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Serum total glycosylation profiling for non-invasive diagnosis of liver cirrhosis in people with chronic hepatitis B

Key Messages

1. A high-throughput assay for quantitative profiling of N-glycans attached to serum glycoproteins has been established.
2. A panel of serum N-glycans were identified as potential biomarkers for diagnosing liver cirrhosis and liver fibrosis.
3. Four glycan peaks of 1341.5, 1829.7, 1933.3, and 2130.3 m/z were all able to detect liver fibrosis and cirrhosis with 85% accuracy.

Introduction

The persistent hepatic inflammation caused by chronic hepatitis B (CHB) infection leads to progressive liver fibrosis, and eventually, liver cirrhosis. Both liver fibrosis and cirrhosis are reversible if treated early. Knowledge of the stage of liver fibrosis is essential for prognostication and decisions about anti-viral treatment.[1,2] Liver biopsy is the gold standard for assessing liver fibrosis based on histological scoring systems.[3] A liver biopsy assessment is recommended whenever anti-viral treatment is considered,[4] but this is an uncomfortable and sometimes risky procedure, so is not suitable for the routine follow-up of CHB patients. Therefore, serum markers that can reliably detect liver cirrhosis are needed, but those currently available are not sufficiently sensitive for effective detection of liver cirrhosis.

In chronic hepatitis C (CHC) infection, serum markers have been used to predict liver fibrosis. It has been suggested that algorithms based on biochemical and haematological markers can correlate with liver fibrosis.[5,6] A commercially available test (FibroTest, BioPredictive SAS, Paris, France) based on a panel of serum protein markers related to liver fibrosis has been developed.[7] Serum-based assays can be used to assess and monitor liver fibrosis in CHC, with area under the 'receiver operator characteristics' curve being 80 to 90%.

In CHB, similar models based on serum biochemical markers have only achieved moderate correlation with liver fibrosis, and show about 50% sensitivity for detecting significant fibrosis.[8] These less encouraging results may be related to CHB's more complicated natural history, as it is characterised by intermittent exacerbations with different disease phases related to the HBeAg status, whereas CHC is generally an indolent, progressive disease.[9]

Considerable evidence indicates that the N-linked carbohydrate side-chains (ie N-glycans) of serum glycoproteins are altered in patients with liver cirrhosis. There are increased degrees of fucosylation of serum proteins (including haptoglobin, alpha1-acid glycoprotein, and cholinesterase) in liver cirrhosis.[10] Hyposialylated variants of haptoglobin, alpha1-antitrypsin and transferrin have been detected in patients with alcoholic cirrhosis.[11]

Glycomics—the study of the global glycan profile—is a relatively new post-genome research area. When N-glycans are released from serum glycoproteins, specific types of N-glycans have been associated with cirrhosis. The unique patterns of these N-glycans have enabled the identification of cirrhosis in patients with chronic liver disease with about 80% accuracy.[12] Liver cirrhosis is the severe, end-stage of liver fibrosis, so it is possible that the aberrant N-glycans appear earlier as liver fibrosis develops, but at lower levels. Thus, the quantitative profiling of N-glycans isolated from all serum glycoprotein may enable early diagnosis and staging of liver fibrosis.

Aims and objectives

1. Establish a high-throughput assay for quantitative profiling of N-glycans attached to serum glycoproteins.

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of the recent injectors reported using new needles for most (>50%) or all injections. About two thirds of the subjects admitted ever sharing injecting equipment, of which 15% admitted to recent sharing (in past 3 months), and 85% tested positive for HCV antibody.

Univariate analyses showed that HCV prevalence was higher in IDUs who were male, older, a longer duration of injection drug use, ever shared needles, and concurrent use of midazolam/triazolam/rohypnol. Multivariate analysis identified duration of injection, recent injection, ever sharing and use of midazolam/triazolam/rohypnol as independent factors associated with HCV seropositivity.

Discussion

Prevalence of HCV antibody was higher in IDUs than reported previously. These results may indicate that (1) all subjects had a definitive history of injection; and (2) sampling bias occurred owing to higher representation of experienced drug users. Follow-up studies are needed to establish infection rates and genotype distributions, which carry clinical and public health implications. In view of a low HIV rate in heroin users, it appears that risk behaviours tend to accumulate over the years, leading to increased HCV infection prevalence. The low HIV prevalence could be associated with a relative decline in high-risk behaviours after the 1980s, though the exact reasons need further exploration.

Conclusions

The HCV antibody prevalence in IDUs was 85%; many of them received methadone treatment. Positive HCV antibody is associated with a long history of injection, though needle-sharing practice is uncommon. Owing to the high prevalence of HCV infection in local drug users, follow-up studies (including HCV RNA tests and genotype analysis) should be useful to determine the clinical and public health implications. Treatment strategy should take into consideration of the high prevalence of HCV infection in local drug users. The discrepancy between the prevalence of HCV (85% in our study) and HIV (less than 1% from surveillance) suggests that behavioural factors alone may not account for the transmission risk in the context of public health.

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AUTHOR INDEX

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