhosis. **Conclusions:** The characteristic ultrastructural changes was found in NAFLD, electron microscopy might be of help in the diagnosis of NAFLD.

Key words: Non-alcoholic fatty liver disease; Ultrastructure; Diagnosis; Electron microscopy

Chinese Library Classification (CLC): R-575.1, R-365 **Document code:** A

Article ID: 1673-6273(2012)14-2703-03

前言

非酒精性脂肪肝病(Non-alcoholic fatty liver disease, NAFLD)是指除外酒精和其他明确的损肝因素所致的,以弥漫性肝细胞大泡性脂肪变为主要特征的临床病理综合征,包括单

纯性脂肪肝以及由其演变的非酒精性脂肪性肝炎(nonalcoholic steatohepatitis, NASH)和肝硬化^[1,2]。随着生活水平的提高和生活方式的改变,近年来NAFLD的发病率不断升高,我国最新的流行病学调查数据显示,上海、广州和香港等发达地区成人NAFLD患病率在15%左右。NASH患者10~15年内肝硬化

* 基金项目:陕西省社发攻关项目(2010K15-03-07),陕西省自然科学基金资助项目(2009JM4006)

作者简介:王爽(1971-),女,实验师,硕士,主要研究方向:超微结构病理

△通讯作者:黄晓峰, E-mail: huangxf@fmmu.edu.cn

(收稿日期:2011-11-28 接受日期:2011-12-24)

发生率高达 15%-25%^[3], 其病因和发病机制尚未完全明了, 解释单纯脂肪变到 NASH 进展最普遍接受的学说是 "二次或多次打击假说(second or multi-hit hypothesis)", 其中游离脂肪酸的代谢障碍是初次打击, 造成肝脏脂肪的过度积聚, 引起脂肪变, 在脂肪肝的基础上加上前炎症因子、脂肪因子、活性氧和内质网应激等二次打击因素, 就可使脂肪肝进一步进展为 NASH^[1,4]。在 NAFLD 不同阶段, 肝活检的主要发现表现为单纯脂肪变、NASH 和肝硬化^[1,2], 但患者肝组织的超微结构特点还不清楚。本研究的目的是阐明 NAFLD 患者肝脏组织的超微结构变化。

1 材料与方法

收集了我校和其他省市送检的 3 例单纯性非酒精性脂肪肝, 16 例 NASH 患者和 4 例 NAFLD 肝硬化患者的肝穿刺组织。16 男 7 女, 平均年龄 35.6±10.2 岁, 无饮酒史或饮酒量 <40 克/每周。进行了精确的病史采集以及不同的实验室检查, 如肝功能检查、血脂分析、酒精摄入确定、血清铜和铁的测定。一部分肝穿刺组织进行了常规 4% 甲醛固定、石蜡包埋、HE 染

色, 并由病理专家进行了光镜检查和诊断; 一部分肝穿刺组织用 2.5% 戊二醛、1% 锇酸双固定、Epon 812 包埋, 超薄切片 70nm 醋酸铀和柠檬酸铅染色后, JEM-2000EX 透射电镜观察。

2 结果

单纯性脂肪肝患者主要表现为大小不等的脂滴沉积、以小脂滴为主, 可互相融合。NASH 患者的肝细胞都可出现大量脂滴积聚, 为大小脂滴混合型、内容物主要为中等电子密度、比较均一的甘油三酯(图 1①), 部分脂滴周围可见磷脂成分, 单纯性脂肪肝和 NASH 患者肝细胞内脂滴有互相融合现象(图 1②)。肝细胞线粒体的超微结构改变包括多形性线粒体, 线粒体基质颗粒增多、线粒体体积增大和嵴的丧失, 线粒体内还可见副晶格样包涵体(图 1③)。部分 NASH 患者肝细胞内可见 Mallory 小体。部分 NASH 患者肝细胞周围可见淋巴细胞浸润。肝血窦 Kupffer 细胞增生不明显, NAFLD 肝硬化患者 Disse 间隙和肝细胞间可见明显胶原纤维增生(图 1④)。

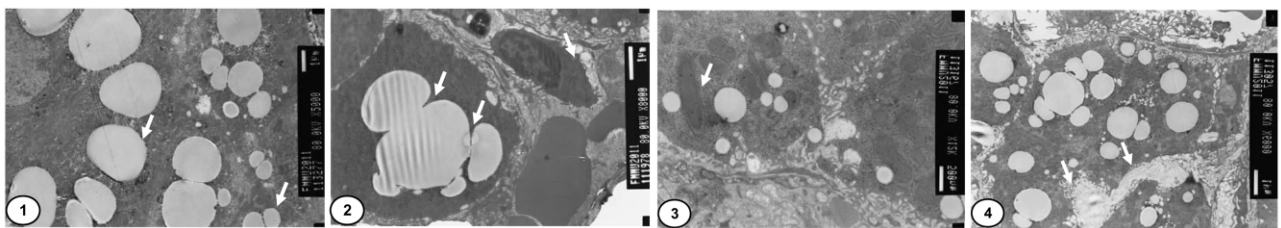


图 1 肝组织超微结构(透射电镜, 标尺 1μm): ①单纯性非酒精性脂肪肝患者肝细胞内大小不等的脂滴沉积, 主要为甘油三酯(↑); ② NASH 患者肝细胞内脂滴互相融合(↑); ③ NASH 患者肝细胞线粒体内的副晶格样包涵体(↑); ④ NAFLD 肝硬化患者肝细胞间可见明显胶原纤维增生(↑)

Fig.1 Ultrastructure of liver tissues (transmission electron microscopy, bar = 1μm): ①The number and size of lipid droplets vary in steatosis which was mainly medium electron dense triglyceride; ②The numerous lipid droplets was found in NASH, and they can fuse each other; ③Paracrystalline inclusion body was found in mitochondria of hepatocytes; ④Increased collagen fibers were found in intercellular spaces of hepatocytes in liver cirrhosis

3 讨论

NAFLD 患者肝组织电镜检查的主要异常发现是肝细胞内大量脂肪沉积以及线粒体的多形性。前期主要是脂滴沉积、内容物主要为甘油三酯, 部分脂滴周围可见磷脂成分。NASH 与线粒体功能障碍有密切关系^[5], 肝细胞线粒体是细胞的能量工厂, 可通过利用脂肪和葡萄糖产生 ATP 或热量。每各肝细胞含有大约 800 个线粒体, 约占整个肝细胞体积的约 18%。线粒体在肝细胞代谢中起重要作用, 是脂肪酸氧化和氧化磷酸化的主要部位。线粒体含有由磷脂双层和蛋白质组成的内膜和外膜。线粒体外膜含大量称为孔蛋白的整合蛋白, 它含有一个相当大的内部通道, 允许分子量 5kD 以下的分子通透。更大的分子只能通过线粒体膜转运蛋白的主动转运穿过外膜。与外面不同, 内膜不含孔蛋白, 具有高度不通透性, 几乎所有离子和分子进出基质都需要特殊的转运蛋白。内膜含有具有 4 种功能的蛋白质: 呼吸链的氧化反应、ATP 合成酶、调节代谢物进出基质的特殊转运蛋白、蛋白质输入机构。基质内含高度浓缩的数百种酶的混合物。这些酶的主要功能包括丙酮酸和脂肪酸氧化、柠檬酸循环。线粒体呼吸链对能量产生相当重要, 由几种多肽组成。大多数呼吸链多肽由细胞核 DNA 编码, 但某些多肽由线粒体 DNA (mitochondrial DNA, mtDNA) 编码。mtDNA 是一种环形双链分子, 位于线粒体基质中。mtDNA 靠近内膜对氧化损伤相当敏感, 缺少组蛋白保护、其 DNA 损伤修复机制不

完全。因此, 影响线粒体完整性的任何因素都可引起线粒体功能的降低。既往研究证明 NASH 患者有明显的脂质过氧化增强^[6]、氧化应激增高^[7]。线粒体畸形、增大和嵴的丧失, 结晶样包涵体的形成都可能对其功能产生影响^[8], 从而引起脂肪酸氧化障碍, 造成脂肪积聚。有报道采用抗氧化剂处理可以改善肝脏的单纯脂肪变和纤维化^[9-11]。这些积聚的脂滴以腺泡 3 区的大泡性脂肪变为主, 同时常有较为特征性的 Disse 间隙和肝细胞周围的纤维化, 尤其是肝细胞周围的纤维化较轻时在光镜下即使经 Masson 三色染色也不容易分辨, 电镜检查则可以发现胶原纤维的增生, 有助于 NAFLD 的诊断。NASH 患者出现的氧化应激、炎症因子的产生都能影响线粒体的代谢、造成脂肪酸氧化障碍^[8,12-18], NASH 患者出现的淋巴细胞浸润可能是机体对肝细胞损伤后产生的局部炎症反应^[19,20]。有研究发现在 NASH 和进展性纤维化患者有更高的中性粒细胞/淋巴细胞比率, 提示该比值是一种新的非侵入性的标志物, 可用来预测病情的进展^[21]。总之, NAFLD 患者的肝组织有较特征性的超微结构特点, 电镜检查有助于 NAFLD 的诊断。

参考文献(References)

- [1] Lewis JR, Mohanty SR. Nonalcoholic Fatty Liver Disease: A Review and Update [J]. Dig Dis Sci, 2010, 55(3):560-578
- [2] Krawczyk M, Bonfrate L, Portincasa P. Nonalcoholic fatty liver disease [J]. Best Pract Res Clin Gastroenterol, 2010, 24(5):695-708
- [3] 中华医学会肝病学会脂肪肝和酒精性肝病学组. 非酒精性脂

- 肪性肝病诊疗指南(2010年1月修订)[J]. 中华内科杂志, 2010, 49(3): 275-278
- The Chinese National Workshop on Fatty Liver and Alcoholic Liver Disease for the Chinese Liver Disease Association. Guidelines for management of alcoholic liver disease: an updated and revised edition [J]. Chin J Inter Med, 2010, 49(3): 275-278
- [4] 黄颖秋. 非酒精性脂肪性肝炎发病机制的研究进展[J]. 世界华人消化杂志, 2009; 17(31): 3181-3188.
- Huang YQ. Recent advances in understanding the pathogenesis of nonalcoholic steatohepatitis [J]. Shijie Huaren Xiaohua Zazhi, 2009, 17(31): 3181-3188
- [5] Serviddio G, Sastre J, Bellanti F, et al. Mitochondrial involvement in non-alcoholic steatohepatitis [J]. Mol Aspects Med, 2008, 29(1-2): 22-35
- [6] Gastaldelli A, Cusi K, Pettiti M, et al. Relationship between hepatic/visceral fat and hepatic insulin resistance in nondiabetic and Type 2 diabetic subjects [J]. Gastroenterology, 2007, 133(2): 496-506
- [7] Chalasani N, Deeg MA, Crabb DW. Systemic levels of lipid peroxidation and its metabolic and dietary correlates in patients with nonalcoholic steatohepatitis [J]. Am J Gastroenterol, 2004, 99(8): 1497-1502
- [8] Sanyal AJ, Campbell SC, Mirshahi F, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities [J]. Gastroenterology, 2001, 120(5): 1183-1192
- [9] Gawrieh S, Opara EC, Koch TR. Oxidative stress in nonalcoholic fatty liver disease: pathogenesis and antioxidant therapies [J]. J Invest Med, 2004, 52(8): 506-514
- [10] Mehta K, Van Thiel DH, Shah N, et al. Nonalcoholic fatty liver disease: pathogenesis and the role of antioxidants [J]. Nutr Rev, 2002, 60(9): 289-293
- [11] Oliveira CP, Gayotto LC, Tatai C et al. Vitamin C and vitamin E in prevention of nonalcoholic fatty liver disease (NAFLD) in choline deficient diet fed rats [J]. Nutr J, 2003, 7(2): 9
- [12] Svegliati-Baroni G, Saccomanno S, van Goor H, et al. Involvement of reactive oxygen species and nitric oxide radicals in activation and proliferation of rat hepatic stellate cells [J]. Liver, 2001, 21(1): 1-12
- [13] Bataller R, Schwabe RF, Choi YH, et al. NADPH oxidase signal transduces angiotensin II in hepatic stellate cells and is critical in hepatic fibrosis [J]. J Clin Invest, 2003, 112(9): 1383-1394
- [14] Tsukamoto H, Rippe R, Niemela O, et al. Roles of oxidative stress in activation of Kupffer and Ito cells in liver fibrogenesis [J]. J Gastroenterol Hepatol, 1995, 10(Suppl 1): S50-S53
- [15] Vignais PV. The superoxide-generating NADPH oxidase: structural aspects and activation mechanism [J]. Cell Mol Life Sci, 2002, 59(9): 1428-1459
- [16] Ledercq IA, Farrell GC, Field J, et al. CYP2E1 and CYP4A as microsomal catalysts of lipid peroxides in murine nonalcoholic steatohepatitis [J]. J Clin Invest, 2005, 105(8): 1067-1075
- [17] Berson A, De Beco V, Letteron P, et al. Steatohepatitis-inducing drugs cause mitochondrial dysfunction and lipid peroxidation in rat hepatocytes [J]. Gastroenterology, 1998, 114(4): 764-774
- [18] Weltman MD, Farrell GC, Hall P, et al. Hepatic cytochrome P450 2E1 is increased in patients with nonalcoholic steatohepatitis [J]. Hepatology, 1998, 27(1): 128-133
- [19] Söderberg C, Marmur J, Eckes K, et al. Microvesicular fat, inter cellular adhesion molecule-1 and regulatory T-lymphocytes are of importance for the inflammatory process in livers with non-alcoholic steatohepatitis [J]. APMIS, 2011, 119(7): 412-420
- [20] Zhan YT, An W. Roles of liver innate immune cells in nonalcoholic fatty liver disease [J]. World J Gastroenterol, 2010, 16(37): 4652-4660
- [21] Alkhouiri N, Morris SG, Campbell C, et al. Neutrophil to lymphocyte ratio: a new marker for predicting steatohepatitis and fibrosis in patients with nonalcoholic fatty liver disease [J]. Liver Int, 2011 Sep 8. doi: 10.1111/j.1478-3231.2011.02639.x.
- (上接第 2697 页)
- [8] Lu Y, Song Z, Zhou X, et al. A 12-month clinical survey of incidence and outcome of acute respiratory distress
- [9] 北京市科委重大项目 MODS 课题组. 1998-2003 年北京地区重症加强治疗病房急性呼吸窘迫综合征的临床流行病学调查 [J]. 中国危重病急救医学, 2007, 19(4): 201-204
- MODS group of major project of Beijing Science and Technology Commission. Clinical epidemiological survey on acute respiratory distress syndrome of ICU in Beijing in 1998-2003 [J]. Chinese Critical Care Medicine, 2007, 19(4): 201-204 (In Chinese)
- [10] 陆再英. 内科学 [M]. 第 7 版. 北京: 人民卫生出版社, 2008: 153.
- Lu Zai-ying. The Seventh Edition of Medicine [M]. Beijing: People's Health Press, 2008: 153 (In Chinese)
- [11] Kato Y, Kudo M, Shinkawa T, et al. Role of O-linked carbohydrate of human urinary trypsin inhibitor on its lysosomal membrane-stabilizing property [J]. Biochem Biophys Res Commun, 1998, 243: 377-383
- [12] 施旂旎, 黄子通, 蒋龙元, 等. 乌司他丁治疗全身炎症反应综合征的临床研究 [J]. 中国急救医学, 2004, 24: 738-739
- Shi Yi-qi, Huang Zi-tong, Jiang Yun-long, et al. Clinical research of ulinastatin on treatment of systemic inflammatory response syndrome. [J]. Chinese Journal of Critical Care Medicine, 2004, 24: 783-789 (In Chinese)
- [13] 林晃纪, 種田, 益造, 他. 乌司他丁对失血所致低血压患者的临床研究 [J]. 急救医学 (日), 1998, 12: 187-192
- Akira Osamu, Hayashi, Taneda, et al. The clinical research of ulinastatin on the patients with hypotension because of blood loss. Emergency Medicine (In Japanese), 1998, 12: 187-192
- [14] Morishita H, Yamakawa T, Matsue T, et al. Novel factor Xa and plasma kallikrein inhibitor activities of the second kunitz-type inhibitory domain of urinary trypsin inhibitor. Thromb Res, 1994, 73, 193-204
- [15] 彭万勇, 陈阳龙, 王琳芳, 等. 乌司他丁治疗严重创伤的临床研究 [J]. 临床外科杂志, 2003, 11: 126-127
- Peng Wan-yong, Chen Yang-long, Wang Lin-fang, et al. The clinical research of ulinastatin on treatment of serious injuries [J]. Clinical Surgery, 2003, 11: 126-127 (In Chinese)
- [16] 邓文锋, 于立新, 余玉明, 等. 乌司他丁减轻移植肾缺血损伤的临床研究 [J]. 中华泌尿外科杂志, 2004, 25: 739-741
- Deng Wen-feng, Yu Wen-xin, Yu yu-ming, et al. The clinical research of ulinastatin on relieving renal allograft hot ischemic injury [J]. Chinese Urology, 2004, 25: 739-741 (In Chinese)
- [17] Sugita T, Watarida S, Katsuyama K, et al. Effect of a human urinary protease inhibitor (ulinastatin) on respiratory function in pediatric patients undergoing cardiopulmonary bypass [J]. J Cardiovasc Surg (Torino), 2002, 43: 437-440
- [18] 徐康清, 孙培吾, 黄文起, 等. 乌司他丁对体外循环心脏手术后肺功能的影响 [J]. 中华胸心血管外科杂志, 2002, 20: 7-9
- Xu Kang-qing, Sun Pei-wu, Huang Wen-qi, et al. The affection of ulinastatin on lung and cardiopulmonary function after bypass [J]. Thoracic and Cardiovascular Surgery, 2002, 20: 7-9 (In Chinese)