Quantitative Assessment of Septa Formation and Progression in CDHFD Rat Model of NASH Using SHG/TPEF Microscopy

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INTRODUCTION

Liver septa formation and progression are commonly observed in the F3 non-cirrhotic and F4 cirrhotic nonalcoholic steatohepatitis(NASH) patients as typical late-stage fibrosis features, which gives crucial information in elucidating the pathogenesis of advanced NASH and evaluating therapeutic agents for this spectrum that has a greater immediate medical need. However, there are very few animal models that within a short time exhibit septae. Also, there are few methods that could quantitatively assess the changes in septal fibrosis.

AIM

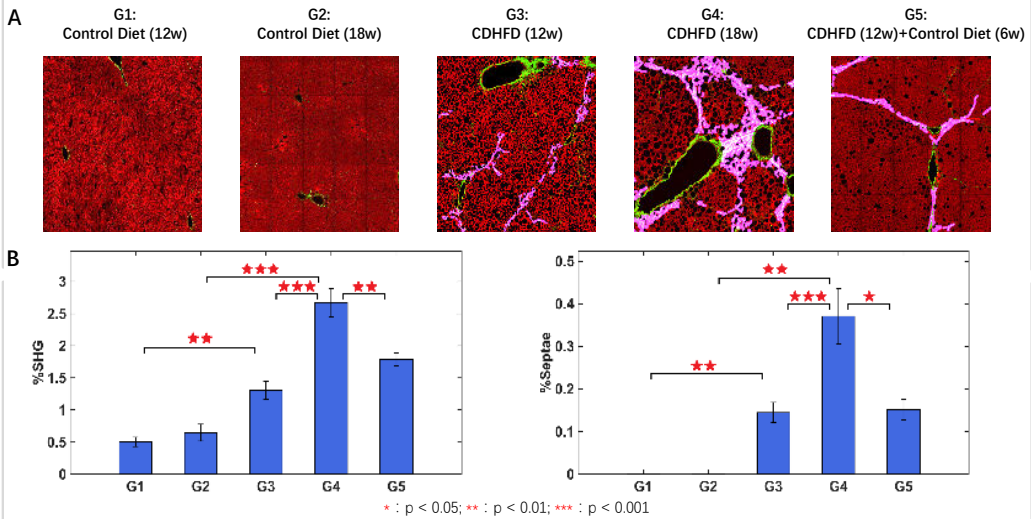
The purpose of this study was to develop an automated platform for septa quantification and evaluate it in a choline-deficient high-fat diet(CDHFD) preclinical rat model of NASH that mimics histological changes in both F3 and F4 patients.

MATERIAL & METHODS

The progression of fibrosis was evaluated using formalin-fixed paraffin-embedded liver tissues from Wistar Han rats fed with CDHFD for 12 and 18 weeks(W). A potential reversal test was conducted by feeding rats with CDHFD for 12W followed by a control diet for an additional 6W.

Second-harmonic generation/two-photon excitation fluorescence (SHG/TPEF) microscopy (Genesis®200, HistoIndex Pte Ltd, Singapore) was used for imaging and quantifying septal collagen in these tissues. An Artificial Intelligence (AI)-based algorithm first recognized the portal tract and central vein zones and then examined each bundle of collagen between the zones using the histological diagnosis of septa in humans as the gold standard reference. Thereafter, the septa formation and progression, on top of the overall steatosis and fibrosis changes, in these animals were quantified and compared among different groups.

RESULTS



The figure shows some representative SHG/TPEF images and the quantitative changes in the study: A. Labeled ROI images for better illustration (SHG in green, TPEF in red and detected septae in pink) and B. The changes of total collagen area and septal collagen area in whole slide images.

A significant increase was observed in %SHG measured overall collagen area for both 12W and 18W CDHFD rats' liver tissues compared to that in the respective control diet groups. In addition, both CDHFD groups exhibited a lot of septae, beginning from delicate ones at 12W, to sturdy progressive ones which even start forming nodules at 18W.

In the reversal test, steatosis decreased significantly compared with both 12W and 18W CDHFD groups. However, despite the fibrosis improvement in fine and distributed collagen fibers, septa reduction in this reversal group was not observed, when compared to the 12W CDHFD group.

CONCLUSION

Septae were formed in the CDHFD rat model after 12W and developed into progressive ones forming nodules in an additional 6W. The reversal test results suggest switching back to a normal diet after septae were formed might only alleviate their progression even though steatosis improvement was significant, just like in humans. Therefore, this model might be useful for the assessment of advanced NASH with septae observed in F3 and F4 patients.

An AI-based SHG/TPEF imaging and analyzing platform could be useful in monitoring liver fibrosis dynamics quantitatively and accurately, especially septal changes in preclinical NASH models.

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REFERENCES

1) Wanxin Sun et al. "Nonlinear optical microscopy: use of second harmonic generation and two-photon microscopy for automated quantitative liver fibrosis studies." Journal of Biomedical Optics 2008
2) Feng Liu, et al. "Automated evaluation of liver fibrosis in thioacetamide, carbon tetrachloride, and bile duct ligation" rodent models using second-harmonic generation/two-photon excited fluorescence microscopy." Laboratory Investigation 2016
3) Feng Liu,et al. "qFIBS: A Novel Automated Technique for Quantitative Evaluation of Fibrosis, Inflammation, Ballooning, and Steatosis in Patients with Non-Alcoholic Steatohepatitis." Hepatology 2020

DISCLOSURES

H.Y and R.M are employees of Takeda Pharmaceuticals and own stock in Takeda. X.T, Q.Y, A.L and G.H are employees of HistoIndex or its subsidiary, X.T holds stock options and G.H owns stock in HistoIndex.

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