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COMPARATIVE EVALUATION OF ZONAL FIBROSIS PATTERNS IN PEDIATRIC AND ADULT METABOLIC DYSFUNCTION-ASSOCIATED STEATOHEPATITIS BIOPSIES USING SECOND HARMONIC GENERATION/TWO PHOTON EXCITATION-BASED qFIBROSIS ANALYSIS

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INTRODUCTION

- Metabolic dysfunction-associated steatotic liver disease (MASLD) is now recognized as the most common chronic liver disease in children, with significant clinical and public health implications
- Pediatric metabolic dysfunction-associated steatohepatitis (MASH) presents unique histopathological features, such as zone 1 centred steatosis, inflammation, and fibrosis, often without the ballooning seen in adult MASH. These differences may affect how fibrosis progresses and how pediatric patients respond to treatment
- Understanding the distinct patterns of fibrosis in pediatric MASH compared to adults is essential for accurate diagnosis, tracking disease progression, and developing effective therapies
- This study uses Second Harmonic Generation/Two-Photon Excitation (SHG/TPE) microscopy and AI-based analysis (qFibrosis) to quantitatively assess and compare fibrosis distribution in pediatric and adult MASH biopsies

AIM

- To evaluate the fibrosis distribution patterns in pediatric MASH liver biopsies using qFibrosis analysis
- To compare these patterns to adult MASH liver biopsies and identify potential differences in fibrosis localization and severity
- Provide insights into how these differences might influence clinical trial designs, patient stratification, and treatment approaches in pediatric MASH

METHOD

- A total of 9 unstained pediatric liver biopsies (ages 8-16 yrs) with MASH were analysed using SHG/TPE microscopy, which allows for imaging of collagen fibres morphology (qFibrosis)
- Each biopsy scanned by the qFibrosis platform provides qFibrosis continuous values, which are then further classified into qFibrosis stages (qF0 to qF4) based on pre-defined cut-offs
- These biopsies were categorized into early (qF0/qF1/qF2, n=3) and advanced fibrosis stages (qF3/qF4, n=6)
- For comparison, 156 adult MASH biopsies from a clinical trial (NCT02855164) were similarly analysed and grouped into early (n=96) and advanced (n=60) fibrosis stages
- Zonal fibrosis distribution (portal, periportal, perisinusoidal [zone 2], pericentral, and central vein regions) was quantified, and differences between pediatric and adult groups were analysed statistically using the Spearman test (p≤0.05 considered significant)
- Fibrosis patterns were visualized using radar plots to highlight areas with significant differences between pediatric and adult biopsies

RESULTS

- **Early Fibrosis Stage (qF0/qF1/qF2) [Figure 1A]:**
  a) Pediatric biopsies showed significantly more perisinusoidal (PS) and portal fibrosis compared to adult biopsies (p=0.03). This suggests a more prominent early fibrotic response in the PS and portal regions in pediatric patients
- **Advanced Fibrosis Stage (qF3/qF4) [Figure 1B]:**
  a) Pediatric biopsies demonstrated significantly less portal fibrosis than adults (p=0.02), but markedly more perisinusoidal fibrosis (p=0.01). This indicates that, while portal fibrosis is less advanced in children at later stages, perisinusoidal fibrosis is more severe, reflecting a distinct progression pattern in pediatric patients
  b) Additionally, pediatric advanced-stage biopsies showed more fibrosis in the periportal (PP) and pericentral (PC) regions compared to adults, although these differences were not statistically significant

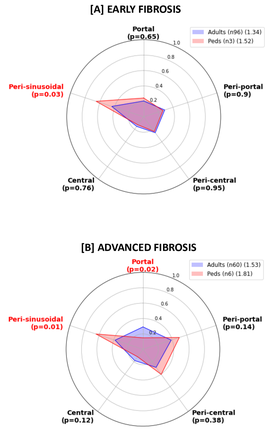

Figure 1: Radar plot illustrating the zonal distribution of fibrosis (according to the NASH-CRN system) in pediatric biopsies categorized as Early (Fig 1A) and advanced fibrosis (Fig 1B) based on their qFibrosis staging, compared with adult biopsies of corresponding qFibrosis stages. Zonal measurements marked in red denote statistically significant differences (p≤0.05)

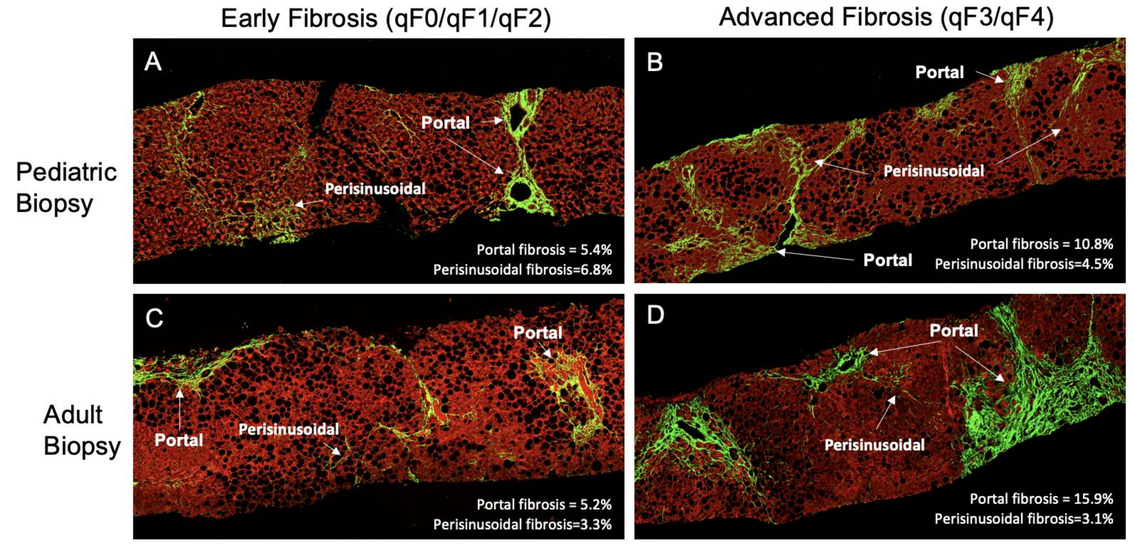

Figure 2. Histological Comparison of Fibrosis in Pediatric and Adult Liver Biopsies Across Early and Advanced Fibrosis Stages Using SHG/TPE Images. In early fibrosis stages, pediatric liver biopsies exhibited significantly higher levels of PS and portal fibrosis (Fig 2A) compared to adult biopsies (Fig 2C). In advanced fibrosis stages, pediatric biopsies (Fig 2B) showed significantly less portal fibrosis than adult samples (Fig 2D), but markedly more perisinusoidal fibrosis

CONCLUSIONS

- This study, although limited by a small sample size of pediatric biopsies, provides quantitative comparison of zonal fibrosis distribution between pediatric and adult MASH, showing significant differences in localization and severity of fibrosis
- Previous studies classify fibrosis distribution in pediatric biopsies into two types; Type 1 NASH: Characterized by steatosis along with ballooning and/or perisinusoidal fibrosis, but lacking features of portal fibrosis; Type 2 NASH: Defined by steatosis with portal inflammation and/or fibrosis, without the presence of ballooning or perisinusoidal fibrosis [1]
- In pediatric patients, perisinusoidal fibrosis is a key feature in both early and advanced stages of disease, while portal fibrosis is less prominent in the advanced stages compared to adults
- Our findings suggest that pediatric MASH may follow a distinct fibrotic progression pathway, which may have implications for assessment of fibrosis dynamics in pediatric MASH trials

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