

Serum level of YKL-40 does not predict advanced liver fibrosis in children with chronic hepatitis B

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Abstract

Purpose: The aim of the study was to evaluate the serum concentration of YKL-40 (human cartilage glycoprotein-39) in the assessment of fibrosis stage in children compared to biopsy and prior to antiviral treatment for chronic hepatitis B.

Material and methods: We determined serum level of YKL-40 (METRA, EIA kit, Quidel Corporation, San Diego, USA) after an overnight fast in 63 children (age range 4-17 years, mean 10 years) with biopsy-verified chronic HBeAg-positive hepatitis B. Fibrosis stage and inflammation grade were assessed in a blinded fashion according to Ishak et al. We defined advanced liver fibrosis as a score >2. Receiver operating characteristics (ROC) analysis was used to calculate the power of the assay to detect advanced liver fibrosis (AccuROC, Canada).

Results: Serum concentration of YKL-40 was significantly higher in patients with chronic hepatitis B compared to controls (n=16) (38.5±19.2 vs 27.9±8.75 ng/mL; p=0.032). The ability of serum YKL-40 to differentiate children with advanced liver fibrosis (n=31; 49.2%) from those with mild fibrosis was not significant (AUC=0.387±0.072, p=0.12). This marker was not a good predictor of histologic inflammation either.

Conclusion: Serum level of YKL-40 does not predict advanced liver fibrosis in children with chronic hepatitis B.

Key words: biomarker, children, chronic hepatitis, HBV, HC gp-39, fibrosis, YKL-40.

Introduction

YKL-40 also known as human cartilage glycoprotein-39 (HC gp-39) or chondrex is a 40 kilodalton glycoprotein first described in whey secretions of nonlactating cows [1]. It is a mammalian member of a chitinase family (18-glycosylhydrolases) [2]. The exact biological functions of YKL-40 are still unknown but its growth factor activities for chondrocytes, synovial cells [3] and fibroblasts [4] have been reported. It also plays a role in angiogenesis by stimulating the migration and reorganization of vascular endothelial cells [5]. Haemodynamic studies have shown that this biomarker is released from the hepatosplanchnic area [6]. The expression of YKL-40 in normal and disease conditions suggest that it is involved in inflammatory processes and in remodelling the extracellular matrix (ECM) [1,2,7]. Subsequent studies have shown that YKL-40 may be used as a potent marker of arthritic disorders [8-10], inflammatory bowel disease [11], purulent meningitis [12], *Streptococcus pneumoniae* bacteremia [13], breast cancer [14] and colorectal cancer [15].

According to Johansen et al. [16] YKL-40 can be secreted by hepatic stellate cell (HSC) which is principal effector cell in liver fibrogenesis. Therefore this glycoprotein can be regarded as biomarker of liver fibrosis.

To our knowledge, serum level of YKL-40 predicting liver fibrosis has not been assessed before in children. Normal serum concentration of this marker is not significantly affected by growth and therefore appear to be more useful in assessing ECM metabolism in paediatric liver diseases [7]. Therefore the serum concentration of this marker was measured in children with chronic hepatitis B and compared to liver histology to determine if measurement of this biochemical test has any clinical usefulness as marker of liver fibrosis.

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Table 1. Initial characteristics of children with chronic hepatitis B

Data of the patients	Mean	SD	Minimum	Maximum
Age (years)	10	3.41	4	17
ALT (IU/L)	84	57	12	312
AST (IU/L)	68	37	27	264
GGT (IU/L)	15	9	3	69
Bilirubin ($\mu\text{mol/L}$)	10.1	4.45	2.74	32.8
YKL-40 (ng/mL)	38.5	19.2	16.2	119.2
Staging	2.55	0.88	1	5
Grading	3.76	1.43	1	8

Normal ranges: ALT – 10-40 IU/L, AST – 10-40 IU/L, GGT – 9-35 IU/L, bilirubin – 1.7-18.8 $\mu\text{mol/L}$

Material and methods

Patients

The study was carried out on 63 consecutive children (mean age 10 years, range 4-17, 41 boys and 22 girls) with biopsy-verified chronic HBeAg positive and HBV DNA positive hepatitis B prior to antiviral therapy. Other causes of chronic liver disease, such as HCV coinfection, autoimmune hepatitis and metabolic liver disorders were excluded. Children with diagnosed liver cirrhosis and evidence of other acute or chronic infections were excluded from this study. Informed consent was obtained from all patients' parents and the protocol was approved by the ethics committee of the Medical University of Bialystok, Poland. As a control group, 16 children (mean age – 10 years) were included without anamnestic, or laboratory signs of organic liver diseases or other systemic diseases. Standard liver tests were measured directly by validated automated methods and included total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl-transpeptidase (GGT). HBsAg and HBeAg were determined by ELISA and HBV DNA – by PCR method.

Measurement of serum YKL-40

YKL-40 was measured in serum samples (obtained after an overnight fast) using commercial kit (METRA, EIA kit, Quidel Corporation, San Diego, USA).

Histological analysis

All children underwent liver biopsy on the day after serum sampling. Liver specimens were fixed in buffered formalin and embedded in paraffin. Histological sections were stained using hematoxylin-eosin, Masson's Goldner, Masson's trichrome and reticulin stains. Fibrosis stage and inflammation grade were assessed in a blinded fashion by a single pathologist without knowledge of the patients' laboratory or clinical data. In order to determine specificity and sensitivity of the assay we arbitrarily defined advanced liver fibrosis as a score >2 and advanced inflammation as a score >3 according to Ishak et al. [17].

Statistics

Serum concentration of biochemical tests were expressed as mean values \pm standard deviation (SD). Statistical analysis was performed with the Mann-Whitney two-sample test for

nonparametric data. The relationship between biochemical tests and liver histology scores was analyzed by the Spearman rank-correlation test for nonparametric data and by the Pearson method for parametric data. Tests were considered statistically significant at $p < 0.05$. Receiver operating characteristics (ROC) analysis (AccuROC, Montreal, Canada) was used to calculate the power of the assay to detect advanced liver fibrosis. Comparison of the area under curve (AUC) was performed using a two-tailed p-test, which compares the AUC to the diagonal line of no information (AUC 0.5) [18].

Results

Patients characteristics

Selected biochemical and histological data are presented in *Tab. 1*.

Serum concentration of YKL-40

Serum concentration of YKL-40 was significantly higher in patients with chronic hepatitis B compared to controls (38.5 ± 19.2 vs 27.9 ± 8.75 ng/mL; $p = 0.032$). There were no significant correlations of YKL-40 with age ($r = 0.059$, $p = 0.65$), ALT ($r = 0.075$, $p = 0.563$), AST ($r = -0.065$, $p = 0.617$), GGT ($r = -0.0062$, $p = 0.96$), bilirubin ($r = 0.045$, $p = 0.728$) or liver fibrosis and inflammation according to Ishak et al. ($r = -0.168$, $p = 0.18$; $r = 0.005$, $p = 0.97$, respectively).

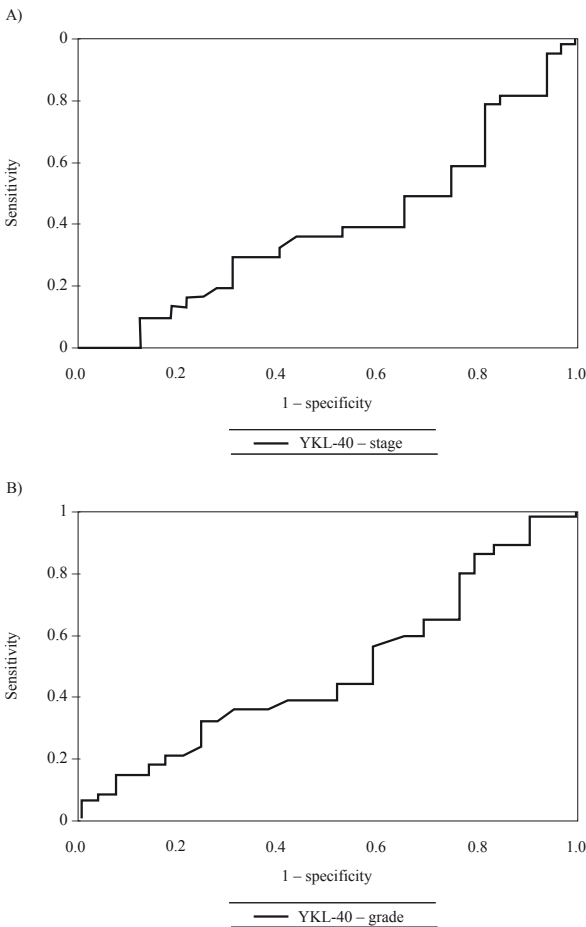
Diagnostic value of YKL-40 for identification of patients with advanced liver fibrosis and inflammation

All the examined children had liver fibrosis – 32 children (50.8%) had mild liver fibrosis: 27 of them – score 2 and 5 – score 1 and 31 children (49.2%) had advanced fibrosis (score >2 according to Ishak et al.): 24 of them – score 3, 4 – score 4 and 2 score 5. Children with advanced liver fibrosis had significantly higher activity of ALT and AST and higher grade of histological inflammation than children with mild fibrosis (*Tab. 2*).

The ability of YKL-40 to differentiate the children with advanced liver fibrosis from those with mild fibrosis was not significant (AUC = 0.387 ± 0.072 , $p = 0.12$) (*Fig. 1A*). The ability of YKL-40 to differentiate children with advanced inflammation ($n = 17$) from those with mild inflammation was not significant either (AUC = 0.488 ± 0.075 , $p = 0.87$) (*Fig. 1B*).

Table 2. Characteristics of 32 children with mild fibrosis and 31 children with advanced liver fibrosis

Data of the patients	Mild fibrosis (n=32) mean±SD	Advanced fibrosis (n=31) mean±SD	p-value
Age (years)	10±3.2	10.4±3.72	0.69
ALT (IU/L)	64±37	106±66	0.003
AST (IU/L)	54±19	82±45	0.02
GGT (IU/L)	14±11	15±8	0.51
Bilirubin (µmol/L)	10.1±5.1	10.1±3.42	0.91
YKL-40 (ng/mL)	42.5±22.6	34.3±14.0	0.12
Grading	3.06±1.07	4.48±1.41	0.0001

Figure 1. Receiver Operating Characteristic Analysis

ROC analysis of YKL-40 in predicting advanced liver fibrosis (A) and inflammation (B) according to Ishak et al. in children with chronic hepatitis B

Discussion

Liver biopsy is still regarded as the standard method to assess fibrosis stage but it is an invasive procedure with possible complications [19]. Furthermore biopsy sampling error can reach 30% for the difference in one stage, when using systems which range from 0 to 4 (cirrhosis). Finally, biopsy only provides static information about the fibrotic process [20,21]. For that reasons there is a clinical need for noninvasive measurement of liver fibrosis both to diagnose significant fibrosis and to

monitor the effects of antiviral or antifibrotic treatment. An ideal serum fibrosis marker should be liver-specific, independent of metabolic alteration, easy to perform, minimally influenced by impaired urinary and biliary excretion, and should reflect fibrosis in all types of chronic liver diseases, correlate with matrix deposition or removal. It should also be sensitive enough to discriminate between different stages of fibrosis and reflect the response to antifibrotic therapy but no single marker fulfills all the criteria sufficiently to merit routine clinical use yet [22,23].

Serum level of extracellular matrix components have been studied in children recently, but almost exclusively in patients with biliary atresia and cystic fibrosis [24-26]. To our knowledge noninvasive markers that predict advanced fibrosis due to chronic hepatitis B in children are lacking, except our previous studies. From the broad panel of matrix-derived serum markers (collagen IV, collagen VI, PIIINP, laminin-2, hyaluronan, MMP-2, TIMP-1, MMP-9/TIMP-1 complex, tenascin - C) the combination of serum hyaluronan and laminin-2 can accurately predict significant liver fibrosis [27]. We have also previously shown that serum TGF beta 1 and cystatin C does not predict advanced liver fibrosis in children with chronic hepatitis B [28,29]. We also found that APRI (aspartate aminotransferase to platelet ratio index) may be an accurate and simple index in predicting advanced liver fibrosis in children [30].

In our present study for the first time we assessed the potent fibrosis marker – YKL-40 in children with chronic hepatitis B and we found its significantly higher level in this group of children than in controls. However, there was no significant correlation of this biomarker with stage of liver fibrosis.

Data regarding assessment of YKL-40 in adults with chronic viral hepatitis are scarce. Our results are in agreement with data presented by Johansen et al. [31] and Nojgaard et al. [32] who also confirmed that YKL-40 level in patients with chronic liver disease were higher than in controls. However, they were not consistent with their other findings, because they showed significant positive correlation of this marker with histological stages. The similar results presented Saitou et al. [33] in patients with chronic hepatitis C, Zheng et al. [34] in patients with hepatic fibrosis due to schistosomiasis japonica and Nojgaard et al. [35] in patients with alcoholic liver disease.

Recently ROC analysis has been recommended to calculate the power of the assays to detect advanced liver fibrosis [18,36]. According to Kelleher et al. [37] in this study we arbitrarily

defined advanced liver fibrosis as a score >2 ("substantial" fibrosis) and mild fibrosis as a score ≤ 2 ("minimal" fibrosis). The ability of YKL-40 to differentiate children with advanced liver fibrosis from those with mild fibrosis was not significant. The studies in adults with chronic hepatitis C were not consistent with our findings. Saitou et al. [33] based on ROC analysis demonstrated that YKL-40 was superior to other serum fibrosis markers (hyaluronan, collagen type IV and PIIINP) in predicting severe fibrosis (stage 2-4) from mild fibrosis (stage 0-1). However, the ability of serum hyaluronan exceeded the ability of YKL-40 to determine fibrosis score 4 from scores 0-3. These data are in keeping with previous results in HIV/HCV co-infected patients with hepatic fibrosis [37].

The lack of correlation between fibrosis marker and liver histology in our study could be explained by findings that liver biopsy is not necessarily a gold standard for assessing liver histology and for that reason noninvasive markers will not have complete concordance with histological staging. It was established that the best correlation was found at the extreme spectra of fibrosis, i.e. low stage of fibrosis and cirrhosis [37]. In the examined group most of the children (51 out of the 63) had moderate disease (Ishak stage 2 and 3) and probable this was a reason for lacking the correlation between YKL-40 and histological staging in our group of children. According to Poynard et al. [38] inadequate liver biopsy rather than inaccuracy of serum markers was more commonly the cause for divergent results between biochemical panel of biomarkers (FibroTest) and biopsy. Other authors suggests that histological scoring systems are not sensitive enough to detect small changes in fibrosis stage and biomarkers may even be more accurate than biopsy in staging disease [39,40].

We conclude that YKL-40 does not predict advanced liver fibrosis in children with chronic hepatitis B but usefulness of this biomarker in this group of children needs to be evaluated in larger studies.

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