Original Article

Predictive accuracy of serum hyaluronic acid as a non-invasive marker of fibrosis in a cohort of multi-transfused Egyptian children with β-thalassaemia major

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ABSTRACT

Background and study aim: Liver disease remains a major cause of morbidity and mortality in patients with β-thalassaemia major (β-TM); therefore, its identification at an early stage is of great significance. Serum hyaluronic acid (HA) is considered as a non-invasive marker that appears early before pathological changes occur. We aim to determine the predictive accuracy of HA in detecting and staging hepatic fibrosis in β-TM patients.

Patients and methods: 30 Egyptian children with β-TM, and 15 age and sex-matched controls were studied. All had abdominal ultrasonography (US), measurement of serum amino-transferases (ALT, AST); hepatitis C, B and human immunodeficiency viruses (HCV, HBV, HIV) sero-markers, serum ferritin and HA. Liver biopsy was done for patients and fibrosis was scaled using Metavir scoring system and liver iron concentration (LIC) was measured.

Results: Twenty patients (67.7%) had sero-markers of HCV, none had HBV or HIV. Serum HA was significantly higher in patients (90.78 ± 28.79 ng/ml) compared to controls (21.1 ± 13.24 ng/ml) with $p < 0.05$. No difference between HCV infected and non-infected patients was detected. Positive significant correlation was detected between serum HA and stages of fibrosis by histopathology and US. No correlation was found between serum HA and age, sex, weight, height, haemoglobin level, platelet count, AST, serum ferritin, necro-inflammatory grade, and LIC.

Conclusions: Serum HA is a valuable non-invasive marker that may contribute to the assessment of liver fibrosis in multi-transfused children and adolescents with β-TM, irrespective of concomitant HCV infection.

Introduction

Liver disease, ending in fibrosis/cirrhosis, remains a major cause of morbidity and mortality in patients with β-thalassaemia major (β-TM). In spite of its clinical relevance, thalassaemia-associated liver damage had been insufficiently characterised [1]. Although, hepatic fibrogenesis has long been thought to be an irreversible process, it is now evident that it is a dynamic process with significant potential for reversal; unlike cirrhosis, which is irreversible [2], therefore, identification of liver fibrosis at an early stage would be of great significance [3]. Liver biopsy is still considered by many investigators the gold standard for assessing liver fibrosis [4]; however, it is inva-sive and can lead to potentially serious complications [5]. The liver normally removes hyaluronic acid (HA) via sinusoidal cell adhesion molecules. This mechanism is impeded in fibrosis, leading to a rise in serum levels of HA. Therefore, serum HA is considered a marker that appears early before pathological changes occur [6].

The findings on ultrasonography (US) in patients with liver disease have been well correlated with their pathological diagnosis made on liver biopsy [7]. Both US and serology have their own advantages and disadvantages in the evaluation of liver fibrosis [6]. Serum markers and imaging methods are increasingly in vogue as non-invasive alternatives to liver biopsy and the development of safe, inexpensive, and reliable non-invasive fibrosis measurement tools remains a research priority in clinical hepatology [8]. To the best of our knowledge, only one Chinese report by Xu and colleagues has addressed the value of HA in β-thalassaemic children, and no other studies have reproduced their findings [3].

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The aim of this work was to prospectively assess the value of serum HA as a non-invasive marker for detection and staging of hepatic fibrosis in a cohort of multi-transfused β-TM Egyptian children and adolescents with or without concomitant chronic hepatitis C virus (HCV) infection by correlating serum HA levels with a simultaneous liver biopsy.

Patients and methods

A prospective cross sectional analytic study enrolling thirty patients with age range between 6 and 18 years and fifteen age- and sex-matched healthy controls was carried out at both the Haematology and Hepatology Units, Cairo University Children Hospital, Cairo, Egypt. This number of patients and controls was considered as a start for a pilot study in our hospital to assess the feasibility of performance and the predictive accuracy of serum HA as a marker for hepatic fibrosis in our patients.

Patients diagnosed as thalassaemia intermedia or having associated extra hepatic disorders including cardiovascular, rheumatic, renal, pulmonary and malignant diseases were excluded. Patients with advanced cirrhosis with evidence of decompensation were also excluded. Fibrosis was excluded in the control group by ultrasound that revealed normal findings in all controls.

The hospital ethics committee approved the study, and parents or guardians of all patients and controls provided written informed consents for participation.

All patients and controls underwent careful history taking and physical examination, abdominal US using Toshiba® Sonolayer SSH-60A apparatus (Toshiba Corporation, Tokyo, Japan) as well as complete blood picture with differential counts, prothrombin time (PT), serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), ferritin, markers of HCV, hepatitis B virus (HBV) and human immune deficiency virus (HIV). Hepatic fibrosis by ultrasonography was categorised into four categories using the scoring system mentioned in Table 1 [9]. Serum HA level was measured by micro enzyme-linked binding protein test (Corgenix; Colorado, United States, under license of Chugai diagnostic science Co.). Percutaneous liver biopsy was performed to all patients using the Menghini aspiration technique [10]. Biopsy samples were examined pathologically by cutting 4-micrometre paraffin serial sections and staining with haematoxylin and eosin, periodic acid Schiff and Masson trichrome. The fibrosis stage and inflammatory grade were assessed using Metavir scoring system where F0; represents no fibrosis, F1; represents fibrosis without septa, F2; represents fibrosis with few septa, F3; represents fibrosis with numerous septa without cirrhosis, and F4 is established cirrhosis [11]. Liver iron concentration (LIC) was measured by atomic absorption spectrometry on lyophilised portions of the needle biopsy [12].

Assay of serum HA

Blood was collected by venipuncture, serum separated by centrifugation and specimens were stored at $-80\,°C$ until the time of thawing and assay. Serum HA was measured by micro enzyme-linked binding protein test according to the manufacturer's

instructions. A 100-μL aliquot of serum or reference solution (dilution 1/10) was added to each hyaluronic-binding protein (HABP)-coated microwell and incubated for 30 min. Subsequently, 100 μL of stop solution (0.36 N sulphuric acid) was added. The HA concentration was assayed by using an enzyme-linked binding protein assay that used HABP as the capture molecule.

HA levels in patient and control samples were determined against a reference curve prepared from the reagent blank (zero ng/mL) and the HA reference solutions provided with the kit (50, 100, 200, 500, 800 ng/mL).

Normal range

Serum samples from 100 healthy blood donors (population A) tested with three HA kit lots. The mean HA value of this population was determined to be 28.5 ng/ml with a standard deviation of 24.0 ng/ml. A normal cut-off value of 75 ng/mL was established based on the 95th percentile of the normal population.

HA normal range for the reagent used = zero − 75 ng/mL as mentioned by the manufacturer (Corgenix; Colorado, United States).

Statistical analysis

Parametric data were expressed as mean ± standard deviation (SD), and non-parametric data were expressed as number and percentage of the total. Comparing the mean ± SD of two groups was done using the paired and unpaired Student's *t* tests. Measuring the mutual correspondence between two values was done using the Spearman correlation coefficient. Probability (*p*) value <0.05 was considered as statistically significant. The sensitivity, positive (PPV) and negative predictive values (NPV) of the tests were calculated. Data were analysed by Microsoft Office 2007 (excel) and statistical package for social science (SPSS) version 19.0.0.

Results

Sixteen males (53.33%) and 14 females (46.67%) were enrolled. Their ages ranged from 8 to 18 years with a mean of 14.07 ± 3.37 years. The baseline characteristics and laboratory findings of patients and controls are illustrated in Table 2.

Twenty patients had markers of HCV infection and ten were negative. None had markers of HBV or HIV infection.

The liver biopsy findings of fibrosis were classified according to Metavir scoring system. The mean LIC was 14.46 ± 6.47 (μg Fe/g dry liver weight).

Table 1
Ultrasound scoring system of fibrosis [9].

	Score 0	Score 1	Score 2	Score 3
Edge	Sharp	Mildly blunted edge	Blunted edge	
Surface	Smooth	Mildly irregular	Irregular	Highly irregular
Parenchymal texture	Fine	Mildly coarse	Coarse	Highly coarse

Table 2
Base line characteristics and laboratory data of patients and controls.

	Patients	Controls
Age (years)	14.07 ± 3.37	12.60 ± 3.20
Age of onset (months)	10.77 ± 3.76	–
Sex (male:female)	16:14	8:7
Duration of blood transfusion (years)	17.23 ± 9.12	–
Duration of iron chelation (years)	15.41 ± 8.79	–
Weight (kg)	33.57 ± 8.32	33.98 ± 20.13
Height (cm)	139.67 ± 11.02	139.87 ± 25.76
Hb (gm/dl)	5.97 ± 1.06	12.11 ± 2.34
Platelets (x10/cmm)	431.50 ± 122.92	300.12 ± 150.31
HCV +ve (%)	67.67	0
HIV +ve (%)	0	0
HBV +ve (%)	0	0
Serum ferritin (ng/ml)	2762.80 ± 1438.95	210.33 ± 150.12
ALT (U/L)	41.13 ± 33.92	15.41 ± 10.22
AST (U/L)	64.60 ± 53.6	16.85 ± 11.17

Data are represented in the form of mean ± SD.