非酒精性脂肪肝病肝组织超微结构特点*

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

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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, fragmentated cristae. The paracrystalline inclusions of mitochondria and Mallory body were also found in NASH. Lymphocytic infiltration is sometimes present in the 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
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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]。随着生活水平的改善和生活方式的改变，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

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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 1 ①)，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 1 ②)。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 1 ④)。

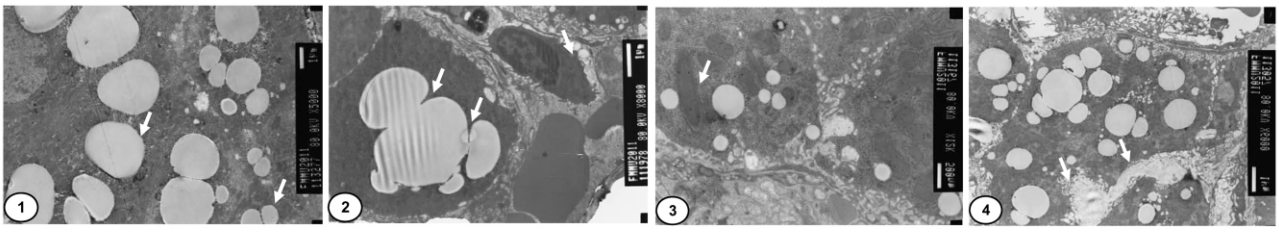

图 1 肝组织超微结构 (透射电镜，标尺 1μm)：①单纯性非酒精性脂肪肝患者肝细胞内大小不等的脂滴沉积，主要为甘油三酯 (↑)；② NASH 患者肝细胞内脂滴互相融合 (↑)；③ NASH 患者肝细胞线粒体内的副晶格样包涵体 (↑)；④ NAFLD 肝硬化患者肝细胞间可见明显胶原纤维增生 (↑)

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，允许分子质量 5kD 以下的所有分子通透。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。

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