Odnos između koncentracije hijaluronske kiseline u serumu i stadija jetrene fibroze kod bolesnika s kroničnim hepatitisom

Relationship between serum hyaluronic acid level and stage of liver fibrosis in patients with chronic hepatitis

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Sažetak

Uvod: Biopsija jetre je zlatni standard u procjeni stadija bolesti jetre, no podložna je brojnim komplikacijama. Predlažu se neke neinvazvne metode kao zamjenski biljezi umjesto biopsije jetre. Prikazano je da koncentracije hijaluronske kiseline (engl. *hyaluronic acid,* HA) rastu sa stadijem težine fibroze jetre. Cilj ovoga istraživanja bio je utvrditi razliku u koncentraciji HA između bolesnika s fibrozom jetre i zdravih ispitanika koji nemaju znakove jetrene bolesti, kao i optimalnu graničnu vrijednost za razlikovanje tih dviju skupina ispitanika u populaciji Irana.

Materijali i metode: Serumska koncentracija HA kod bolesnika s kroničnim hepatitisom (N = 62) i kontrolnih ispitanika (N = 20) uspoređena je metodom ELISA, a stadij fibroze određen je prema modificiranom Knodellovu indeksu.

Rezultati: Srednja vrijednost koncentracije HA u serumu bolesnika (113,4 ± 59,2 ng/mL) bila je viša od one kod kontrolne skupine (46,0 ± 10,5 ng/mL; P < 0,001). Bolesnici s višim stupnjem i stadijem nekrotičnih oštećenja jetre imali su više koncentracije HA u serumu. Razlike između različitih stadija jetrene fibroze i koncentracije HA u serumu bile su statistički značajne kod svih stadija fibroze u usporedbi s kontrolnom skupinom (P < 0,05). Granična vrijednost koncentracije HA u serumu (59,5 ng/mL) pokazala je dobru osjetljivost (80,3%) i specifičnost (85%).

Zaključak: Postoji jaka korelacija između koncentracije HA u serumu i stupnja nekrotičnih oštećenja jetre. Naši rezultati ukazuju na to da je povećanje koncentracija HA u serumu iznad prediktivne vrijednosti povezano s jetrenom fibrozom te se može rabiti kao neinvazivni indeks razlikovanja između bolesnika s jetrenom fibrozom i zdravih ispitanika.

Ključne riječi: kronični hepatitis; stupanj upale jetre; stupanj jetrene fibroze; hijaluronska kiselina

Abstract

Background: Liver biopsy is the gold standard for assessing liver disease stage, but it is prone to some complications. Noninvasive methods have been proposed as surrogate markers for liver biopsy. Serum hyaluronic acid (HA) level has been shown to increase with the development of liver fibrosis. The aim of this study was to determine differences in HA concentration between patients with liver fibrosis and healthy individuals without any signs of liver disease, and the optimal cut-off value for discrimination between the two study groups of Iranian population.

Materials and methods: Serum HA levels in chronic hepatitis patients (N = 62) and controls (N = 20) were compared by ELISA, while the stage of fibrosis was assessed by the modified Knodell score.

Results: The mean serum HA concentrations was higher in patients (113.4 ± 59.2 ng/mL) than in the control group (46.0 ± 10.5 ng/mL; P < 0.001). Patients with higher stages and grades of liver necroinflammatory injuries had higher serum HA concentrations. Differences in serum HA concentrations were statistically significant in all fibrosis stages as compared to healthy controls (P < 0.05). The cut-off point of serum HA concentration identified (59.5 ng/mL) showed a reasonably good sensitivity (82.3%) and specificity (85%).

Conclusions: There was a strong correlation between serum HA concentration and the grade of liver necroinflammatory injury. Our findings suggested serum HA concentration increases above the predictive value to be associated with liver fibrosis and could be used as a noninvasive marker to discriminate between patients with liver fibrosis and healthy individuals.

Key words: chronic hepatitis; hepatic inflammation grade; hepatic fibrosis stage; hyaluronic acid

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Uvod

Jetrena fibroza obilježena je pojačanim odlaganjem izvanstaničnog matriksa (engl. *extracellular matrix,* ECM) uključujući molekularnu i histološku preraspodjelu raznih vrsta kolagena, glikoproteina i proteoglikana/glikozaminoglikana (1,2). Odlaganje ECM u Disseovu prostoru jetre (perisinusoidna fibroza), stvaranje (nepotpunih) subendotelnih bazalnih membrana i onemogućavanje funkcioniranja hepatocita iz okolnog matriksa ne nanose štetu samo protoku krvi kroz organ, nego i biosintetičkoj funkciji hepatocita i sposobnosti uklanjanja tih i ostalih stanica. Stoga je postavljanje dijagnoze, praćenje kliničke slike i terapije fibrogeneze klinički vrlo važno (3).

Značajna sastavnica u kliničkoj obradi jetrene fibroze je klinička procjena težine bolesti. Histologija jetre često se smatra zlatnim standardom za određivanje težine upale i jetrene fibroze. Međutim, biopsija jetre je invazivan postupak koji može izazvati neželjene pojave kao što su bol u 20% do 30% slučajeva, veće komplikacije u 0,5% slučajeva, pa čak i smrt. Zbog komplikacija izazvanih postupkom i činjenice da bolesnici rijetko prihvaćaju biopsiju, troškovi takvih postupaka su visoki (4). Stoga se zamjenski biljezi jetrene fibroze mogu smatrati primjerenima za smanjenje broja postupaka biopsije jetre kod bolesnika s hepatitisom.

Među brojnim serumskim biljezima jetrene fibroze, hijaluronska kiselina (engl. *hyaluronic acid*, HA) je pokazatelj ciroze jetre i značajno doprinosi neželjenom ishodu kroničnih jetrenih bolesti. HA je nerazgranati anionski polisaharid velike molekularne težine i vrlo važna sastavnica izvanstaničnih matriksa (5). U jetri HA uglavnom sintetiziraju jetrene zvjezdaste stanice, a razgrađuju ju sinusoidne endotelne stanice. Koncentracija HA u serumu izravno raste s napredovanjem kronične jetrene bolesti. Iako je HA uzajamno povezana s fibrozom, nije izravno povezana s upalom i nekrozom (6-8). Kod bolesnika s kroničnim virusom hepatitisa C (engl. *chronic hepatitis C virus*, HCV) koncentracije HA rastu s višim stadijem fibroze jetre. Štoviše, kod bolesnika oboljelih od ciroze, koncentracija HA je u uzajamnoj vezi s kliničkom težinom bolesti (9,10).

Postoji nekoliko objavljenih istraživanja (11,12) provedenih na populaciji Irana o odnosu između serumske koncentracije HA i stadija jetrene fibroze ili stupnja upale kod bolesnika s kroničnim hepatitisom. Ta su istraživanja provedena na bolesnicima s kroničnim hepatitisom B, a značenje takvih biljega kod bolesnika s kroničnim hepatitisom C i autoimunim hepatitisom (engl. *autoimmune hepatitis*, AIH), naročito u području sjevernog Irana, još je uvijek nejasan. U njima se jetrena fibroza procjenjivala Ishakovom ljestvicom (13) u biopsiji jetre pa nema podataka o odnosu između serumske koncentracije HA i nekrotičnih oštećenja jetre prema Knodellovu indeksu (14). Stoga je naš cilj bio utvrditi razlike u koncentracijama HA između bo-

Introduction

Liver fibrosis is characterized by excess deposition of extracellular matrix (ECM) involving molecular and histological rearrangement of various types of collagens, glycoproteins and proteoglycans/glycosaminoglycans (1,2). Deposition of ECM in the liver spaces of Disse (perisinusoidal fibrosis), the generation of (incomplete) subendothelial basement membranes, and strangulation of hepatocytes by the surrounding matrix impair not only the blood flow through the organ, but also the biosynthetic function of hepatocytes and the clearance capability of these and other cells. Thus, the diagnosis, follow-up and therapeutic monitoring of fibrogenesis are of great clinical importance (3).

An important component of the management of hepatic fibrosis is clinical assessment of the disease severity. Liver histology is frequently considered the gold standard for establishing the severity of hepatic necroinflammation and fibrosis. However, liver biopsy is an invasive procedure that may cause undesirable events such as pain in 20% to 30% of cases, major complications in 0.5%, and even death. In addition to the complications derived from the procedure and frequently poor patient acceptance, the direct cost of such procedures is high (4). Thus, identification of surrogate markers of liver fibrosis would be useful to reduce the number of liver biopsies in patients with hepatitis.

Among various serum markers of hepatic fibrosis, hyaluronic acid (HA) is a hallmark of liver cirrhosis and contributes significantly to the deleterious outcome of chronic liver diseases. HA is an unbranched high-molecular weight anionic polysaccharide that forms a critical component of extracellular matrices (5). In the liver, HA is mostly synthesized by the hepatic stellate cells and is degraded by the sinusoidal endothelial cells. Serum HA levels increase directly with the progression of chronic hepatic disease. Although HA has been correlated with fibrosis, it is not truly associated with inflammation and necrosis (6-8). In patients with chronic hepatitis C virus (HCV), HA levels increase with the development of liver fibrosis. Moreover, in patients with cirrhosis, HA levels correlate with clinical severity (9,10).

In Iranian population, there are few published studies (11,12) concerning the relationship between serum HA levels and liver fibrosis stage or inflammation grade in patients with chronic hepatitis. The published studies were performed in chronic hepatitis B patients; therefore the value of such markers in patients with chronic hepatitis C and autoimmune hepatitis (AIH) – especially in the north of Iran – remain unclear. In addition, the published studied used the Ishak scoring system (13) to assess liver fibrosis in liver biopsy and there is no report concerning the relationship between HA serum levels and liver necroin-

lesnika s jetrenom fibrozom (bolesnici s kroničnim hepatitisom B, C i AIH) i zdravih ispitanika koji nemaju znakova jetrene bolesti, te naći optimalnu graničnu vrijednost za razlikovanje između dviju skupina ispitanika iz sjevernog Irana. Kao dodatak ovom istraživanju u promatranje je uključena histološka procjena rezultata biopsije jetre prema modificiranom Knodellovu indeksu.

Materijali i metode

Ispitanici

U ovom retrospektivnom istraživanju parova sudjelovala su je 62 bolesnika (35 muškaraca i 27 žena; medijan dobi (raspon): 35 (15-65) godina), među kojima je 35 bolesnika imalo hepatitis B, 14 hepatitis C i 13 je imao AIH. Ispitanici su odabirani u razdoblju od svibnja 2007. do srpnja 2008. iz niza bolesnika upućenih u Centar za gastroenterološka istraživanja u Tabrizu i na Sveučilište medicinskih znanosti u Gorganu (Gonbadu). Bolesnici su uključeni u istraživanje ako su bili pozitivni na HBs-Ag ili na antitijela virusa hepatitisa C (HCV) te im je aktivnost trajno povišenih serumskih aminotransferaza bila 1,5 puta viša od gornje granice referentnog raspona barem zadnjih 6 mjeseci. Bolesnicima s AIH dijagnoza je postavljena prema protokolu Međunarodne skupine za autoimuni hepatitis (engl. *International Autoimmune Hepatitis Group Report*) (15). Za određivanje indeksa jetrene fibroze, svim je bolesnicima napravljena biopsija jetre kao dio standardnog dijagnostičkog postupka te su raspoređeni u podskupine prema rezultatima indeksa histološke aktivnosti (engl. *histological activity index,* HAI).

Bolesnici s poviješću gastrointestinalnog krvarenja i nekih kroničnih jetrenih bolesti (Wilsonova bolest, hemokromatoza, nedostatak alfa$_1$-antitripsina, karcinom jetre), aktivnom intravenskom zlouporabom droge i transplatiranom jetrom bili su izuzeti iz istraživanja.

Kontrolni serumi za određivanje koncentracije HA dobiveni su od 20 zdravih dobrovoljnih davatelja koji su upućeni na Sveučilište medicinskih znanosti u Tabrizu (10 žena i 10 muškaraca; medijan dobi (raspon): 41 (20-69) godina). Budući da je kod zdravih ispitanika aktivnost serumske aminotransferaze i alkalne fosfataze (ALP) bila u granicama normale, oni nisu trebali proći biopsiju jetre. Prema podacima iz upitnika, ispitanici nisu imali povijest gastrointestinalnog krvarenja niti neke od kroničnih jetrenih bolesti, nisu nikada pušili niti uzimali alkohol, u obitelji nisu imali slučajeve hepatitisa niti jetrenih bolesti, nisu aktivno intravenski uzimali drogu niti im je transplatirana jetra. Osobe koje su pušile (> 1 cigareta/dan) i pile alkohol (> 5 g/dan) razvrstane su kao pušači i osobe koje uživaju alkohol.

flammatory injuries according to the modified Knodell score system (14). Therefore, we aimed to determine differences in the concentrations of HA between patients with liver fibrosis (chronic hepatitis B, C and AIH patients) and healthy individuals without any signs of liver disease, as well as the optimal cut-off value for discrimination between the two study groups from the north of Iran. Moreover, histological evaluation of liver biopsies according to the modified Knodell score system was considered in the present study.

Materials and Methods

Subjects

Sixty-two patients (35 men and 27 women; age median (range): 35 (15-65) years) were enrolled in this case-control, retrospective study. Hepatitis B was present in 35, hepatitis C in 14 and AIH in 13 patients. Patients were selected from May 2007 till July 2008 among patients referred to Gastroenterology Research Center of Tabriz and Gorgan (Gonbad) University of Medical Sciences for diagnostic work-up. Patients were included in the study if they were positive for serum hepatitis B surface antigen (HBs-Ag) or hepatitis C virus (HCV) antibodies, and had persistently elevated serum aminotransferases greater than 1.5-fold upper limit of the reference range for at least six months. AIH patients were diagnosed according to the International Autoimmune Hepatitis Group Report protocol (15). For assessment of liver fibrosis scores, all patients underwent liver biopsy as part of the regular diagnostic procedure and were subclassified according to the histological activity index (HAI) score.

Patients with a history of gastrointestinal bleeding and chronic liver disease (Wilson's disease, hemochromatosis, alpha$_1$-antitrypsin deficiency, biliary disease, hepatocellular carcinoma), active intravenous drug abuse, and liver transplantation were excluded.

Control sera for HA determination were obtained from 20 healthy volunteers referred to the Tabriz University of Medical Sciences (10 women and 10 men; age median (range): 41 (20-69) years). These healthy subjects had normal serum levels of aminotransferases and alkaline phosphatase (ALP), therefore did not have to undergo liver biopsy. Also, these persons had no history of gastrointestinal bleeding and chronic liver disease, smoking (never smoker), alcohol intake (never drinker), no family history of hepatitis and liver disease, and no active intravenous drug abuse and liver transplantation, according to the information collected by use of a questionnaire. The subjects that smoked (> 1 cigarette/day) and drunk alcohol (> 5 g/day) were classified as smokers and alcohol drinkers.

Svi bolesnici su pismeno dali svoj obaviješteni pristanak da se njihovi podaci rabe u znanstvene svrhe. Istraživanje je odobrio Etički odbor Medicinskog sveučilišta u Tabrizu.

Metode

Uzorci krvi (5 mL) sakupljani su ujutro natašte. Serum je odvojen (na 2500 x g 5 minuta) u roku od jednog sata od uzimanja krvi. Na alikvotima svakog seruma napravljene su standardne pretrage funkcije jetre uključujući određivanje aktivnosti aspartat-aminotransferaze (AST), alanin-aminotransferaze (ALT), alkalne fosfataze (ALP) i serološka dijagnostika hepatitisa komercijalno dostupnim testovima. Ukratko, aktivnosti AST i ALT izmjerene su kako su opisali Reitman i Frankel kolorimetrijskim testom (Ziestchem kit, Tehran, Iran) s 2,4 DNPH (2,4 dinitrofenilhidrazon), a aktivnost ALP testirana je pomoću PNPP (engl. *para-nitrophenylphosphate*) kao supstratom (Ziestchem kit, Tehran, Iran) na spektrofotometru Apel (PD303S, Japan).

Serološki biljezi hepatitisa analizirani su na čitaču ELISA mikrotitarskih pločica (Norahan Fajr, Iran) i priborom opisanim u nastavku. Analizirani su slijedeći serumski biljezi za AIH: antinuklearna antitijela (ANA; Biazyme ELISA kit, Birmingham, UK) i anti-LKM-1 (antitijela na mikrosome jetre i bubrega) (engl. *type 1 liver and kidney microsomes*, LKM-1, Euroimmun ELISA kit, Njemačka), serološki biljezi hepatitisa B bili su: hepatitis B površinski antigen (engl. *hepatitis B surface antigen*, HBsAg; Diakey, Shinjin Medics Inc. ELISA kit, Koreja), antitijelo na HBsAg (engl. *hepatitis B surface antibody*, HBsAb; Diakey, Shinjin Medics Inc. ELISA kit, Koreja) i antitijelo na HBc (engl. *hepatitis B core antibody*, HBcAb; Dia-Pro ELISA kit, Italija). Serološki biljeg hepatitisa C bilo je antitijelo na hepatitis C virus (HCV) (engl. *HCV antibody*, HCVAb; Diakey, Shinjin Medics Inc. ELISA kit, Koreja). Za otkrivanje HCV u serumu rabio se reagens za lančanu reakciju polimeraze (engl. *polymerase chain reaction,* PCR) za određivanje HCV (PCR kit za određivanje HCV) koja se temelji na umnažanju specifične cDNA (engl. *complemetary DNA*) reverznom transkriptazom od 5' kraja HCV genoma. Izolacija RNA napravljena je priborom Accuprep viral RNA extraction kit (Bioneer Corporation, Koreja) prema uputama proizvođača. Za PCR umnažanje korišten je komercijalni kit (PCR kit, Roche Diagnostics, Mannheim, Njemačka) na uređaju Eppendorf Thermocycler (Mastercycler Personal) prema uputama proizvođača. Rabljene su dvije početnice (Sinagene, Tehran, Iran) sa sekvencama kako slijedi: za prvu početnicu: 5'-GCA GAA AGC GTC TAG CCA TGG CGT-3' i za drugu početnicu: 5'-CTC GCA AGC ACC CTA TCA GGC AGT-3'.

Ostatak uzoraka krvi pohranjen je na -20 °C. Isti postupak proveden je s uzorcima kontrolnih ispitanika.

Koncentracija HA u serumu odredila se na čitaču ELISA mikrotitarskih pločica (Immunoscan, Lab System, Švicarska) i priborom HA-ELISA Kit (HA-test, broj: K-1200, Echelon Bioscience Inc., SAD). Metoda HA-ELISA je kompe-

All patients gave a written informed consent to use these data for scientific purposes and the study was approved by the Tabriz Medical University Ethics Committee.

Blood sampling and analysis

Blood samples (5 mL) were collected after an overnight fast. Serum was separated (at 2500 x g for 5 minutes) within one hour of blood collection. Standard liver function tests, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline-phosphatase (ALP) and hepatitis serology were performed using commercially available kits. Briefly, AST and ALT were measured according to Reitman and Frankel by colorimetric test (Ziestchem kit, Tehran, Iran) with 2,4 DNPH (2,4 dinitrophenylhydrazone) and ALP was assayed with PNPP (para-nitrophenylphosphate) as a substrate (Ziestchem kit, Tehran, Iran) by Apel spectrophotometer (PD303S, Japan).

The hepatitis serology markers were analyzed on an ELISA reader (Norahan Fajr, Iran) using the respective kits. The following serum markers were analyzed for AIH: antinuclear antibody (ANA; Biazyme ELISA kit, Birmingham, UK) and anti-LKM-1 (LKM-1: type 1 liver and kidney microsomes, Euroimmun ELISA kit, Germany); hepatitis B serology markers: hepatitis B surface antigen (HBsAg; Diakey, Shinjin Medics Inc. ELISA kit, Korea), hepatitis B surface antibody (HBsAb; Diakey, Shinjin Medics Inc. ELISA kit, Korea) and hepatitis B core antibody (HBcAb; Dia-Pro ELISA kit, Italy); and hepatitis C serology marker: HCV antibody (HCVAb; Diakey, Shinjin Medics Inc. ELISA kit, Korea). The HCV PCR kit was used to detect HCV in sera based on the amplification of the specific cDNA reverse transcribed from the 5' region of the HCV genome. For RNA extraction we used Accuprep viral RNA extraction kit (Bioneer Corporation, Korea), according to the manufacturer's instructions. The PCR was carried out with the PCR kit (Roche Diagnostics, Mannheim, Germany) by an Eppendorf Thermocycler (Mastercycler Personal) according to the manufacturer's instructions. Two primers (Sinagene, Tehran, Iran) with the following sequences were used: first primer sequence 5'-GCA GAA AGC GTC TAG CCA TGG CGT-3' and second primer sequence 5'-CTC GCA AGC ACC CTA TCA GGC AGT-3'.

The rest of blood samples were stored at -20 °C. Control samples were dealt with in the same manner.

Serum HA was assayed using an ELISA reader (Immunoscan, Lab System, Switzerland) and HA-ELISA Kit (HA-test, product number: K-1200, Echelon Bioscience Inc, USA). The HA-ELISA method was a competitive ELISA assay in which the colorimetric signal was inversely proportional to the amount of HA present in the sample. The concentration of HA in samples was determined from a standard curve using the reagent blank (0 ng HA/mL) and HA reference solutions (50, 100, 200, 400, 800 and 1600 ng HA/mL). Serum HA concentrations were determined in

titivna ELISA metoda u kojoj je kolorimetrijski signal bio obrnuto proporcionalan koncentraciji HA u uzorku. Koncentracija HA u uzorcima određena je iz kalibracijske krivulje sa slijepom probom (0 ng HA/mL) i kalibratorima HA različitih koncentracija (50, 100, 200, 400, 800 i 1600 ng HA/mL). Koncentracija HA u serumu određena je u jednoj analitičkoj seriji unutar jednog radnog dana. Varijabilnost unutar serije izražena kao koeficijent varijacije (CV) za tu metodu prema navodu proizvođača iznosi 5%.

Histološka procjena oštećenja jetre

Kod 62 bolesnika napravljena je perkutana biopsija jetre (biopsijskom iglom Tru-Cut broj 16, uz kontrolu ultrazvukom tipa B) kako bi se procijenila prisutnost i težina jetrene bolesti. Uzorci biopsije jetre bili su fiksirani 12 sati u 10%-tnoj otopini formalina i stavljeni u parafin. Presjeci tkiva obojani su hematoksilin-eozinom, Massonovom trikromnom bojom i retikulinskom bojom za histološki pregled i za određivanje stupnja oštećenja jetre.
U uzorcima dobivenim biopsijom jetre određen je stupanj težine bolesti i stadij proširenosti prema modificiranom Knodellovu indeksu koji označava stupanj upale (14). Bodovna ljestvica od 0-18 temeljena je na zbroju bodova svih četiriju indeksa:

1) periportalni ili periseptalni *interface* hepatitis (*piecemal* nekroze, bodovi 0-4);
2) konfluirajuća nekroza (bodovi, 0-6);
3) žarišna (mjestimična) litična nekroza, apoptoza, žarišna upala (bodovi 0-4);
4) portalna upala (bodovi 0-4).

Stadij fibroze određen je temeljem slijedećih uputa:

- stadij 0 – nema fibroze;
- stadij 1 – fibrozno širenje nekih portalnih područja s kraćim fibroznim septumima ili bez njih;
- stadij 2 – fibrozno širenje većine portalnih područja s kraćim fibroznim septumima ili bez njih;
- stadij 3 – fibrozno širenje većine portalnih područja s mjestimičnim portalno-portalnim (P-P) premoštenjima;
- stadij 4 – fibrozno širenje portalnih područja s izrazitim premoštenjima [portalno-portalnim (P-P) kao i portalno-centralnim (P-C)];
- stadij 5 – izrazito premoštavanje (P-P i/ili P-C) s mjestimičnim kvržicama (nepotpuna ciroza);
- stadij 6 – vjerojatna ili potvrđena ciroza (15).

Statistička analiza

Statistička analiza napravljena je statističkim programskim paketom SPSS 12.0 (SPSS for Windows 12.0, SPSS, Chicago, IL, SAD). Podaci su izraženi kao srednja vrijednost ± SD ili medijan i raspon, a vrijednost $P < 0,05$ se smatrala statistički značanjom. Razlike u koncentraciji HA u serumu bolesnika s različitim tipovima kroničnog hepatitione analytical batch in one working day. The intra-assay variability (coefficient of variation, CV) of the procedure declared by the manufacturer was 5%.

Histological assessment of liver damage

Sixty-two patients underwent percutaneous liver biopsy (with a Trucut needle number 16 guided by B type ultrasound) to assess the presence and severity of liver disease. Biopsy fragments were fixed in 10% formalin solution for 12 hours and embedded in paraffin. Sections were stained with hematoxylin-eosin, Masson's trichrome and reticulin stain to establish the histological diagnosis and the extent of liver lesions.
Specimens were graded and staged according to the modified Knodell scoring system to determine inflammation grade (14). The grading system scored 0-18 and was based on the sum of four indices:

1) periportal or periseptal interface hepatitis (piecemeal necrosis, score 0-4);
2) confluent necrosis (score 0-6);
3) focal (spotty) lytic necrosis, apoptosis, and focal inflammation (score 0-4); and
4) portal inflammation (score 0-4).

Fibrosis was scored as follows:

- stage 0 – no fibrosis;
- stage 1 – fibrous expansion of some portal areas, with or without short fibrous septa;
- stage 2 – fibrous expansion of most portal areas, with or without short fibrous septa;
- stage 3 – fibrous expansion of most portal areas with occasional portal to portal (P-P) bridging;
- stage 4 – fibrous expansion of portal areas with marked bridging [portal to portal (P-P) as well as portal to central (P-C)];
- stage 5 – marked bridging (P-P and/or P-C) with occasional nodules (incomplete cirrhosis); and
- stage 6 – probable or definite cirrhosis (15).

Statistical analysis

The SPSS 12.0 statistical package (SPSS for Windows 12.0, SPSS, Chicago, IL, USA) was used on statistical analysis. Data were expressed as mean ± SD, or median and range, and P less than 0.05 was considered statistically significant. The mean patient serum HA levels between different types of chronic hepatitis and healthy controls were compared with analysis of variance (ANOVA). For multiple comparisons, we used the post-hoc, least square differences (LSD) method to compare HA levels of different fibrosis stages with those in control subjects. The correlation between serum HA level, liver fibrosis stage and inflammation grade was calculated by Spearman's correlation coefficient (r_S).

sa i kontrolnih ispitanika analizirani su analizom varijance ANOVA. Za višestruke usporedbe rabili smo *post hoc* test najmanje značajne razlike (engl. *least square differences*, LSD) kako bismo usporedili koncentracije HA kod različitih stadija fibroze s vrijednostima kontrolne skupine. Korelacija između koncentracija HA u serumu, stadija jetrene fibroze i stupnja upale izražena je Spearmanovim koeficijentom korelacije (r_S).

Kako bi se procijenila dijagnostička točnost koncentracije HA za razlikovanje bolesnika s jetrenom fibrozom i zdravih ispitanika, načinjena je ROC analiza (engl. *receiver operating characteristic*) te za usporedbu izračunata površina ispod krivulje (engl. *area under the curve*, AUC) (16). Vrijednost AUC od 1 je značajka idealnog testa, dok vrijednost 0,5 ukazuje na test bez dijagnostičke vrijednosti (17,18).

Rezultati

Značajke bolesnika i kontrolnih ispitanika uključenih u ovo istraživanje prikazane su u tablici 1., gdje se vidi da je kod bolesnika koncentracija HA u serumu bila viša nego u skupini kontrolnih ispitanika (P < 0,001). 56,4% bolesnika imalo je kronični hepatitis B, 22,6% kronični hepatitis

To assess the diagnostic accuracy of HA for differentiating chronic hepatitis patients with severe liver fibrosis from healthy individuals, we plotted the receiver operating characteristic curve (ROC) and calculated the area under the curves (AUC) (16). An AUC of 1.0 is characteristic of an ideal test, whereas 0.5 indicates a test of no diagnostic value (17,18).

Results

Characteristics of the patients and control subjects included in the study are summarized in Table 1. As shown in this table, the patients had higher serum HA levels than the control group (P < 0.001). Hepatitis serology revealed that 56.4% of patients were suffering from chronic hepatitis B, 22.6% from chronic hepatitis C and 20.9% from AIH. Histological examination of the liver for fibrosis scoring revealed 35% of patients to have significant fibrosis (stage ≥3).

The mean serum HA concentrations in patients with HBV, HCV, AIH and healthy controls are presented in Table 2. These results showed statistically increased serum HA levels in patients as compared with healthy controls

Tablica 1. Značajke bolesnika i kontrolne skupine

Table1. Characteristics of the patient and control groups

Controls (N = 20)	Patients (N = 62)	Variables
10 / 10	35 / 27	Male/Female (N)
41.0 (20-69)	35.0 (15-65)	Age in years, median (range)
0	7	Smoking (N)
0	1	Alcohol intake (N)
0	5	Family history of Hepatitis (N)
0	1	Family history of Chronic Liver disease (N)
0	2	Drug Abuse (N)
46.0 ± 10.5	113.4 ± 59.2*	Hyaluronic acid ng/mL (mean ± SD)

* P < 0.001; Mann-Whitney U test

Tablica 2. Usporedba koncentracije HA kod bolesnika s različitim tipovima hepatitisa sa kontrolnom skupinom.

Table 2. Comparison of hyaluronic acid serum concentration in different types of chronic hepatitis and control group.

Groups	Hyaluronic acid (ng/mL)	Range (ng/mL)	P (*vs.* control)
Chronic hepatitis B (N = 35)	108.0 ± 58.7	45.0-285.0	< 0.001
Chronic hepatitis C (N = 14)	122.4 ± 62	46.0-245.0	< 0.001
Autoimmune hepatitis (N = 13)	117.4 ± 60.9	490-241.0	< 0.001
Control (N = 20)	46.0 ± 10.5	27.0-62.0	-
ANOVA; post hoc LSD method			

C i 20,9% AIH. Histološkim pregledom jetre za bodovanje fibroze otkriveno je da 35% bolesnika ima teži stadij fibroze (stadij ≥3).

Koncentracija HA u serumu kod bolesnika s HBV, HCV i AIH prikazana je u tablici 2. Ovi rezultati pokazuju statistički značajno povišene koncentracije HA u serumu bolesnika u usporedbi s kontrolnom skupinom (P < 0,001). Usporedba serumske koncentracije HA analizom varijance ANOVA i *post hoc* LSD testom pokazala je da nema statistički značajnih razlika među podskupinama s različitim tipovima hepatitisa (HBV u usporedbi s HCV: P = 0,400; HBV u usporedbi s AIH: P = 0,599; HCV u usporedbi s AIH: P = 0,803).

Tablica 3. prikazuje koncentracije HA u serumu kod različitih stadija jetrene fibroze. Razlike u koncentraciji HA u serumu dobivene ANOVA *post hoc* LSD testom kod bolesnika u usporedbi s kontrolnom skupinom bile su statistički značajne (P < 0,001) kod gotovo svih stadija jetrene fibroze, osim stadija 0 jetrene fibroze gdje se serumska koncentracija HA nije statistički značajno razlikovala (P = 0,439) od one u kontrolnoj skupini. Istodobno provedene višestruke usporedbe koncentracija HA u serumu kod svih 6 stadija jetrene fibroze pokazale su statistički značajne razlike serumske koncentracije HA za sve stadije jetrene fibroze na razini značajnosti < 0,001, uz tri iznimke: usporedba stadija 0 sa stadijem 1 (P = 0,003), stadija 1 sa stadijem 2 (P = 0,006) i stadija 2 sa stadijem 3 (P = 0,040). Samo je jedan bolesnik imao stadij 6 jetrene fibroze, stoga njegove rezultate nismo statistički uspoređivali sa skupinom zdravih ispitanika. Bolesnici s višim stadijem jetrene fibroze imali su više koncentracije HA u serumu. Ova je korelacija bila linearna i vrlo snažna (r_S = 0,850; P < 0,001) (Slika 1.).

Korelaciju koncentracije HA u serumu sa stupnjem upale (r_S = 0,685; P < 0,001) prikazana je na slici 2. Bolesnici s višim stupnjem upale imaju višu koncentraciju HA u serumu.

(P < 0.001). Comparison of serum HA concentrations by ANOVA *post hoc* LSD performed in these diseases simultaneously showed that serum HA concentrations did not differ statistically significantly among chronic hepatitis subtypes (HBV *vs.* HCV: P = 0.400; HBV *vs.* AIH: P = 0.599; and HCV *vs.* AIH: P = 0.803).

Table 3 shows serum HA levels in various chronic hepatitis stages. Differences in serum levels of HA were statistically significant in almost all stages of hepatic fibrosis (P < 0.001) compared with the healthy group, with the exception of patients with stage 0 (no fibrosis; P = 0.439). In addition, when multiple comparisons of serum HA concentrations in six stages of liver fibrosis were simultaneously performed, differences in serum HA concentrations in all stages of liver fibrosis were statistically significant at the significance level of 0.001, however, with three exceptions: stage 0 *vs.* stage 1 (P = 0.003); stage 1 *vs.* stage 2 (P = 0.006); and stage 2 *vs.* stage 3 (P = 0.040). We had only one patient with stage 6 of liver fibrosis, therefore this single patient could not compare statistically with the healthy group.

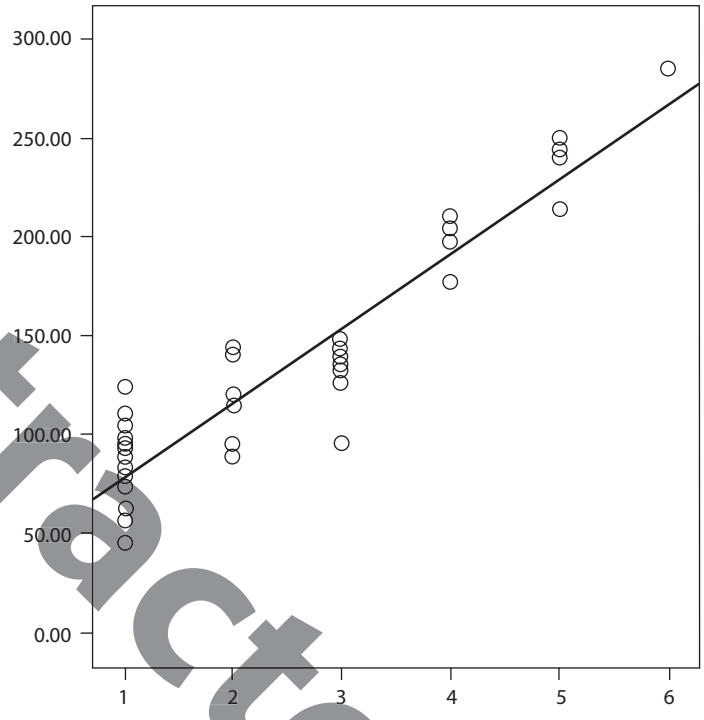
Patients with higher stages of liver fibrosis had higher serum HA concentrations. As illustrated in Figure 1, this correlation was linear and very strong (r_S = 0.850; P < 0.001). Figure 2 presents correlation of serum HA with inflammation grade (r_S = 0.685; P < 0.001). The patients with higher inflammation grades had higher serum HA concentrations.

Table 4 shows the cut-off point, sensitivity and specificity of serum HA concentrations. These data were obtained by ROC analysis and the cut-off point was selected according to the maximal sensitivity and specificity values.

Tablica 3. Usporedbe koncentracije HA u serumu kod bolesnika u šest stadija fibroze jetre s kontrolnom grupom

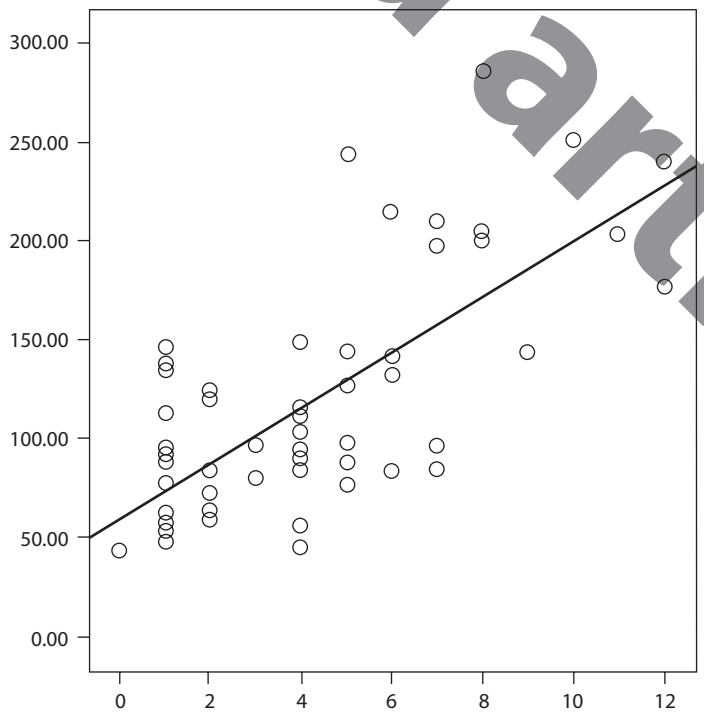
Table 3. Comparisons of HA concentrations between patient subgroups (six stages of liver fibrosis) and control group

Fibrosis Stage / HA level	Hyaluronic acid (ng/mL)	Range (ng/mL)	P (*vs.* control)
Fibrosis stage 0 (N = 10)	53.6 ± 6.4	45.0-63.0	0.439
Fibrosis stage 1 (N = 19)	81.8 ± 21.5	46.0-125.0	< 0.001
Fibrosis stage 2 (N = 11)	106.7 ± 22.7	78.0-145.0	< 0.001
Fibrosis stage 3 (N = 9)	128.6 ± 24.9	78.0-149.0	< 0.001
Fibrosis stage 4 (N = 8)	172.0 ± 51.2	85.0-210.0	< 0.001
Fibrosis stage 5 (N = 4)	238.0 ± 15.9	215.0-251.0	< 0.001
Control (N = 20)	46.0 ± 10.5	27.0-62.0	-
ANOVA; post hoc LSD method			



Slika 1. Korelacija između koncentracija HA u serumu i stadija fibroze prema rezultatima biopsije jetre. Korelacije između tih parametara izračunate su Spearmenovim koeficijentom korelacije (r_S = 0,850, P < 0,001).

Figure 1. Correlation between serum HA concentrations and stage of fibrosis in liver biopsies. Correlations between these parameters were calculated by the Spearman correlation coefficient (r_S = 0.850; P < 0.001).



Slika 2. Korelacija između srednje vrijednosti koncentracija HA u serumu i stupnja upale prema rezultatima biopsije jetre (r_S = 0,685, P < 0,001).

Figure 2. Correlation between mean serum HA concentrations and grade of inflammation in liver biopsies (r_S=0.685; P<0.001).

Tablica 4. ROC analiza koncentracije HA u serumu za razlikovanje bolesnika s fibrozom jetre i kontrolnih ispitanika

Table 4. ROC curve of hyaluronic acid serum level to discriminate patients with liver fibrosis and control group

Index	AUC (CI = 95%)	P	Cut-off point	Sensitivity	Specificity
HA (ng/mL)	0.932 (0.881-0.983)	< 0.001	59.5	82.3%	85%

Rezultati ROC analize (granična vrijednost, osjetljivost i specifičnost) prikazani su u tablici 4. Granična vrijednost je odabrana prema najvećoj vrijednosti osjetljivosti i specifičnosti.

Rasprava

Postoji nekoliko istraživanja (11,12) provedenih na populaciji Irana o povezanosti serumske koncentracije HA i nekrotičnih oštećenja jetre kod bolesnika s kroničnim hepatitisom, a niti jedno od njih nije provedeno na populaciji sjevernog Irana. Stoga je ovo istraživanje provedeno u dvije sjeverne pokrajine Irana (Istočni Azerbejdžan i Golestan) kako bi se naglasila povezanost koncentracije HA u serumu i nekrotičnih oštećenja jetre, te istražilo može li se na temelju koncentracije HA u serumu razlikovati između fibroze jetre bolesnika s kroničnim hepatitisom i zdravih osoba.

Koncentracija HA u serumu bila je značajno viša kod bolesnika nego kod zdravih ispitanika (P < 0,001). Naši se podaci slažu s radovima drugih autora koji također izvještavaju o povišenoj koncentraciji HA u serumu kod kronične bolesti jetre, poglavito kod bolesnika s cirozom (19-22). U ranijim istraživanjima provedenim na populaciji Irana u bolesnika s kroničnim hepatitisom B (11,12) izmjerena je viša koncentracija HA u serumu nego kod zdravih ispitanika, no čini se da se koncentracija HA u serumu iz našeg istraživanja razlikuje od one iz ranijih istraživanja. Bolesnici pušači i oni koji su uzimali alkohol imali su više koncentracije HA u serumu, što je ukazivalo na mogućnost da pušenje i unos alkohola mogu promijeniti jetreni metabolizam HA (22).

Koncentracije HA u serumu bile su povišene u skupini bolesnika s HBV, HCV i AIH u odnosu na kontrolnu skupinu. McHutchinson i sur. izvijestili su da je koncentracija HA u serumu kod bolesnika s HCV viša nego kod kontrolnih ispitanika te da su najviše vrijednosti koncentracije izmjerene kod bolesnika s cirozom (23).

Pronašli smo statistički značajnu razliku u koncentraciji HA u svim stadijima jetrene fibroze u odnosu na kontrolnu skupinu, osim u bolesnika sa stadijem 0 (bez fibroze; P = 0,439). Usporedba koncentracija HA u serumu kod raznih stadija fibroze jetre otkrila je da su razlike između svih stadija statistički značajne (P < 0,05) i da bolesnici s višim stadijem jetrene fibroze imaju višu koncentraciju HA u se-

Discussion

In Iranian population, there are few reports (11,12) on the relationship between HA serum concentrations and liver necroinflammatory injuries in patients with chronic hepatitis, while there is no report on this topic, particularly in the north of Iran. Therefore, this study was conducted in two northern provinces of Iran (East Azerbaijan and Golestan) to highlight the relationship between HA serum concentrations and liver necroinflammatory injuries and to explore whether serum HA concentrations could discriminate between liver fibrosis in patients with chronic hepatitis and healthy individuals.

The serum HA concentrations were significantly higher in patients than in healthy controls (P < 0.001). Our data are consistent with the work of other groups that have reported increased levels of serum HA in chronic liver disease, particularly in patients with cirrhosis (19-22). In previously published studies in Iranian chronic hepatitis B patients (11,12), higher serum HA concentrations as compared with healthy subjects were recorded, but it seems that serum HA concentrations in our patients were different from those in previous studies. The patients reported to be smokers and alcohol drinkers had higher serum HA concentrations, suggesting that smoking and alcohol intake could possibly change the HA metabolism in the liver (22).

There was a rise in serum HA concentrations in HBV, HCV and AIH patients. McHutchinson *et al*. report that serum HA concentration was higher in HCV patients than in healthy controls and the highest levels were observed in cirrhotic patients (23). The serum HA concentrations and different stages of liver fibrosis yielded significant differences *versus* healthy controls. An exception was observed in stage 0 of liver fibrosis. In this stage of liver fibrosis, differences in serum HA concentrations as compared with healthy controls were not statistically significant (P = 0.439), suggesting that this group may have better HA metabolism in the liver than the other groups with liver fibrosis. Comparison of serum HA concentrations in various stages of liver fibrosis altogether revealed that differences were statistically significant in all stages of liver fibrosis (P < 0.05), while patients in higher stages of liver fibrosis had higher serum HA concentrations. This suggested poor liver HA metabolism irrespective of the liver

rumu. To ukazuje na poremećen jetreni metabolizam HA, bez obzira na stupanj oštećenja jetre. Murawaki i sur. (24) su mjerili koncentracije HA u serumu bolesnika oboljelih od kroničnog hepatitisa C i ustanovili visoke koncentracije HA u serumu, pri čemu su razlike među bolesnicima s različitim stadijima jetrene fibroze bile statistički značajne; autori su zaključili kako bi određivanje koncentracije HA u serumu mogao biti od kliničkog značenja za procjenu stupnja težine i stadija bolesti kod tih bolesnika.

Postoji snažna pozitivna korelacija između koncentracije HA u serumu i stadija jetrene fibroze. Takva ja korelacija ustanovljena između srednje vrijednosti koncentracije HA u serumu i stupnja upale jetre; bolesnici s višim stupnjevima upale imali su više koncentracije HA u serumu. Korner i sur. izvješćuju kako među brojnim serumskim parametrima (kao što su laminin, A1-apolipoprotein i protrombinsko vrijeme) koncentracija HA najjače korelira s nekrotičnim oštećenjem jetre te se može rabiti za dugoročno praćenje napredovanja bolesti (25). Kupcova i sur. (26) izvješćuju da koncentracija HA raste u ranijim stadijima kronične bolesti jetre te da se najviše koncentracije mogu izmjeriti kod aktivne ciroze jetre i aktivnog kroničnog hepatitisa. U svom istraživanju provedenom u 141 bolesnika s kroničnim hepatitisom Tao i sur. (27) su utvrdili kako samo u 14,16% bolesnika koncentracija HA nije u skladu s njihovim histološkim stadijem jetrene fibroze te da je koncentracija HA u serumu bila u pozitivnoj korelaciji sa stupnjem upale. U drugom istraživanju koje su proveli Pilette i sur. (28) koncentracije HA u serumu bile su u najvišoj korelaciji s histološkim indeksima. U svojem su istraživanju Zhang i sur. (29) zaključili da serumska koncentracija HA raste s povišenjem stadija jetrene fibroze, dok Zheng i sur. (30) izvješćuju da su koncentracije HA u serumu porasle s težim stupnjem upale. Cai i sur. (31) opisuju kako su koncentracije HA u serumu bile u korelaciji s histopatološkim oštećenjima jetre i stupnjem upale.

Granična vrijednost od 59,5 ng/mL HA za razlikovanje između bolesnika s jetrenom fibrozom i zdravih ispitanika pokazala je dobru osjetljivost i specifičnost. AUC koncentracije HA u serumu kod analize krivuljom ROC bila je 0,932, što znači da se koncentracija HA može rabiti kao kriterij razlikovanja bolesnika s jetrenom fibrozom od zdravih osoba koje nemaju znakove bolesti. Slični su rezultati dobiveni u drugim istraživanjima za dijagnozu jetrene fibroze kod iste granične vrijednosti (odnosno 50 ng/mL) (23,32).

Nekoliko bi mehanizama moglo doprinositi povišenoj koncentraciji HA u serumu kod tih bolesnika. U početnom stadiju bolesti pojačana proizvodnja HA u aktiviranim zvjezdastim stanicama jetre mogla bi biti odgovorna za povišenje njene koncentracije u serumu (33). Kasnije, u naprednim stadijima bolesti kada se uspostavi sinusoidna kapilarizacija jetre i ciroza, smanjena razgradnja HA koju provode sinusoidne endotelne stanice može biti uzrokom

damage severity. Murawaki *et al*. measured serum HA levels in chronic hepatitis C patients and reported that patients had high serum levels of HA and the differences in serum HA levels between various stages of liver fibrosis in patients were statistically significant, concluding that serum HA testing would be clinically useful for assessing staging and grading in these patients (24).

There was strong positive correlation between the mean serum HA and liver fibrosis stages. Such a correlation was found between the mean serum HA and liver inflammation grades, and patients with higher grades of inflammation had higher serum HA concentrations. Korner *et al*. report that among various serum parameters (such as laminin, A1-apolipoprotein, and prothrombin time), HA correlated most strongly with liver necroinflammatory injuries and can be used for long-term monitoring of disease progression (25). Kupcova *et al*. (26) report that HA concentration increases in early stages of chronic liver disease, with highest concentrations recorded in active cirrhosis and chronic active hepatitis. In a report by Tao *et al*. (27), only 14.16% of 141 chronic hepatitis patients had serum HA concentrations inconsistent with histological stage of their hepatic fibrosis and serum HA levels positively correlated with the grade of inflammation. In another study by Pilette *et al*. (28), serum concentrations of HA showed highest correlation with histological indices. In another study, Zheng *et al.* (29) found serum HA concentrations to increase in higher stages of liver fibrosis, whereas Zheng *et al.* (30) report that serum HA increased as the grade of inflammation aggravated. Cai *et al*. (31) report that serum HA levels were in correlation with liver histopathologic lesions and inflammation grades.

A cut-off point of 59.5 ng/HA mL for differentiation between patients with liver fibrosis and healthy individuals showed good sensitivity and specificity. The AUC of serum HA in ROC analysis was 0.932. It means that HA can discriminate patients with liver fibrosis and healthy individuals without any signs of liver disease. Similar results were obtained elsewhere for the diagnosis of liver fibrosis at the same cut-off value (i.e. 60 ng/mL) (23,32).

Several mechanisms may contribute to the elevation of serum HA levels in these patients. At the beginning of disease development, the enhancement of HA production by the activated hepatic stellate cells may be responsible for the increase in its serum levels (33). Later, in advanced stages of the disease when hepatic sinusoid capillarization and cirrhosis are established, reduced degradation by sinusoidal endothelial cells may be the cause of greater HA increments (34). In early stages of liver fibrosis (stages 0-2) and inflammation grades (grades 0-5; Fig. 2), serum HA concentrations were high and the highest levels were observed in liver fibrosis stages ≥3 and inflammation grade ≥6. It seems that progression of liver fibrosis (and inflammation) was accompanied by impairment in the liver

povišenja koncentracije HA (34). Kao što smo pokazali u ovom istraživanju, u ranom stadiju jetrene fibroze (stadiji 0-2) i stupnjevima upale (stupnjevi 0-5; slika 2.) koncentracije HA u serumu bile su visoke, a najviše vrijednosti koncentracije HA u serumu izmjerene su kod fibroze stadija ≥ 3 i stupnja upale ≥ 6. Čini se da je napredovanje jetrene fibroze (i upale) bilo udruženo s poremećajem funkcije endotelnih stanica jetre, a time i sa smanjenom razgradnjom ovog heteropolisaharida, što naposljetku rezultira povišenjem koncentracije HA u serumu.

U ovom smo istraživanju primijetili da je koncentracija HA u serumu kod bolesnika s kroničnim hepatitisom – u populaciji sjevernog Irana – viša nego kod kontrolnih ispitanika, no čini se da se koncentracije razlikuju od onih iz ranijih istraživanja koja su provedena na području srednjeg i južnog Irana. Stoga su potrebna daljnja istraživanja kako bi se utvrdila točna razlika u serumskoj koncentraciji HA među populacijama raznih dijelova Irana.

Zaključujemo kako rezultati ovoga istraživanja ukazuju na to da se na temelju mjerenja koncentracije HA u serumu mogu razlikovati bolesnici s jetrenom fibrozom od zdravih ispitanika i da ta mjerenja mogu pružiti podatke o progresivnim promjenama u jetri sa smanjenom funkcijom endotelnih stanica jetre. Čini se da je serumska koncentracija HA uglavnom pokazatelj smanjene funkcije jetrenih endotelnih stanica kod kronične bolesti jetre te da je povezana s histopatološkim promjenama kod kronične bolesti jetre prema modificiranom Knodellovu indeksu. Nadalje, čini se da je povišenje koncentracije HA u serumu iznad granične vrijednosti (59,5 ng/mL) povezano s jetrenom fibrozom. Stoga se određivanje koncentracije HA u serumu može smatrati dodatnim kliničkim postupkom procjene jetrene fibroze u slučajevima kada je biopsija jetre kontraindicirana i ne može se provesti.

Naše je istraživanje imalo stanovita ograničenja. Ono ispituje koncentraciju HA u serumu u jednoj vremenskoj točki u životu bolesnika oboljelih od kronične jetrene bolesti. Kada bi se ovi bolesnici pratili i kada bi se biopsija jetre ponovila tijekom protokola liječenja, mogli bismo dobiti bolji uvid u metabolizam HA. Potrebno je daljnje prospektivno istraživanje kako bi se dobio cjelovit uvid u koncept metabolizma HA. S obzirom da naša kontrolna skupina nije bila ujednačena sa skupinom bolesnika prema statusu pušenja i unosu alkohola, moguće je da su pušenje i unos alkohola kod ispitanika sa bolešću jetre dodatno utjecali na koncentraciju HA u serumu.

Zahvala

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endothelial cell function and reduced degradation of this heteropolysaccharide, eventually resulting in elevation of serum HA concentrations.

In this study we observed that serum HA levels in chronic hepatitis patients from north Iran were higher than those in healthy control subjects, but the levels seemed to differ from those recorded in previous studies carried out in central and southern provinces of Iran. Therefore, additional studies are needed to establish the exact differences in serum HA levels in Iranian population from different regions.

In conclusion, our study results suggest that measurement of serum HA concentrations can discriminate between liver fibrosis patients and healthy individuals, and can give information about progressive changes in the liver with a reduced function of liver endothelial cells. Serum HA concentration seems to be an expression of mainly reduced functional capacity of liver endothelial cells in chronic liver disease and is associated with histopathologic changes in chronic liver disease according to the modified Knodell scoring system; an increase in serum HA concentration above the predictive value of 59.5 ng/mL is associated with liver fibrosis. Therefore, serum HA levels could be determined as an additional clinical tool for evaluation of liver fibrosis, when liver biopsy is contraindicated and impossible to perform.

Our study had some limitations. Serum HA concentration was measured at a single time point during the life of patients with chronic liver disease. Had the study included patient follow up and liver biopsy repeated during the treatment protocol, a better insight into the metabolism of HA would have been obtained. Additional prospective study is needed to get a complete concept of HA metabolism. In addition, our groups were not matched according to smoking and alcohol intake and those variables could have influenced HA concentrations in patient group.

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