Background/Aim

Digital image analysis on stained liver biopsies reveals significant correlation of collagen proportionate area (CPA) with traditional semi-quantitative staging systems. Histological staining may introduce operator-dependent variations and collagen progression dynamics have not been considered. New second harmonic generation/two-photon excited fluorescence (SHG/TPEF) microscopy, Genesis®, can be used to observe collagen and other morphology features such as steatosis, inflammation, and ballooning without staining. The images obtained from unstained slides are more consistent in image quality compared to images obtained from stained slides, and this reproducibility is particularly useful for pathological assessment in multi-centre clinical studies. By combining these techniques, morphometry information can be quantitated in a more sensitive and more reproducible manner.

Methods

Unstained slides from 101 NAFLD and NASH patients were staged using NASH-CRN and ISHAK systems, and were also imaged and analysed using Genesis®200 (Histoindex Pte Ltd.). A total of 100 morphology features in fibrosis were generated from these stain-free images with fully automated algorithms. For comparison, digitized images of Sirius red stained sections were also acquired to calculate the collagen proportionate area (CPA).

Results

Both staging systems of fibrosis were more significantly correlated with SHG collagen features (NASH CRN: r = 0.905, p<0.0001; Ishak: r = 0.930, p<0.0001) than CPA staining (NASH CRN: r = 0.481, p<0.001; Ishak: r = 0.542, p<0.001); we found the same results correlating SHG collagen features and CPA with clinical data (e.g. SHG × AST: r = 0.40, CPA × AST: r<0.3).

Results - continued

Figure 1. Comparison of Sirius red stained images with SHG/TPEF images. The progression of hepatic fibrosis can be quantified by specific SHG parameters. Here, a feature called long string, is illustrated.

Figure 2. Collagen string features match both NASH CRN and Ishak scores. (A-C) Changes of collagen proportional area (CPA), number of long strings and perimeter of strings with NASH CRN. (D-F) Changes of CPA, number of long strings and perimeter of strings with the modified Ishak system. On each box, the central mark is the median, the edges of the box are the 25th and 75th percentiles, the whiskers extend to the most extreme data points not considered outliers, and outliers are plotted individually.

Figure 3. Correlation between combined index with NASH CRN and the modified Ishak staging systems.

Figure 4. Comparison of capability to differentiate mild fibrosis (stage 0, 1 and 2) using CPA and combined index approaches. Combined index showed much higher distinctions between stages 0, 1, and 2.

Figure 5. Illustration of fully automated, and fully quantitative assessment on fibrosis, steatosis, ballooning, and inflammation on same slide specimen using non-staining Genesis®200 imaging system.

Conclusions

Compared with traditional stained image analysis, quantitative assessment using SHG collagen features recorded by SHG/TPEF microscopy may be more robust for evaluating fibrosis in NAFLD.

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