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Structural changes of fibrinogen as a consequence of cirrhosis

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Abbreviations: IGFBP-1, insulin-like growth factor binding protein-1; IGF, insulin-like growth factor; PBS, phosphate buffered saline; 2,4-DNP, 2,4-dinitrophenylhydrazine; CD, circular dichroism; Man, mannose; Fuc, fucose; GlcNAc, N-acetylglucosamine; Gal, galactose; Glc, glucose; CBB, Coomassie Brilliant Blue; INR, international normalized ratio of prothrombin time; FFP, Fresh Frozen Plasma

Abstract

Cirrhosis is a disease which may develop as a consequence of various conditions. In advanced liver disease, blood coagulation can be seriously affected. Portal hypertension, vascular abnormalities and/or a dysbalance in coagulation factors may result in bleeding disorders or in the development of thrombosis. Fibrinogen is the main protein involved in clot formation and wound healing. The aim of this work was to analyse the glycosylation pattern of the isolated fibrinogen molecules by lectin-based protein microarray, together with the carbonylation pattern of the individual fibrinogen chains, possible changes in the molecular secondary and tertiary structure and reactivity with the insulin-like growth factor-binding protein 1 (IGFBP-1) in patients with cirrhosis. The results pointed to an increase in several carbohydrate moieties: tri/tetra-antennary structures, Gal β-1,4 GlcNAc, terminal α-2,3 Sia and α-1,3 Man, and a decrease in core α-1,6 Fuc and bi-antennary galactosylated N-glycans with bisecting GlcNAc. Fibrinogen Aα chain was the most susceptible to carbonylation, followed by the Bβ chain. Cirrhosis induced additional protein carbonylation, mostly on the α chain. Spectrofluorimetry and CD spectrometry detected reduction in the α-helix content, protein unfolding and/or appearance of modified amino acid residues in cirrhosis. The amount of complexes which fibrinogen forms with IGFBP-1, another factor involved in wound healing was significantly greater in patients with cirrhosis than in healthy individuals. A more detailed knowledge of individual molecules in coagulation process may contribute to deeper understanding of coagulopathies and the results of this study offer additional information on the possible mechanisms involved in impaired coagulation due to cirrhosis.

Keywords: Fibrinogen; Glycosylation; Carbonylation; Protein structure; Protein interaction

1. Introduction

Cirrhosis is a disease which may develop as a consequence of various conditions such as viral infection, alcohol abuse, metabolic disorder, autoimmunity, congenital disease and may also be of the unknown origin [1]. Risk factors for developing cirrhosis are regular alcohol consumption, age over 50, male gender and metabolic syndrome [2]. In the advanced liver disease, blood coagulation can be seriously affected, as both pro- and anti-coagulation factors may become progressively reduced primarily due to the reduced synthetic capacity of the liver. Most bleeding complications which develop in cirrhosis are due to portal hypertension and vascular abnormalities [3]. A dysbalance in coagulation factors and a subsequent loss of endogenous anticoagulant mechanisms may account for the excessive formation of thrombus. It is not uncommon to diagnose hypercoagulable state at one site, developed from the local endothelial dysfunction, together with a systemic hypocoagulable condition [4,5].

Oxidative stress contributes to both initiation and progression of liver disease [6]. Fibrinogen or coagulation factor I, is the main protein involved in the clot formation and wound healing. It consists of two α, β and γ chains [7]. This protein is much more susceptible to oxidation than

other major plasma proteins such as albumin, transferrin or immunoglobulins [8]. Protein carbonylation increases in cirrhosis [9] and it effects fibrinogen structure and function [10]. Recent results have shown that modifications which occur on fibrinogen due to cirrhosis may contribute to the development of thrombotic complications [11].

Another important post-translational modification of proteins is glycosylation. Glycosylation influences ligand-receptor recognition, protein-protein interaction and protein turnover [12]. The carbohydrate units on proteins contribute to protein stability and dynamics [12-14]. Thus, changes in protein saccharide moieties are expected to affect its function. It was already shown that structural changes of glycans on fibrinogen occur due to ageing and influence clotting time [15]. In general, glycosylation may change as a result of certain pathology, such as cancer or liver disease. Common to most liver diseases seems to be an increased fucosylation, branching and the quantity of the bisecting N-acetylglucosamine, GlcNAc [16]. Fibrinogen in patients in the advanced stage of cirrhosis is characterised by the low concentration, increased oxidation, hypersialyation, delayed conversion to fibrin and decreased fibrin permeability [17].

The aim of this work was to analyse the glycosylation pattern of fibrinogen molecules by lectin-based protein microarray, together with the carbonylation pattern of the individual fibrinogen chains, possible changes in the molecular secondary and tertiary structure and the reactivity with IGFBP-1in patients with cirrhosis. IGFBP-1 participates in wound healing by both IGF dependent [18] and independent actions [19]. The first one is responsible for the IGF delivery to the site of injury and the second exploits interaction between the RGD domain in IGFBP-1 and $\alpha_5\beta_1$integrin on cell surfaces initiating cell signalling. Fibrinogen was shown to associate with IGFBP-1 *in vivo* and its glyco-oxidation reduces the amount of complexes [20].

2. Materials and methods

2.1 Samples

Blood samples were collected from 20 healthy volunteers (9 males, 11 females, age 41-73 years) and 20 patients with advanced cirrhosis (12 males, 8 females, age 43-72 years) who were diagnosed and followed-up in the Clinical-Medical Center "Bežanijska kosa". The patients (n = 12) were hospitalised due to pain under the right rib, loss of body weight, increased temperature, nausea, vomiting, general weakness, hepatosplenomegaly and ascites. In 6 patients, the main reason for hospitalisation was variceal bleeding from esophagus and anemia (one patient had portal vein thrombosis). Two patients were hospitalised due to ulcer perforation and stomach pain. Blood was taken from all patients upon their admission to the hospital, prior to any treatment. The diagnosis of cirrhosis was made on the basis of clinical, biochemical and pathohistological parameters using standard techniques including ultrasound, endoscopy and microscopy. The etiology was recognised as excessive alcohol consumption (n = 17), chronic hepatitis C infection (n = 1) and miscellaneous causes (n = 2). All cases were classified as Child score C, decompensated. The concentration of albumin in patients was 26.0 (20.15-37.80) g/l,

total bilirubin 84.5 (40.04-209.10) μM, conjugated bilirubin 25.0 (9.84-117.76) μM and INR 1.8 (0.99-2.15). All patients were hospitalised and subjected to the following therapy: Hepa-Merz amp. (3x2 units) and Hepasol (8 %). Additionally, patients who had active bleeding received transfusion of the concentrated erythrocytes if their haemoglobin concentration was below 75 g/l and heamtocrit below 0.26, and FFP in a dose of 10-15 ml/kg of body weight per day. Cryoprecipitate (7-10 units per day) was administrated to the patients who, besides active bleeding, had fibrinogen level below 2 g/l. Patients with platelet count bellow $20x10^9$ /l received preparation of concentrated thrombocytes. Patients with INR above 2.5 received vitamin K (10-40 mg per day) and FFP, while those with albumin below 26 g/l received 20 % albumin solution (100 ml per day). The exclusion criteria for patients were the following: the presence of congenital coagulation disorder, recent infection, HIV positive status and malignancy. The study was approved by Ethical Committees of the CMC "Bežanijska kosa" and the Institute INEP, and informed consent was obtained from patients for the use of their samples in this research. Blood samples from volunteers were obtained in the morning, after at least 12 h of fasting and the plasma separated within 1 h. Determination of protein concentrations and isolation of fibrinogen were performed on the same day.

2.2 Determination of protein concentrations

The concentration of total proteins was determined by the Biuret reagent (Human GmbH, Wiesbaden, Germany), fibrinogen by the Fowell reagent (Alfapanon, Novi Sad, Serbia) and IGFBP-1 by the ELISA test (Abcam, Cambridge, UK).

2.3 Isolation of fibrinogen from plasma

Fibrinogen was isolated from plasma according to the modified published procedure [21], using ethanol. After the first precipitation step, fibrinogen was dissolved in 10 mM PBS and the precipitation repeated once more in order to obtain highly purified fibrinogen. The purity of the isolated fibrinogen was assessed by the reducing SDS and native PAGE, followed by the CBB staining. The concentration of the isolated fibrinogen was determined using both Fowell reagent and the bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific, Waltham, USA). The choice of the method was made taking into consideration that fibrinogen had to be determined both in plasma and in the preparation of the isolated fibrinogen. An optical based coagulometer could not be employed, as this method is based on the coagulation process involving many coagulation factors present in plasma and absent in the preparation of the isolated fibrinogen. Thus, it was considered that chemical reaction for determination of fibrinogen would be more appropriate, possibly less affected by the altered milieu (plasma *vs* fibrinogen in the buffer solution).

2.4 Analysis of fibrinogen glycosylation

Lectin-based protein microarray was used for the assessment of fibrinogen glycosylation. Individual fibrinogen samples (0.1 mg/ml in PBS) were spotted on the Nexterion slide E (Schott, Jena, Germany) using a spotter sciFLEXARRAYER S1 and piezo dipense capillary PDC 90 (Scienion AG, Berlin,Germany) with the fixed drop volume of 480 pl, at 10 ^{o}C and 50 % humidity. Each sample was spotted in triplicate into 15 identical subarrays. After incubation at 4 ^{o}C for 2h, the remaining free epoxy groups on slides were blocked with 3% bovine serum albumin in PBS for 1 h, the slide washed with PBST (PBS with 0.1 % TWEEN-20) and 15 biotinylated lectins (Vector, Burlingame, USA) applied at the concentration of 25 µg/ml in PBST (Table 1). After incubation with lectins for 1h, the slide was washed with PBST and exposed to CF647-streptavidin conjugate (Biotium, Hayward, USA, 0.5 µg/ml in PBST) at room temperature for 15 min. The slide was thoroughly washed, dried and scanned in InnoScan®710 laser fluorescent scanner (Innopsys, Carbonne, France) at a wavelength of 635 nm. Images were processed by the Mapix 5.5.0 software (Innopsys) and the results corrected for the background signal. Signal-to-noise ratio was above 3 in the case of all lectins.

Table 1. The list of lectins used in the lectin-based protein microarray

Lectin	**Carbohydrate specificity**
Griffonia simplicifolia lectin-II (GSL-II)	Agalactosylated tetra/triantennary N-type
Galanthus nivalis lectin (GNL)	High-mannose type N-glycans, Man α1,3 Man
Maackia amurensis lectin-I (MAL-I)	Gal (β-1,4) GlcNAc
Hippeastrum hybrid lectin (HHL)	High-mannose type N-glycans, Man α1,3 Man, Man α1,6 Man
Narcissus Pseudonarcissus Lectin (NPL)	Polymannose structures containing (α-1,6) linkages
Ricinus communis agglutinin-I (RCA-I)	Gal β1,4 GlcNAc
Lens culinaris agglutinin (LCA)	α-D-Glc, α-D-Man in N-glycans with Fuc α1,6 GlcNAc
Aleuria aurantia lectin (AAL)	Fuc α1,6 GlcNAc, Fuc α1,3 (Gal β1,4) GlcNAc
Phaseolus vulgaris leukoagglutinin (PHA-L)	Tri-/tetraantennary complex type N-glycans
Concanavalin A (ConA)	High-mannose type N-glycans, Man α1,6 (Man α1,3) Man
Pholiota squarrosa lectin(PhoSL)	Core α1–6-fucosylated *N*-glycans
Sambucus nigra agglutinin (SNA)	Terminal α2,6 Sia bound to Gal or GalNAc
Phaseolus vulgaris erythtroagglutinin (PHA-E)	Bi/tri-antennary complex type N-glycans with terminal Gal and bisecting GlcNAc
Wheat germ agglutinin (WGA)	GlcNAc β1,4 GlcNAc
Maackia amurensis lectin-II (MAL-II)	Terminal α2,3 Sia bound to Gal 1,4 GlcNAc

2.5 Analysis of fibrinogen carbonylation

In order to determine a degree of fibrinogen carbonylation and investigate which chains are prone to oxidation, 6 pools of the isolated fibrinogen (2 mg/ml), were analysed: 3 from each

study group (healthy individuals or patients with cirrhosis). Pools were made by using equal quantities of fibrinogen isolated from 6-7 persons. Fibrinogen carbonyl groups were derivatised with 2,4-DNP, [22] and the samples analysed by the reducing SDS PAGE on 10 % gels [23]. Proteins were transferred to the nitrocellulose membrane, stained with the Ponceau S and subjected to immunoblotting using rabbit anti-DNP antibody (Sigma, Steinheim, Germany), HRP-conjugated secondary anti-rabbit IgG antibody (AbD Serotec, Kidlington, UK) and the ECL reagent (Pierce Biotechnology, Rockford, USA). Proteins were visualised by autoradiography. Densitometric analysis was done using the Image Master TotalLab software (Amersham BioSciences, Buckinghamshire, UK).

2.6 Analysis of fibrinogen by spectrofluorimetry

Six pools of the isolated fibrinogen samples (40 nM, made as explained in the section 2.5) were analysed by spectrofluorimetry. Fluorescence spectra were obtained on the FluoroMax®-4 spectrofluorimeter (HORIBA Scientifc, Japan), with the width of the excitation and emission slits adjusted at 5 nm. Spectra were recorded in 2 ranges: 290-400 nm (after the excitation at 280 nm) and 310-400 nm (after the excitation at 295 nm). Spectra were corrected for the background signal originating from the buffer.

2.7 Analysis of fibrinogen by CD spectroscopy

CD measurements were performed on the Jasco J-815 spectropolarimeter (Jasco, Tokyo, Japan). Far-UV CD spectra of 6 pooled fibrinogen samples (made as explained in the section 2.5), at protein concentration of 1μM, were recorded in the range of 185-260 nm, at a scan speed of 50 nm/min, using a cell with an optical path length of 0.1 mm and with an accumulation of three scans. Near-UV CD spectra of the same solutions were recorded in the range of 250-350 nm, at a scan speed of 50 nm/min, using a cell with an optical path length of 10 mm and with an accumulation of two scans.

2.8 Analysis of the interaction between fibrinogen and IGFBP-1

In order to detect the interaction between fibrinogen and IGFBP-1, individual fibrinogen samples (0.4 mg/ml) were subjected to the native PAGE on 8 % gels and immunoblotting using rabbit anti-IGFBP-1 (AbD Serotec) or goat anti-fibrinogen (Abcam) primary antibody. Biotinylated secondary anti-goat IgG antibody, coupled with the HRP-conjugated avidin (Vector) or HRP-conjugated secondary anti-rabbit IgG antibody (AbD Serotec) and the ECL reagent (Pierce Biotechnology) were applied for the immunodetection. Densitometric analysis was performed using the Image Master Total Lab software (Amersham BioSciences). The IGFBP-1 immunoblotting was performed first, then the membrane was stripped, fibrinogen detected and the results presented as the ratio of IGFBP-1 to fibrinogen signals.

2.9. Statistical analysis

All data are presented as medians with the percentile rank (2.5^{th}– 97.5^{th}). In the case of the normally distributed data, the statistical difference between the groups was analysed by the Student's *t*-test. Mann-Whitney *U* test was performed for the non-normally distributed data.

3. Results and discussion

Determination of the protein concentration revealed that total proteins in plasma were significantly reduced in patients with cirrhosis, the concentration of fibrinogen remained in a similar range as in healthy persons, whereas the concentration IGFBP-1 increased more than twice in patients (Table 2).

Table 2. The concentrations of proteins in plasma samples obtained from healthy persons and the patients with cirrhosis (medians with the percentile rank, 2.5^{th} – 97.5^{th})

Protein concentrations	**Healthy persons (n = 20)**	**Patients with cirrhosis (n = 20)**
Total proteins (g/l)	75.3 (67.42-80.67)	53.8 (45.80-64.25) $p = 5.344 \times 10^{-7}$
Fibrinogen (g/l)	3.3 (2.45-4.79)	3.8 (2.22-6.15) p = 0.119
IGFBP-1 (µg/l)	15.3 (1.12-42.97)	37.7 (9.21-108.27) p = 0.005

These results are in agreement with the already published data, as hypoproteinemia is a common feature in patients with cirrhosis, while fibrinogen may remain within the reference range or decrease in the advanced stages of cirrhosis [24-25]. Since the fibrinogen level increases in inflammatory states [26] and cirrhosis may be accompanied by inflammation, the final concentration of fibrinogen is a result of the equilibrium between two opposing processes (inflammation and reduced synthetic capacity of the liver). An increase in IGFBP-1 concentration due to cirrhosis was detected in several studies [27-30].

Although plasma concentrations of fibrinogen in two study groups were similar, the difference between samples became evident during the isolation of fibrinogen. Fibrinogen from healthy persons was almost quantitatively precipitated by ethanol, resulting in the concentration of 3.1 (2.13-4.78) mg/ml, when normalized to 1 ml of plasma. By using two methods to determine the concentration of isolated fibrinogen, a specific one (Fowell) and a general for proteins (BCA), we could conclude that the isolated protein was just fibrinogen. The recovery of fibrinogen was 93 (87.2-99.0) %. The purity of the preparation was confirmed by the reducing SDS and native PAGE (Figure 1). On the contrary, fibrinogen from the patients with cirrhosis was rarely precipitated quantitatively, resulting in the concentration of 2.5 (0.80-4.44) mg/ml. The recovery of fibrinogen was 67 (36.4-72.1) % and the preparation contained only fibrinogen (Figure 1).

The difference in the efficiency of fibrinogen isolation between these two groups was significant ($p = 0.001$). Such difference pointed to the altered plasma milieu due to cirrhosis, which disabled complete precipitation of the protein by the procedure optimized for the isolation of fibrinogen from plasma originating from healthy persons.

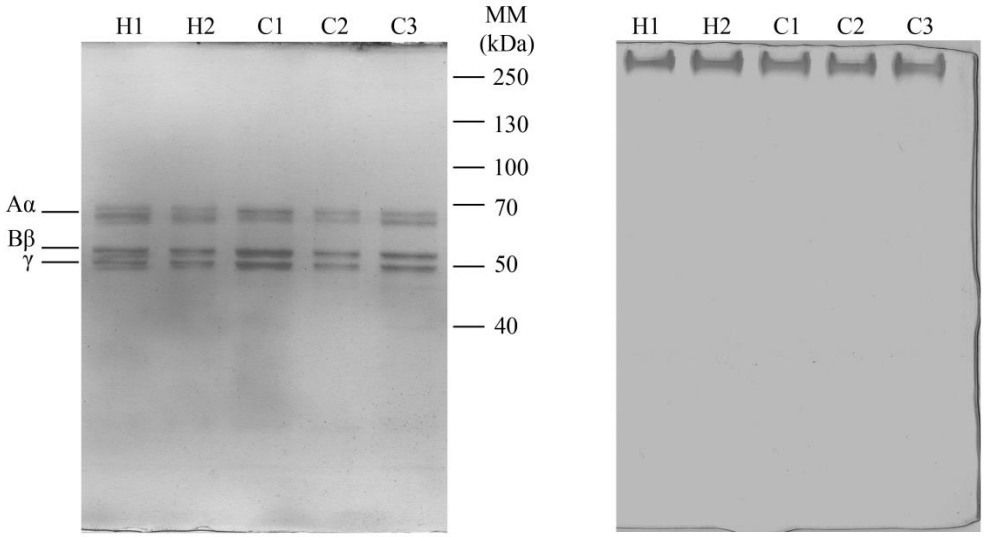

Figure 1. The CBB staining of the isolated fibrinogen resolved by the reducing SDS PAGE on 10 % gel (left panel) and native PAGE on 8 % gel (right panel). Five isolated fibrinogen samples (representative) are shown: 2 from healthy persons (H1-2) and 3 from patients with cirrhosis (C1-3).

The assessment of the glycosylation pattern of fibrinogen by the lectin-based protein microarray revealed that cirrhosis induced changes in fibrinogen glycosylation. As it can be seen in Table 3, significant differences (compared to the healthy persons) were observed with lectins PhoSL, MAL-I, MAL-II, GNL, PHA-L and PHA-E. The results pointed to an increase in several carbohydrate moieties: tri/tetra-antennary structures, Galβ-1,4GlcNAc, terminal α-2,3 Sia and α-1,3 Man. On the other hand, the core α-1,6Fuc and bi-antennary galactosylated N-glycans with the bisecting GlcNAc were reduced in cirrhosis.

An increase of Sia residues on fibrinogen due to the liver diseases was previously reported [31]. A similar increase of terminal Sia residues and glycan branching was detected on transferrin in patients with liver cancer [32]. Sia is negatively charged and fibrin clots obtained from the patients with cirrhosis have higher density [11]. Sia residues are the binding sites for calcium ions. This interaction may facilitate clot formation [33], but can also induce an increase in total calcium in plasma (free plus protein-bound), contributing to an overall hypercalcemia, a well-known feature of the advanced chronic liver disease [34-35] and a risk factor for development of thrombosis. Increased fucosylation was detected in the patients with liver disease; in whole serum, on α_1-acid glycoprotein or haptoglobin [36-38]. An increase in α-1,6 Fuc bound to GlcNAc was also detected in this study, but the change was below the statistical significance.

Reduction in the core Fuc on fibrinogen suggests that glycosylation of different proteins may be differently affected by cirrhosis. The importance of glycans on fibrinogen for its proper function was already documented. Deglycosylation or modified glycosylation alters its coagulation properties possibly leading to a complete dysfunction [39-43]. The altered glycosylation of fibrinogen due to ageing affects its clotting time [15].

Table 3.The reactivity of the isolated fibrinogen obtained from healthy persons and the patients with cirrhosis with lectins and IGFBP-1 (medians with the percentile rank, 2.5^{th} – 97.5^{th})

Reactivity of fibrinogen with lectins (arbitrary fluorescence units)	**Healthy persons (n = 20)**	**Patients with cirrhosis (n = 20)**
GSL-II	1.9 (1.433-2.023)	2.0 (1.80-2.09) p = 0.070
GNL	1.8 (1.29-1.99)	1.9 (1.81-1.93) p = 0.033
MAL-I	1.0 (0.79-1.32)	1.2 (1.03-1.26) p = 0.016
HHL	1.2 (0.95-1.35)	1.2 (1.10-1.27) p = 0.732
NPL	1.9 (1.47-2.22)	2.0 (1.87-2.12) p = 0.569
RCA-I	3.2 (3.12-3.28)	3.2 (2.90-3.23) p = 0.094
LCA	2.3 (2.11-2.60)	2.3 (2.18-2.41) p = 0.972
AAL	2.2 (2.00-2.51)	2.3 (2.12-2.44) p = 0.539
PHA-L	1.5 (1.29-1.77)	1.7 (1.61-1.79) p = 0.004
ConA	3.1 (3.04-3.18)	3.1 (2.89-3.17) p = 0.452
PhoSL	2.4 (2.28-2.45)	2.3 (2.19-2.38) p = 0.010
SNA	3.1 (3.02-3.11)	3.0 (2.89-3.09) p = 0.073
PHA-E	2.5 (2.40-2.71)	2.5 (2.26-2.59) p = 0.046
WGA	2.3 (2.08-2.58)	2.3 (1.99-2.38) p = 0.269
MAL-II	1.7 (1.52-2.02)	2.0 (1.63-2.11) p = 0.007
Reactivity of fibrinogen with IGFBP-1 (ratio of densitometric signals)		
IGFBP-1/fibrinogen	0.1 (0.04-0.19)	0.2 (0.03-0.28) p = 0.044

The assessment of the carbonylation pattern of fibrinogen revealed that the Aα chain was the most susceptible to carbonylation followed by the Bβ chain (Figure 2). There was no DNP immunoreactive signal detected on the γ chain. The same type of results was obtained with fibrinogen originating from the either study group. Xu and co-workers [44] reported that α chain of fibrinogen is the most susceptible chain to interaction with the reactive oxygen species *in vitro*, followed by the β chain. Our finding that α and β chains of fibrinogen are primarily (and perhaps exclusively) oxidatively modified *in vivo* in patients with cirrhosis may contribute to the better understanding of changes which accompany coagulation process, since α and β chains contain sites for the cleavage by thrombin. Upon the release of fibrinopeptides A and B by thrombin, polymerisation sites are liberated to participate in the initial end-to-middle association of fibrin monomers and the formation of double-stranded fibrils, as well as for the lateral associations to create multi-stranded fibrils. Additionally, the αC domains are important as they can mutually interact favouring the lateral growth of fibrin fibrils [7,10,45-46]. The data obtained both *in vivo* and *in vitro* have shown that fibrinogen carbonylation affects its function [10,44,47-49]. When results on fibrinogen carbonylation were compared between healthy persons and the patients with cirrhosis in this study (densitometric signals normalized to the protein concentrations), it was found that cirrhosis induced additional protein carbonylation, mostly on the α chain. The intensity of densitometric signals in the patients was 38.5 (25.40-51.58) and in healthy persons 17.3 (16.81-34.60), which is also in agreement with the published data [11].

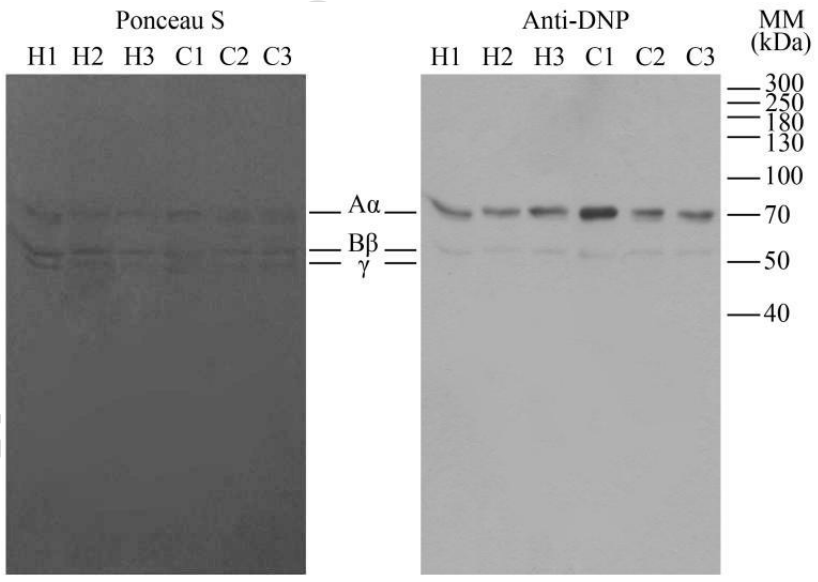

Figure 2. The Ponceau S staining (left panel) and immunoblot with anti-DNP antibody (right panel) of the fibrinogen chains derivatised with DNP and resolved by the reducing SDS PAGE on a 10 % gel. Six pools of the isolated fibrinogen were analysed: 3 from each study group (healthy persons, H1-3 and the patients with cirrhosis, C1-3).

In order to investigate possible changes of the fibrinogen structure due to cirrhosis, several experiments were performed (Figure 3). Far-UV CD spectra of fibrinogen displayed typical signal for the α-helix (negative bands at 209 and 222 nm), as it is its dominant secondary structure. Reduction in the α-helix content in fibrinogen originating from the patients with cirrhosis compared to the healthy persons was observed (Figure 3A). Becatti and co-workers [48] reported a reduction in the fibrinogen α-helix structure due to oxidation in the patients with myocardial infarction, which resulted in the formation of denser clots. Judging from the data from our study and some other published results [11], it is possible that similar structure-function relationship can be attributed to fibrinogen in patients with cirrhosis. Near-UV CD spectra showed slight differences between two groups (Figure 3B), suggesting the possible change in the protein tertiary structure. Spectrofluorimetric analysis (Figure 3C and 3D), revealed a reduction in the intrinsic fluorescence of fibrinogen from the patients, confirming that its tryptophan residues resided in the altered surrounding. This feature can be a consequence of either protein unfolding or the appearance of modified amino acid residues. Based on these results, it is plausible to suspect that structural changes of fibrinogen can be attributed to the changes in the glycosylation and/or oxidation, but this was not definitely confirmed.

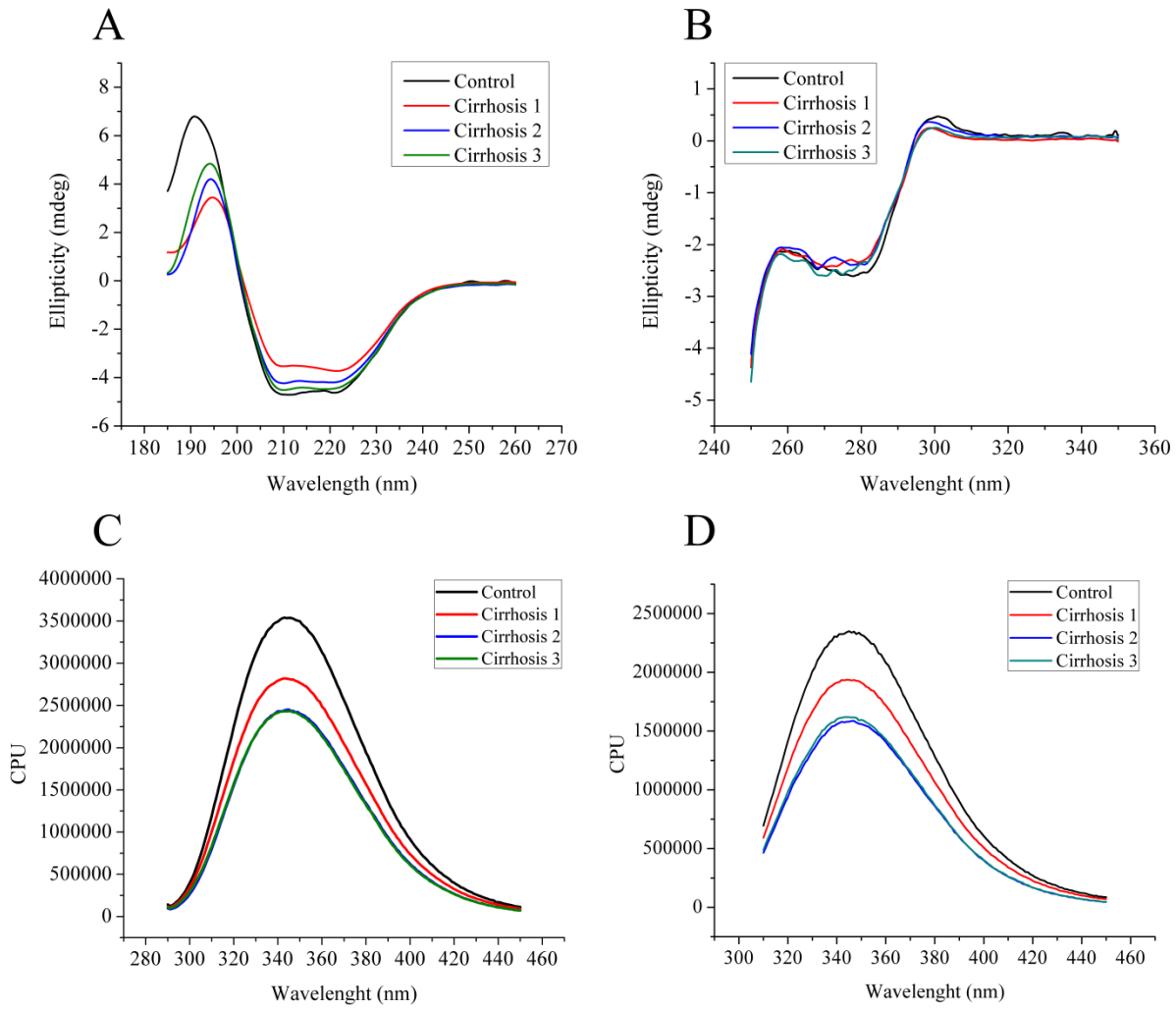

Figure 3. The analysis of the secondary and tertiary structure of fibrinogen: far-UV CD spectra (A), near-UV CD spectra (B), fluorescence spectra after excitation at 280 nm (C), or 295 nm (D). The results for 4 pools of the isolated fibrinogen are shown: one from healthy persons (Control) and 3 from the patients with cirrhosis (Cirrhosis 1-3).

Investigation of fibrinogen reactivity with the IGFBP-1 demonstrated that the amount of IGFBP-1/fibrinogen complexes was significantly greater in the patients with cirrhosis than in healthy individuals (Table 3 and Figure 4). Taking into account that the concentration of IGFBP-1 was much higher in patients (Table 2), the finding is not unexpected. However, the increase in the IGFBP-1 level in patients is greater than the increase in the amount of complexes (2.5 times compared to 1.5 times), suggesting that other factors besides the IGFBP-1 concentration influenced the formation of complexes. The reduced affinity of fibrinogen for IGFBP-1, due to its structural modification, may be one such factor.

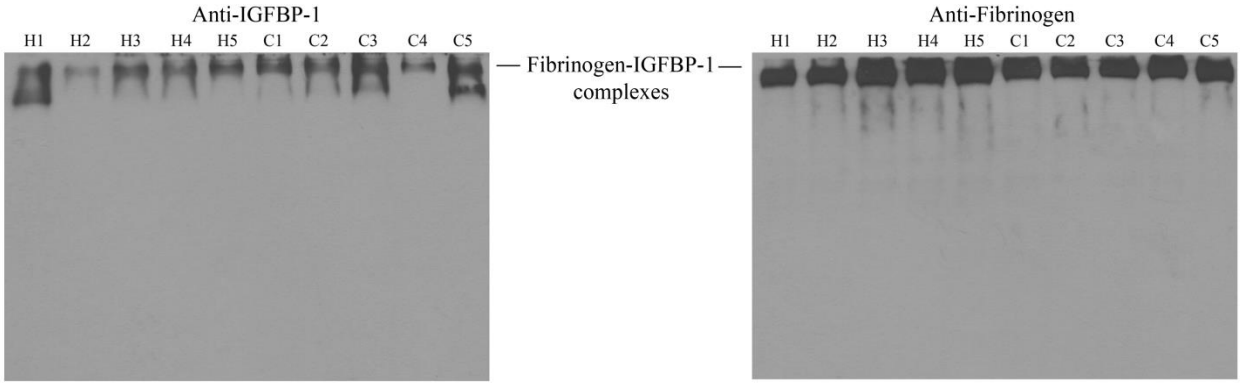

Figure 4. The immunoblot analysis of the fibrinogen/IGFBP-1 complexes subjected to the native PAGE on a 8 % gel with anti-IGFBP-1 (left panel) or anti-fibrinogen antibody (right panel). Results for 10 individual (representative) samples of the isolated fibrinogen are shown: 5 from each study group (healthy persons, H1-5 and the patients with cirrhosis, C1-5).

As mentioned in the Introduction, IGFBP-1 has beneficial effects on the injured tissue either by delivering IGF-I [50] or by directly interacting with cells, affecting their mobility [51], secretion of other growth factors [52] or contributing to the wound healing [19,53]. In cirrhosis, however, the increased IGFBP-1 concentration at the site of injury is, perhaps, not beneficial as its action may contribute to thrombosis. The similar hypothesis was proposed by Ramos-Mozo and co-workers [18] who discovered the increased IGFBP-1 concentration in patients with the abdominal aortic aneurysm as a risk factor.

Conclusions

The results of the study presented in this article pointed to significant changes in fibrinogen glycosylation, carbonylation, secondary and tertiary structure, as well as its reactivity with IGFBP-1 in the patients with cirrhosis, complementing results of other researchers on the coagulation anomalies due to cirrhosis [11,54]. A more detailed knowledge on the events which affect individual molecules in coagulation process may contribute to a deeper understanding of coagulopathies and, possibly, creation of better therapeutic approaches.

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Highlights

- Multiantennary glycans, GlcNAc, Sia and Man increase on fibrinogen in cirrhosis
- Cirrhosis induces additional protein carbonylation, mostly on α chain
- Reduction in α-helix content in fibrinogen occurs in cirrhosis
- Fibrinogen forms more complexes with IGFBP-1 in cirrhosis