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***Basic Study***

**insulin-like growth factor-1 mRNA isoforms and insulin-like growth factor-1 receptor mRNA expression in chronic hepatitis C**

Kasprzak A *et al*. Expression of IGF-1 mRNA isoforms in HCV infection

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**Abstract**

**AIM:** to evaluate the expression of different insulin-like growth factor (IGF)-1 mRNA isoforms and IGF-1 receptor (IGF-1R) mRNA in hepatitis C virus (HCV)-infected livers.

**METHODS**: Thirty-four liver biopsy specimens from chronic hepatitis C (CH-C) patients were obtained before anti-viral therapy. Inflammatory activity (grading) and advancement of fibrosis (staging) were evaluated using a modified point scale of METAVIR. The samples were analyzed using quantitative real-time PCR technique. From fragments of liver biopsies and control liver, divided and ground in liquid nitrogen, RNA was isolated using RNeasy Fibrous Tissue Mini Kit according to the manufacturer’s instruction. Expression levels of IGF-1 mRNA isoforms (IGF-1A, IGF-1B, IGF-1C, P1, P2), and IGF-1R mRNA were determined through normalization of copy numbers in samples as related to reference genes: [glyceraldehyde-3-phosphate dehydrogenase](http://www.ncbi.nlm.nih.gov/gene/2597) and hydroxymethylbilane synthase. Results on liver expression of the IGF-1 mRNA isoforms and IGF-1R transcript were compared to histological alterations in liver biopsies and with selected clinical data in the patients. The statistical analysis was performed using Statistica PL v. 9 software.

**RESULTS**: The study showed differences in quantitative expression of IGF-1 mRNA variants in HCV-infected livers, as compared to the control. Higher relative expression of total IGF-1 mRNA and of IGF-1 mRNAs isoforms (P1, A, and C) in HCV-infected livers as compared to the control were detected. Within both groups expression of IGF-1A mRNA isoform significantly prevailed over expressions of B and C isoforms. Expression of P1 mRNA was higher than that of P2 only in CH-C. Very high positive correlations were detected between reciprocal expressions of IGF-1 mRNA isoforms P1 and P2 (*r =* 0.876). Expression of P1 and P2 mRNA correlated with IGF-1A mRNA (*r =* 0.891; *r =* 0.821, respectively), with IGF-1B mRNA (*r =* 0.854; *r =* 0.813, respectively), and with IGF-1C mRNA (*r =* 0.839; *r =* 0.741, respectively). Expression of IGF-1A mRNA significantly correlated with isoform B and C mRNA (*r =* 0.956; *r =* 0.869, respectively), and B with C isoforms (*r =* 0.868) (*P <* 0.05 in all cases). Lower expression of IGF-1A and B transcripts was noted in the more advanced liver grading (G2) as compared to G1. Multiple negative correlations were detected between expression of various IGF-1 transcripts and clinical data (*e.g.,* AFP, HCV RNA, steatosis, grading, staging). Expression of IGF-1R mRNA manifested positive correlation with grading and HCV-RNA.

**Conclusion:** Differences in quantitative expression of IGF-1 mRNA isoforms in HCV-infected livers, as compared to the control, suggest that HCV may induce alteration of *IGF-1* splicing profile.

**Key words**: chronic hepatitis C; insulin-like growth factor-1 receptor; insulin-like growth factor-1 mRNA isoforms; quantitative polymerase chain reaction

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**Core tip:** hepatitis C virus (HCV) may induce alteration of insulin-like growth factor (IGF)-1 splicing profile. A quantitative polymerase chain reaction analysis has confirmed higher relative expression of total IGF-1 mRNA and of IGF-1 mRNAs isoforms P1, A, and C in HCV-infected livers as compared to the control. Increase in inflammatory activity (grading) of HCV-infected livers was linked to decreased IGF-1 mRNA expression, an altered profile of mRNA isoforms and to an increase in IGF-1R mRNA expression. Decreased expression level of IGF-1 mRNA isoforms, and an increased liver expression of IGF-1R mRNA, associated with indicators of liver damage (*e.g.,* grading, staging, steatosis, liver serum enzyme activity) may be of a prognostic significance.

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**INTRODUCTION**

Hepatitis C virus (HCV) is the major causative agent of hepatocellular carcinoma (HCC), mainly through indirect pathways: chronic inflammation, cell deaths, and proliferation[1,2]. Function of the insulin-like growth factor (IGF) system in promotion of cell growth and neoplastic transformation were previously described[3,4]. The IGF ligands (IGF-1 and -2) bind to the receptors which include: IGF-1 receptor (IGF-1R), IGF-2R, insulin receptor and hybrid receptors (IGF-1R/insulin receptor)[3]. Involvement of IGF receptors in maintenance of the transformed hepatocyte phenotype was also described[5-9]. Most of the studies documented an increase in IGF-1R expression, starting at the preneoplastic lesions up to a developed HCC[5,9,10]. There are also studies available where no significant difference was detected in expression of IGF-1R mRNA between HCC and a control[8]. Similarly, an increase in expression of IGF-1R could not be noted in culture of human hepatocytes[11]. In a few studies on patients with liver cirrhosis and with chronic hepatitis C (CH-C) the amounts of IGF-1R transcript were found to increase as compared to the normal liver[12,13]. A more thorough recognition of the role played by IGF system in hepatic carcinogenesis is thought to improve HCC therapy[3,9,14].

The six exons of *IGF-1* are alternatively spliced into multiple transcripts, encoding specific circulating and tissue-specific isoforms of the IGF-1 peptide. At the 5’end of the gene, different promoters (P1 and P2) in combination with alternative transcription start sites and differential splicing generate the mutually exclusive class 1 and class 2 IGF-1 isoforms[15-17]. At the 3’ end of the gene, alternative splicing gives rise to at least three subsets of RNA transcripts, each encoding three distinct C-terminal portions of the unique E-peptide as well as the 3’-UTR[15,18,19]. Exon 3 encodes parts of the signal peptide and the mature peptide common to all isoforms, while exon 4 encodes the rest of the mature peptide and the proximal part of the E domain. Composition of nucleotides in exons 5 and 6 determine formation of isoforms: A (Ea), B (Eb) and C (Ec) within classes 1 and 2[20]. The biochemical mechanism which controls use of *IGF-1* promoters 1 or 2 in the alternate splicing remains poorly recognized[16,21-23]. Studies on human liver RNA demonstrated that IGF-1 transcript, undergoing an alternate splicing contains exons 3 and 4, 49 bp of exon 5 and exon 6 (exon 4-5-6)[24]. A role of *IGF-1* alternate splicing was best recognized in the skeletal muscular tissue[25-28] and in nervous tissue[29,30]. A differential profile of IGF-1 mRNA isoforms was demonstrated in different tumors[31-34]. It still remains unknown if HCV and its oncogenic proteins (C, NS3, NS5A) *in vivo* may induce alterations in the profile of hepatic IGF-1 gene expression[35].

This study aimed at evaluation of expression of various IGF-1 mRNA isoforms (P1, P2, 1A, 1B, 1C) and IGF-1R mRNA in chronically HCV-infected livers. Herein we examined if IGF-1 alternative splicing is associated with the degree of liver damage (grading and staging) caused by HCV virus. Results on liver expression of the IGF-1 mRNA isoforms and IGF-1R transcript were compared to histological alterations in liver biopsies and with selected clinical data in the patients. Data about changes in IGF-1 alternative splicing in CH-C were not published up to now. The relationship between liver expression of mRNA IGF-1 isoforms and progression of CH-C to HCC is unclear.

**MATERIAL AND METHODS**

***Patients***

The examined group consisted of 34 patients (age of 18-63 years; 18 men and 16 women) with CH-C, diagnosed and treated in the Department of Infectious Diseases, Poznan University of Medical Sciences in Poznan in the years of 2010-2012. Patients were referred to an anti-viral treatment and were not treated previously. Infections with other hepatotropic viruses (HBV, HCMV, EBV) or other reasons of liver damage were excluded (*e.g.,* alcohol abuse, autoimmune hepatitis, NASH, drugs, history of anti-cancer therapy). Patients with diabetes mellitus, kidney failure or any hormones disturbances were not included in the group. In the study we used basic clinical data on HCV-infected patients as well as other results involving biochemical tests on peripheral blood, results of ELISA tests (glucose, insulin, estradiol) and histopathological examination of liver biopsies. Presence of HCV-specific antibodies was tested using chemiluminescence taking advantage of ARCHITECT Anti-HCV kits (ABBOTT, Wiesbaden, Germany) in ARCHITECT and 2000 analysers (ABBOTT). Infection with HCV was confirmed estimating serum HCV-RNA, by application of GeneProof HEPATITIS C VIRUS HCV tests (GeneProofa.s., Brno, Czech Republic) manifesting sensitivity of 50 IU/ml. In all patients genotype of HCV was estimated (VERSANT HCV GENOTYPE 2.0 ASSAY, LiPA).

Negative tissue control (*n* = 7) (patient’s age of 35-72 years; 4 men, 3 women) involved liver fragments with no morphological traits of organ pathology, perioperatively sampled from vicinity of the dissected focal lesion in the liver and a single liver biopsy taken from a patient to diagnose reasons for elevated aminotransferase activity. The latter patient proved to be HCV-negative (absence of specific antibodies and of HCV RNA). The remaining control material originated also from HCV- and HBV-negative patients. Age and sex of the patient were known. The control material was obtained from the Chair and Department of General Surgery, Gastroenterological Oncology and Plastic Surgery, Poznan University of Medical Sciences in Poznan.

***Tissue material***

Liver biopsy was done in all cases as a routine procedure before antiviral therapy. Basing on USG tests and alpha fetoprotein (AFP) levels, in none of the patients neoplastic growth (HCC) was suspected. Written informed consent was obtained from every patient before liver biopsy, and approval for the study was granted by the institution’s Ethical Committee (no. 22/09). The excised liver fragment (HCV-infected and control) was divided, cutting off its terminal 0.5 cm fragment, which was immersed in RNA Stabilization Solution (RNAlater®, Applied Biosystems) at -80 °C until use. The remaining part of the fragments obtained from patients was fixed in a buffered 10% solution of formalin and embedded in paraffin. ~5µm-thick preparations were stained with hematoxylin and eosin and silver impregnated using standard techniques. Inflammatory activity (grading) and advancement of fibrosis (staging) were evaluated using a modified point scale of METAVIR[36]. This score is composed of two-letter and two-number scoring system: histological activity (grading: G0 - no activity, G1 - mild activity, G2 - moderate activity, G3 - severe activity) and fibrosis (staging: S0 – no fibrosis, S1 – portal fibrosis without septa, S2 – portal fibrosis with rare septa, S3 – numerous septa without cirrhosis, S4 – cirrhosis).Fatty degeneration of liver was evaluated using point scale, in which grade 0 corresponded to absence of fatty degeneration while grades 1 and 2 corresponded to, respectively, < 30% and ≥ 30%-70% hepatocytes showing traits of fatty degeneration.

***Technique of quantitative real-time PCR***

From fragments of liver biopsies and control liver, divided and ground in liquid nitrogen, RNA was isolated using RNeasy Fibrous Tissue Mini Kit (QIAGEN) according to the manufacturer’s instruction. In course of the procedure traces of DNA contamination were eliminated using DNases. Finally, total RNA was dissolved in RNase-free water. Quality of RNA preparations was consecutively checked using electrophoresis in an agar-formaldehyde gel. RNA content was quantitated by spectrophotometry. Every RNA sample was subjected to additional digestion with DNase using RNase-Free DNase Set (QIAGEN) in order to avoid contamination with genomic DNA. Subsequently, 1μg RNA from every sample was subjected to reverse transcription using QuantiTect Reverse Transcription Kit (QIAGEN) and cDNA was obtained. Analysis of gene expression was performed using primers specific for individual mRNA IGF-1 isoforms as previously described[34] and selected reference genes: GAPDH ([glyceraldehyde-3-phosphate dehydrogenase](http://www.ncbi.nlm.nih.gov/gene/2597)) and HMBS (hydroxymethylbilane synthase)[37]. The primer sequences for IGF-1R were: forward 5’-GGGAATGGAGTGCTGTATG-3’, reverse 5’-CACAGAAGCTTCGTTGAGAA-3’, GAPDH forward 5’-AAGGTCGGAGTCAACGGATTT-3’, reverse 5’-ACCAGAGTTAAAAGCAGCCCTG-3’, HMBS forward 5'-TGCAACGGCGGAAGAAAA-3’, reverse 5'-ACGAGGCTTTCAATGTTGCC-3’. The reactions were performed in a final volume of 10 μl. Each of the samples contained 15 ng/μl cDNA and a mixture of reagents forming the SYBR Green PCR master mix (Applied Biosystems, United Kingdom), each of the primer’s pair at the concentration of 0.25mM. The reaction was conducted using an automated fluorimeter (Rotor-Gene 6000, Corbett Research). The PCR program was as followed: (1) Preliminary denaturation, 95 °C, 10 min; (2) Denaturation, 95 °C, 10-15 s; (3) Primer annealing, 53-67 °C, 15-35 s; and (4) Elongation, 72 °C, 15-40 s. The number of cycles was 40-50. The initial quantity of the product was calculated in relation to the standard curve. Presence of an appropriate product was evaluated by determination of the melting point for a specific PCR product. All samples were amplified in duplicate or triplicate and in case that results varied more than 15% the reactions were repeated. Expression levels of IGF-1 mRNA isoforms (IGF-1A, IGF-1B, IGF-1C, P1, P2), and IGF-1R mRNA were determined through normalization of copy numbers in samples as related to reference genes (housekeeping genes). In the normalization reference genes were accepted to include GAPDH and HMBS gene, specific for the liver, according to literature data[37].

***Statistical analysis***

At the first stage of statistical analysis consistency of all the results with normal distribution of Gauss was verified using the Shapiro-Wilk test. Subsequently, parameters of descriptive statistics were calculated (arithmetic mean, standard deviation, median value, minimum and maximum value). The data related to quantitative gene expression of IGF-1 mRNA isoforms, IGF-1R mRNA were compared to data for normal livers (negative control) using Mann-Whitney’s test (a non-parametric test for unlinked variables for two groups). In cases of linked variables the Wilcoxon test was used. For comparing more than two groups Kruskal-Wallis test was employed and, the multiple comparison Dunn test was also used. For comparison of the percentage shares of IGF-1 mRNA isoforms the test of differences between two structural indices was employed. Pearson’s correlation and Spearman’s rank correlation were used to correlate values of variables. Effect of age was also analysed in strength of correlation between IGF-1 mRNA isoforms and IGF-1R transcript and also selected clinical data. The results were thought to be statistically significant at *P <* 0.05. The statistical analysis was performed using Statistica PL v. 9 software (Statsoft, Inc., Tulsa, OK, United States).

**RESULTS**

***Expression of mRNA class 1 (I) and 2 (II) as related to the used promoter P1 or P2***

Both among the HCV-infected patients and in the control, transcription of IGF-1 from the first promoter (P1) (class 1) prevailed. In CH-C group the shares of transcripts class 1 (from P1) (76.4%) and those of class 2 (from P2) (23.6%) were similar to those in the control (62.7% and 37.3%, respectively) (*P* > 0.05) (Figure 1).

***Expression of mRNA isoforms: IGF-1A, IGF-1B and IGF-1C – shares in percents***

In CH-C group expression of IGF-1A (A) splicing form of mRNA prevailed (92% of all transcripts). Similarly in the control group the highest expression was manifested by the A isoform, followed by B and C (Figure 2). Expression of A isoform was significantly higher in CH-C group than in the control (92% *vs* 85%), while expression of isoform B was lower than in the control (5.2% *vs* 12.9%). No significant differences could be demonstrated between the two compared groups in shares of expressed P1 and P2 transcripts (Figure 3).

***Quantitative analysis of IGF-1 mRNA isoforms in CH-C group and in the control***

Detailed analysis of relative expression manifested by each of IGF-1 mRNA isoform, normalized against reference genes (GAPDH and HMBS), demonstrated a significantly higher expression of IGF-1A and IGF-1C mRNA isoforms, of P1 transcripts (class 1) and of total IGF-1 mRNA in CH-C group as compared to the control. A significantly higher expression of all isoforms and classes of IGF-1 mRNA and of total IGF-1 was detected in women as compared to men (Table 1).

***Analysis of IGF-1 mRNA isoforms in HCV-infected livers as related to activity of inflammation (grading) and of liver fibrosis (staging)***

A liver with more intense inflammatory lesions (grading 2, G2) contained lower expression of all IGF-1 isoforms as compared to their expression in a liver with G1, except of IGF-1C isoform. No significant differences could be disclosed between expression of all IGF-1 mRNA isoforms and different stages of fibrosis (*P* > 0.05 in all the cases) (Table 2).

***Expression of IGF-1R mRNA***

In CH-C group expression of IGF-1R mRNA was significantly lower than in the control. No sex-related differences were detected in expression of IGF-1R transcript (Table 1). Expression of IGF-1R mRNA was higher in livers with higher grading (G2) as compared to livers with G1 (Table 2).

***Expression of various transcripts of IGF-1 and IGF-1R vs the clinical data***

**Isoforms of IGF-1 mRNA:** Expression of total IGF-1 mRNA and of all mRNA isoforms manifested a very strong negative correlation with patient’s age and BMI value. Expression of all isoforms demonstrated also negative correlations with the liver steatosis. Highly negative correlations were detected also with activity of ALT, and slightly less pronounced ones (also negative) with activity of AST. Expression of mRNA isoforms A, B, P1 and P2 showed negative correlations with liver grading. Very poor correlation was detected between liver fibrosis and expression of P2 mRNA. Also for expression of P2 mRNA a relatively poor negative correlation was documented with AFP concentration and HCV viral load. Similarly low were the negative correlations between HCV viral load and expression of IGF-1A mRNA and total IGF-1 mRNA in HCV-infected liver (Table 3).

**Transcripts of IGF-1R:** Expression of IGF-1R mRNA manifested poor positive correlations with age of HCV-infected patients. Moreover, positive correlations were detected between tissue expression of IGF-1R mRNA and grading and HCV-RNA (Table 3).

***Reciprocal correlations between expressions of IGF-1 mRNA isoforms and IGF-1R mRNA***

Very high positive Spearman’s correlations were detected between reciprocal expressions of IGF-1 mRNA isoforms P1 and P2 (*r =* 0.876), P1 and A (*r =* 0.891), P1 and B (*r =* 0.854), P1 and C (*r =* 0.839); P2 and A (*r =* 0.821), P2 and B (*r =* 0.813), P2 and C (*r =* 0.741); A and B (*r =* 0.956), A and C (*r =* 0.869), B and C (*r =* 0.868) (*P <* 0.05 in all cases) in patients with HCV infection. In livers with CH-C additionally significant weak negative correlations were detected between expression of IGF-1R mRNA and quantities of mRNAs for isoforms of IGF-1A (*r =* -0.397), IGF-1B mRNAs (*r =* -0.419) and for total expression of IGF-1 mRNA (*r =* -0.397) (*P <* 0.05 in all cases).

In control livers we confirmed very high positive Spearman’s correlations between reciprocal expression of all IGF-1 mRNA isoforms (data not shown). No significant relationships were detected between expression of IGF-1 mRNA isoforms and IGF-1R mRNA in control livers (data not shown).

**DISCUSSION**

Studies on expression of various IGF-1 mRNA isoforms have been performed for the first time on livers with CH-C. Our results have pointed to prevalent expression of mRNA from P1 promoter of *IGF-1* both on the control liver and in liver with chronic HCV infection. This confirms involvement of P1 in production of 60-65%, and of P2 in production of approximate 25% IGF-1 transcripts, originally demonstrated in rat liver[17,22]. The percentage shares of the remaining IGF-1 mRNA isoforms (A, B and C) in the control and in HCV-infected liver demonstrated also prevalence of mRNA isoform A over expression of mRNAs of the remaining isoforms. In the literature data there are no references to such results. In studies on human papilloma virus (HPV)-positive and –negative tissues of uterine cervix carcinoma a significant prevalence was demonstrated of IGF-1B share over remaining isoforms in tissues with uterine cervix carcinoma as compared to the remaining stages of carcinogenesis, suggesting that this form of the transcript may lead to formation of the peptide Eb with strongly mitogenic properties[33].

Analysis of relative values of expression manifested by various IGF-1 mRNA isoforms in livers in the two examined groups has confirmed quantitative prevalence of isoform A over the remaining variants of IGF-1 transcripts. Prevalence of expression for IGF-1A over IGF-1B was documented also by Othsuki *et al*[38] in such organs as uterus, ovaries, liver and kidneys in mice. The authors accentuated organ-specific control of transcription manifested by the gene in the course of development. The prevalence (as high as tenfold) of IGF-1A over IGF-1B mRNA transcrips, both in *in vivo* conditions (human liver) and in culture (*hepatoma* cells, macrophage-like cells and fibroblasts) using RT-PCR technique was demonstrated also by the team of Nagaoka[39]. Dominant expression of IGF-1A transcripts among other transcripts in the liver itself was described also by other authors[16,40].

A detailed quantitative analysis has confirmed higher expression of mRNAs for A and C isoforms (and of total IGF-1 mRNA) in our patients with HCV infection as compared to the control. Expression of all studied IGF-1 transcripts has also been significantly higher in women as compared to men with HCV infection. Koczorowska *et al*[33] demonstrated quantitatively higher expression of total IGF-1 mRNA in precancerous stages in uterine cervix and activity of both gene promoters at the stage of intraepithelial neoplasia. Brokaw *et al*[31] demonstrated a significant relationship between higher expression of IGF-1A isoform expression and progression of ovarian carcinoma. Nevertheless, the role of IGF-1 isoform A remains unclear. It is argued that the arising from it peptide Ea may exert both mitogenic effects[41], and it can inhibit growth of neoplastic cells[42].

The quantitative prevalence of expression manifested by IGF-1 isoforms A and C in HCV-infected livers, as compared to the healthy organ may point to influence of HCV on alterations in splicing profile of the gene in humans. Our other observations suggest influence of the mainly nonstructural proteins (NS3 and NS5A) on an increase of IGF-1 protein expression in HCV-infected livers[35] and on augmentation of IGF-1 P1 and P2 mRNA expression mRNA (unpublished data). Another explanation may involve a compensatory increase in production of IGF-1 mRNA (particularly that of IGF-1A isoform) under effect of locally acting growth factors, proinflammatory cytokines, an increased regeneration of the organ in CH-C. An increased production of two principal IGF-1 transcripts in rabbit skeletal muscle, *i.e*., muscle L.IGF-1 (resembling the isoform A in liver) and of mRNA mechano-growth factor (MGF, isoform IGF-1B in rabbits) (a homologue of IGF-1C in humans) was detected under stretching and electrical stimulation of a muscle[43]. Mechanisms of differential IGF-1 mRNA stability were also described[44,45], including effects of various RNA-binding proteins (*e.g.,* of Hu and hnRNP families) on this process[46]. In patients analyzed in this study hepatic expression of IGF-1 mRNA isoforms has been significantly lower upon higher activity of inflammation. The relationships have not been so spectacular as related to fibrosis. Possibly, we have examined an insufficient number of patients with a more advanced staging (3 patients with staging 3, one with staging 4). The studies have documented also a negative relationship between expression of all IGF-1 mRNA isoforms and steatosis and ALT activity. The results indicate a coexistence of the more pronounced inflammatory/necrotic lesions in the liver and a lower hepatic production of IGF-1 mRNA isoforms. Since literature data contains no references to the role of IGF-1 mRNA isoforms in progression of HCV-related hepatic diseases, the results seem to be of pioneer character. The other few reports on the subject concerned mainly human tumors[31-34]. In the case of *hepatoma* and HCV-associated HCC, expression of IGF-1 mRNA was studied but with no references to specific isoforms[8,13,47]. Su *et al*[47], using Northern blotting demonstrated numerous IGF-1 transcripts of various size in *hepatoma* cells and in lines of other neoplastic cells (HepG2, Huh-7, PLC/PRF/5 and Hep3B). Each fragment of neoplastic tissue showed lower expression of IGF-1 mRNA, as compared to control tissue. Using quantitative technique of real-time RT-PCR (similarly to this study) Tovar *et al*[8] demonstrated a decrease in expression of IGF-1 mRNA expression at the early stages of HCV infection, as compared to control but with no significant differences between preliminary and advanced stages of HCC in patients. Stefano *et al*[13], using RT-PCR technique demonstrated a comparable quantity of IGF-1 mRNA in patients with CH-C and in the control. Another study on cultured rat hepatocytes, using also the RT-PCR technique demonstrated 50-fold increase in expression of IGF-1B mRNA isoform (a homologue of human isoform C) in cells of obese animals as compared to hepatocytes of lean individuals[48]. The team of Armakolas *et al*[32], examining alternative splicing of IGF-1 in prostate carcinoma demonstrated in the cells overexpression of IGF-1C (MGF), suggesting role of the IGF-1 isoform in stimulation of cell proliferation.

Expression of IGF-1R transcript proved to be lower in our HCV-infected livers than that in the control, which has been accompanied by an increase in at least a portion of studied IGF-1 transcripts in the liver. It is known from earlier studies that even if normal liver represents an organ with the highest production of IGF-1, it contains an almost undetectable levels of IGF-1R mRNA[10]. Moreover, marked expression of the receptor used to be noted mainly in Kupffer cells, vascular endothelium, stellate cells but not in hepatocytes[49,50]. In this study, expression of IGF-1R mRNA in CH-C group has manifested positive correlation with grading. Therefore, it seems that local production of IGF-1R mRNA does not increase until HCV-associated hepatic lesions become pronounced, which was noted to be accompanied by a reduced production of total IGF-1 mRNA (and of its certain isoforms). A lowered expression of IGF-1, coexisting with an increased production of IGF-1R in uterine carcinoma was also described[33]. Whereas, in studies on more advanced stages of liver carcinogenesis an increase in expression of IGF-1R was detected already in preneoplastic focal lesions in liver, in HCC itself and in cell lines of human *hepatoma*[5,10]. No such increase was detected in culture of human hepatocytes[11]. Studies of Price *et al*[6] on the rat model demonstrated higher expression of IGF-1R mRNA in the control liver as compared to HCC and it was only cooperation of two proteins: IGF-1 and hepatocyte growth factor-scatter factor (HGF-SF) which stimulated mitogenesis of hepatocytes in the animals. In patients with liver cirrhosis and CH-C increased amounts of IGF-1R transcript were detected as compared to normal liver[12,13]. Tovar *et al*[8] in the material of human HCC failed to detect a significant difference in expression of IGF-1R mRNA in hepatocellular carcinoma as compared to the control.

Differences in quantitative expression of IGF-1 mRNA isoforms in HCV-infected livers, as compared to the control, suggest that HCV may induce alteration of *IGF-1* splicing profile. Increase in grading of HCV-infected livers was linked to decreased IGF-1 mRNA expression, an altered profile of mRNA isoforms and to an increase in IGF-1R mRNA expression. Demonstration of an increased tissue expression of IGF-1R mRNA and the decreased expression level of IGF-1 mRNA isoforms, accentuated in line with increasing liver damage may be of a prognostic significance.

**comments**

***Background***

Insulin-like growth factor 1 (IGF-1) represents a well-recognized proproliferative factor.

***Research frontiers***

No studies are available on the role played by local expression involving various IGF-1 mRNA isoforms in chronic hepatitis C *in vivo*.

***Innovations and breakthroughs***

A quantitative polymerase chain reaction analysis used in the study has confirmed higher expression of total IGF-1 mRNA and of IGF-1 mRNAs isoforms A and C in hepatitis C virus-infected livers as compared to the control.

***Applications***

Demonstration of the decreased expression level of IGF-1 mRNA isoforms, and an increased tissue expression of IGF-1R mRNA, associated with indicators of liver damage (*e.g.,* grading, staging, steatosis, liver serum enzyme activity) may be of a prognostic significance.

***Peer review***

This paper is really interesting but it presents important clinical limitations, mostly related to the low number of patients with chronic hepatitis C investigated, to the low prevalence old patients with a severe forms of the disease and to the insufficient number of controls.

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**Figure 1 Shares (%) of studied insulin-like growth factor-1 mRNA isoforms of class 1 (I) and class 2 (II) (depending upon employed promoter P1 or P2) as related to the total mRNA for both insulin-like growth factor-1 promoters (100%) in chronic hepatitis C patients and in the control.** Igf: insulin-like growth factor; CH-C: Chronic hepatitis C.



**Figure 2 Shares (%) of splicing mRNA isoforms for insulin-like growth factor-1 (1A, 1B, 1C) as related to the total amount of mRNA for all three insulin-like growth factor-1 isoforms (100%) in Chronic hepatitis C patients and in the control.** IGF: insulin-like growth factor; CH-C: Chronic hepatitis C.

b



b

**Figure 3 Comparison of shares (%) in expression of insulin-like growth factor-1 mRNA isoforms in patients chronically infected with hepatitis C virus and in the control.** b*P <* 0.01 *vs* control.

**Table 1 Quantitative expression of insulin-like growth factor-1 mRNA isoforms, total mRNA insulin-like growth factor-1 and insulin-like growth factor-1R mRNA (mean ± SD) normalized in relation to housekeeping genes in chronic hepatitis C and in control organ**

|  |  |  |  |
| --- | --- | --- | --- |
| **mRNA** | **Control** **(*n =* 7)** | **CH-C** **(*n =* 34)** | **CH-C** |
| **Women (*n =* 16)** | **Men****(*n =* 18)** |
| class 1 (P1) | 0.19 ± 0.21 | 0.45 ± 0.45 | 0.63 ± 0.59 | 0.28 ± 0.17 |
| *P =* 0.025 | *P =* 0.003 |
| class 2 (P2) | 0.08 ± 0.07 | 0.15 ± 0.17 | 0.20 ± 0.15 | 0.11 ± 0.18 |
| *P =* 0.164 | *P =* 0.006 |
| IGF-1A | 1.30 ± 1.31 | 5.28 ± 9.94 | 7.88 ± 13.83 | 2.97 ± 3.32 |
|  | *P =* 0.009 | *P =* 0.027 |
| IGF-1B | 0.18 ± 0.18 | 0.28 ± 0.40 | 0.35 ± 0.45 | 0.22 ± 0.35 |
|  | *P =* 0.486 | *P =* 0.042 |
| IGF-1C | 0.04 ± 0.05 | 0.21 ± 0.57 | 0.35 ± 0.82 | 0.08 ± 0.06 |
|  | *P =* 0.031 | *P =* 0.046 |
| total IGF-1 | 1.52 ± 1.52 | 5.77 ± 10.85 | 8.58 ± 15.09 | 3.26 ± 3.70 |
|  | *P =* 0.014 | *P =* 0.027 |
| IGF-1R | 0.26 ± 0.18 | 0.14 ± 0.04 | 0.14 ± 0.05 | 0.14 ± 0.04 |
|  | *P =* 0.016 | *P =* 0.295 |

IGF-1: insulin-like growth factor 1; CH-C: Chronic hepatitis C.

**Table 2** **Tissue expression of insulin-like growth factor-1 mRNA isoforms and insulin-like growth factor-1R mRNA (mean ± SD) as related to grading and staging in hepatitis C virus-infected livers**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **mRNA** | **Grading1 1 (*n =* 15)** | **Grading 2****(*n =* 16)** | **Staging1 1****(*n =* 21)** | **Staging 2****(*n =* 8)** |
| class 1 (P1) | 0.63 ± 0.61 | 0.33 ± 0.20 | 0.50 ± 0.55 | 0.41 ± 0.23 |
|  | *P =* 0.037 | *P =* 0.905 |
| class 2 (P2) | 0.25 ± 0.22 | 0.09 ± 0.06 | 0.19 ± 0.20 | 0.11 ± 0.07 |
|  | *P =* 0.009 | *P =* 0.549 |
| IGF-1A | 8.67 ± 14.34 | 2.42 ± 1.45 | 6.55 ± 12.47 | 3.01 ± 1.77 |
|  | *P =* 0.008 | *P =* 0.943 |
| IGF-1B | 0.44 ± 0.55 | 0.15 ± 0.10 | 0.34 ± 0.49 | 0.18 ± 0.12 |
|  | *P =* 0.012 | *P =* 0.720 |
| IGF-1C | 0.36 ± 0.84 | 0.08 ± 0.06 | 0.27 ± 0.72 | 0.09 ± 0.07 |
|  | *P =* 0.093 | *P =* 0.582 |
| total IGF-1 | 9.47 ± 15.66 | 2.65 ± 1.58 | 7.16 ± 13.61 | 3.29 ± 1.91 |
|  | *P =* 0.006 | *P =* 0.830 |
| IGF-1R | 0.12 ± 0.04 | 0.16 ± 0.03 | 0.14 ± 0.04 | 0.16 ± 0.04 |
|  | *P =* 0.025 | *P =* 0.198 |

1parameters evaluated in a semi-quantitative scale (see Material and Methods). IGF-1: insulin-like growth factor 1.

**Table 3 Values of Spearman’s coefficient for correlation between expression of insulin-like growth factor-1, insulin-like growth factor-1R mRNAs and clinical data in hepatitis C virus-infected patients**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **class 1 (P1)** | **class 2 (P2)** | **IGF-1A** | **IGF-1B** | **IGF-1C** | **total IGF-1** | **IGF-1R** |
| age (yr) | **-0.545** | **-0.644** | **-0.582** | **-0.654** | **-0.532** | **-0.596** | **0.372** |
| BMI | **-0.527** | **-0.452** | **-0.468** | **-0.414** | **-0.489** | **-0.468** | 0.060 |
| grading | **-0.431** | **-0.569** | **-0.446** | **-0.430** | -0.285 | **-0.457** | **0.456** |
| staging | -0.223 | **-0.346** | -0.182 | -0.187 | -0.160 | -0.190 | 0.264 |
| steatosis (%) | **-0.415** | **-0.571** | **-0.412** | **-0.408** | **-0.454** | **-0.422** | 0.125 |
| ALT (U/l) | **-0.538** | **-0.616** | **-0.540** | **-0.577** | **-0.533** | **-0.550** | 0.181 |
| AST (U/l) | **-0.434** | **-0.569** | **-0.378** | **-0.400** | **-0.342** | **-0.392** | 0.212 |
| AFP (ng/ml) | -0.291 | **-0.390** | -0.168 | -0.271 | -0.240 | -0.180 | 0.311 |
| HCV RNA (IU/ml) | -0.330 | **-0.388** | **-0.357** | -0.244 | -0.096 | **-0.345** | **0.403** |
| total protein (g/dl) | 0.302 | 0.251 | 0.324 | **0.375** | **0.366** | 0.319 | 0.141 |
| albumins (g/dl) | 0.293 | **0.381** | 0.303 | **0.349** | 0.222 | 0.295 | 0.123 |
| gammaglobulins (g/dl) | -0.061 | -0.164 | 0.039 | 0.045 | 0.174 | 0.044 | 0.138 |
| blood platelets (G/l) | 0.273 | **0.372** | 0.235 | 0.265 | 0.138 | 0.232 | -0.099 |
| cholesterol (mg/dl) | **0.386** | 0.286 | **0.409** | **0.405** | 0.372 | **0.404** | 0.048 |
| GGTP (U/l) | -0.384 | **-0.603** | -0.284 | -0.399 | -0.367 | -0.307 | 0.330 |
| Blood glucose (mg/dl) | -0.083 | -0.226 | -0.190 | -0.112 | -0.084 | -0.201 | 0.152 |
| HOMA-IR | -0.067 | -0.157 | -0.237 | -0.140 | -0.184 | -0.234 | -0.019 |
| Insulin (µU/ml) | -0.020 | -0.082 | -0.145 | -0.079 | -0.125 | -0.135 | -0.045 |

bold numbers indicate values of *r* coefficient for which the *P* < 0.05. IGF-1: insulin-like growth factor 1.