Oxidative stress-induced growth inhibitor 1 in alcohol-induced liver cirrhosis

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INTRODUCTION

Human oxidative stress-induced growth inhibitor 1 (OSGIN1), otherwise known as Bone marrow Derived Growth Factor (BDGI or OKL38) is a protein belonging to the OKL38 protein family. The aim of the study was to investigate the levels of this protein depending on the severity of alcohol-induced liver cirrhosis.

Materials and method. The study group consisted of 60 patients: 30 patients with cirrhosis in the P-Ch A and B stage and 30 in the P-Ch C stage. The control group consisted of 18 healthy individuals without liver diseases, who did not abuse alcohol. Oxidative stress induced growth inhibitor 1 (OSGIN1), fibroblast growth factor 1 (FGF1) and fibroblast growth factor 21 (FGF21) were determined in blood serum using enzyme-linked immunosorbent assay (ELISA) kits. All absorbance readings were conducted using an Epoch Microplate Spectrophotometer (BioTek Instrumentals, Inc., Winooski, VT, USA). OSGIN1, FGF1 and FGF21 concentrations were determined using Sandwich enzyme immunoassay kits (by Cloud Clone Corp., Katy, TX, USA). Statistica 13.3 (TIBCO Software, Inc.) was used for data analysis.

Results. The concentration of OSGIN1 was 0.028 ± 0.017 in the control group which increased with the advancement of liver cirrhosis (stage of Pugh-Child): 0.075 ± 0.098 in the P-Ch A + B group and 0.121 ± 0.134 in the P-Ch C stage. Multiple comparison tests confirmed statistically significant differences in OSGIN1 concentration between the control group and P-Ch C (p <0.02). Significant correlations were noted between OSGIN1 and FGF1 (r = 0.39; p = 0.004) and between OSGIN1 and FGF21 (r = 0.53; p <0.0001).

Conclusions. The study revealed that the level of OSGIN1 increased significantly in the P-Ch C stage of liver cirrhosis. It is possible that OSGIN1 may be used for the non-invasive diagnosis of ALD, but its possible diagnostic value is still very uncertain.

Key words
liver cirrhosis, alcoholic liver disease, fibroblast growth factor 21, Oxidative stress induced growth inhibitor 1, fibroblast growth factor 1
The progression mechanism of alcohol-induced liver cirrhosis is not yet completely understood [7]; however, it should be emphasized that selective autophagy plays an important role in the pathogenesis in this type of disease. The damaged mitochondria are selectively removed by mitophagy and this, in turn, may reduce cellular ROS accumulation in alcoholic liver disease (ALD) [8, 9].

OSGIN1 regulates apoptosis by inducing cytochrome c (cyt-c) release from mitochondria, which may be a key process in the initiation of hepatocyte apoptosis in the progression of non-alcoholic fatty liver disease (NAFLD) [10]. It is important to investigate the relationships between progression of liver cirrhosis and OSGIN1 levels. In this context, it should be emphasized that the phenomenon of apoptosis has been reported in both experimental and clinical alcoholic liver disease [11].

The importance of OSGIN1 expression in the course of alcohol liver cirrhosis (ALD) has not been yet studied; therefore, the aim of the current study was to investigate the levels of this protein depending on the severity of alcohol-induced liver cirrhosis.

Controlled variables of the study included serum levels of FGF-1 and FGF-21. In previous studies, FGF-21 has been considered as a potential marker of the development and progression of non-alcoholic fatty liver disease (NAFLD) [12, 13].

MATERIALS AND METHOD

The study group consisted of 60 patients: 30 patients with cirrhosis in the Pugh-Child A and B stages and 30 patients in the Pugh-Child C stage. The control group consisted of 18 healthy individuals without liver disease, who did not abuse alcohol. Patient’s detailed demographic and clinical characteristics are presented in Tables 1 and 2.

Patients with liver cirrhosis were characterized by higher total bilirubin, alanine aminotransferase, aspartate aminotransferase and C-reactive protein levels, and significantly lower albumin levels than the control group (Tab. 2).

Biochemical measurements. After centrifugation, blood serum was collected for analysis. Oxidative stress-induced growth inhibitor 1 (OSGIN1), fibroblast growth factor 1 (FGF1) and fibroblast growth factor 21 (FGF21) were determined using enzyme-linked immunosorbent assay (ELISA) kits. All absorbance readings were conducted using Epoch Microplate Spectrophotometer (BioTek Instrumentals, Inc., Winooski, VT, USA). OSGIN1, FGF1 and FGF21 concentrations were measured in the standard curve.

Patients’ clinical characteristics

Table 1. Characteristics of control and study groups – demographic and clinical parameters

|                  | Control group (n=18) | Study group (Liver cirrhosis) | P     |
|------------------|----------------------|------------------------------|-------|
| Age (years)      | 44.3±15.2            | 57.3±13.1                    | 0.71  |
| Percentage of males (%) | 66.7 % | 76.8 % | 82.35% | 0.48 |
| Body weight (kg) | 69.2±9.1             | 79.5±11.6                    | 0.82  |
| Height (cm)      | 172±6.6              | 175.8±6.7                    | 0.39  |
| Duration of alcohol abuse (years) | - 22.2±11.2       | 23.7±8.5                     | 0.99  |
| Desophageal varices (%) | - 50.1% | 94.1% | <0.0001 |
| Encéphalopathy (%) | - 28.6% | 82.4% | <0.0001 |
| Ascites (%)      | - 42.8%              | 88.2%                        | <0.0001 |

Table 2. Patients’ clinical characteristics

|                  | Control group (Liver cirrhosis) | P     |
|------------------|------------------------------|-------|
| Total bilirubin (mg/dl) | 0.8±0.6 | 3.4±3.4 | 12.2±10.1 | <0.0001 |
| INR               | - 1.49±0.43                | 1.99±0.62 | 0.04 |
| Alanine aminotransferase-ALT (U/l) | 18.7±8.2 | 29.6±19.9 | 39.2±19.6 | 0.007 |
| Aspartate aminotransferase-AST (U/l) | 19.1±6.4 | 76.5±41.2 | 93.9±51.8 | <0.0001 |
| Albumina (g/dl)   | - 3.1±0.67                 | 2.4±0.52 | 0.0002 |
| Total protein (g/dl) | 6.5±0.5 | 6.8±1.1 | 5.7±0.8 | 0.01 |
| Urea (mg/dl)      | - 27.7±15                  | 68.4±43.9 | 0.07 |
| Platelets(G/l)    | 229.6±34.3                | 178.6±129.7 | 129.6±78.2 | 0.0004 |
| Mean corpuscular volume (fl) | 85.5±4.2 | 89.6±11.6 | 93.4±8.2 | 0.0002 |
| Sodium (mmol/l)   | 141±2.9                   | 134.3±4.5 | 131.8±4.7 | 0.0001 |
| Potassium (mmol/l) | 4.5±0.6       | 3.9±0.55 | 4.2±0.9 | 0.13 |
| C-reactive protein (mg/l) | 2.8±1.9 | 10.4±9.72 | 37.5±31.1 | <0.0001 |

Statistical analysis. Statistica 13.3 (TIBCO Software, Inc.) was used for data analysis. Continuous variables were expressed as the mean±standard deviation (SD). Before calculations, variables were checked for normality using the Shapiro-Wilk test. To compare the results between more than two groups, one-way ANOVA and Kruskal-Wallis tests were used, depending on distribution. Correlations among variables were tested using the Pearson’s and Spearman’s correlation tests, depending on distribution. Qualitative variables are shown as indicators of structure (percentage). For intergroup comparisons, the χ² test was used. For all tests, p<0.05 was considered as statistically significant.
Table 3. Concentrations of selected markers depending on the stage of cirrhosis

|                    | Control group | Study group: Liver cirrhosis |                  |                  |
|--------------------|---------------|-----------------------------|------------------|------------------|
|                    |               |                             | Pugh-Child A+B   | Pugh-Child C     |
| OSGIN-1            | 0.028±0.017   | 0.075±0.098                 | 0.121±0.134      | 0.02             |
| FGF-1              | 39.4±40.4     | 155.2±173.5                 | 147.8±157.4      | 0.03             |
| FGF-21             | 14.3±6.9      | 48.8±67.6                   | 37.8±41.8        | 0.01             |

Table 4. Correlations between FGF1, FGF21 and OSGIN1

|                  | Correlation coefficient | r - Spearman test |
|------------------|-------------------------|-------------------|
| FGF1 & OSGIN1    | r = 0.39                | p = 0.004         |
| FGF21 & OSGIN1   | r = 0.53                | p < 0.0001        |

Table 5. Independent factors associated with oxidative stress induced growth inhibitor 1 (OSGIN-1) concentration (multiple regression)

|                  | Beta | SE with Beta | B | SE with B | p           |
|------------------|------|--------------|---|-----------|-------------|
| Pugh-Child stage | 0.21 | 0.11         | 0.017 | 0.0084 | 0.0498 |
| FGF21            | 0.65 | 0.11         | 0.001 | 0.0002 | < 0.0001 |

Model: R = 0.73; R^2 = 0.53; R^2 = 0.51; p < 0.0001

Figure 1. Concentration of oxidative stress induced growth inhibitor 1 (OSGIN-1) associated with linked to the stage of alcoholic liver cirrhosis

RESULTS

The concentration of OSGIN1 was 0.028±0.017 in the control group which increased with the advancement of liver cirrhosis (stage of Pugh-Child): 0.075±0.098 in the P-Ch A + B group and 0.121±0.134 in the P-Ch C stage. Multiple comparison tests confirmed statistically significant differences in OSGIN1 concentration between control and the P-Ch C group (p = 0.02). In turn, the concentration of FGF-1 was the lowest in the control group, 39.4±40.4 and increased in patients with cirrhosis to the value of 155.2±173.5 in the P-Ch A + B group and 147.8±157.4 in the P-Ch C group (p = 0.03). A similar trend was noted in case of FGF-21. Its concentration in the control group was 14.3±6.9, in the P-Ch A + B group – 48.8±67.6, and in the P-Ch C group – 37.8±41.8 (p = 0.01).

DISCUSSION

Alcohol-induced liver cirrhosis is a significant therapeutic and diagnostic problem [14–17] and it is therefore important to search for non-invasive methods for assessing the severity of liver disease, and estimating mortality in alcoholic liver diseases. Such possibilities are facilitated through biochemical diagnostics of blood serum. Previous reports have suggested the possibility of using selected interleukins as biochemical markers of cirrhosis. Nandeesha et al. suggested that one of the predictors of fibrosis in alcoholic cirrhosis may include elevated interleukin-6 [18]. Yang et al. investigated serum levels of FGF-21 in diagnosing non-alcoholic steatohepatitis (NASH) and the critical stage of non-alcoholic fatty liver disease (NAFLD) [19]. In a recent study, Wagner-Skacel et al. showed that increased FGF21 levels in patients correlated with recent alcohol consumption [20]. Remmler et al. indicated that the serum level of IL-6 and Model of End-Stage Liver Disease (MELD) scores are associated with mortality in patients with end-stage liver disease, evaluated for liver transplantation [21]. Apoptosis has been described in chronic and acute liver diseases. This being taken into account, Lasso et al. showed that alcoholic liver disease patients have functional and phenotypical changes in cytotoxic lymphocytes which circulate in the blood. They suggested that these changes could be related to the progression of liver injury [22].

The results obtained in the current study indicate that the level of oxidative stress-induced growth inhibitor 1 (OSGIN-1) increases with the advancement of ALD, and was more than twice as high in the Pugh-Child A + B stage compared to the control group. In the most advanced stage of alcoholic cirrhosis (Pugh-Child C), OSGIN-1 levels were almost twice as high as in Pugh-Child A + B. It should be noted that in comparison to the fibroblast growth factors 1 (FGF-1) and FGF-21 determined in the study, the expression of OSGIN-1 was more evenly compared to the severity of cirrhosis determined by the Pugh-Child scale. The levels of FGF-1 and FGF-21 clearly escalated already in the Pugh-Child A + B stage – with over threefold increases. In contrast, the differences in the levels of fibroblast growth factors between Pugh-Child A + B and Pugh-Child C were minimal. It is worth mentioning that positive correlations were observed between OSGIN1 and FGF1 (r = 0.39; p = 0.004) and between OSGIN1 and FGF21 (r = 0.53; p < 0.0001). There were no significant correlations between OSGIN-1 and other laboratory and clinical parameters.

In the developed multiple regression model, independent variables related to the OSGIN1concentration included the P-Ch category and FGF21 concentration. This model was statistically significant (p < 0.0001) and accounted for approximately half of all the variation (adjusted R^2 = 0.51).
course of both NAFLD and ALD; however, some reports suggest the possibility of using this factor in the diagnosis of neoplastic diseases. Ong et al. suggested that OSGN1/OKL38 may be used in diagnosis, prognosis, and treatment of kidney cancer [23]. It was shown that the absence of OSGN1/OKL38 could lead to the progression or development of hepatocellular carcinoma (HCC). The reduced expression level of OKL38 protein correlated with high tumour stages in HCC (p=0.004) [24]. Hu et al. stated that OSGN1/OKL38 may play an important role in tumorigenesis. These authors also showed that OSGN1/OKL38 is essential in the growth regulation and differentiation of breast epithelial cells during pregnancy [25]. Taking these results into account, it is worth assessing the usefulness of determining OSGN1 expression as an early marker of ‘oncological anxiety’, especially in patients with stages B and C of Child–Pugh.

The few reports so far indicate the possibility of pharmacological modulation of the OSGN1 level. This may offer the prospect of therapeutic use of certain molecules, possibly also in the treatment of ALD associated liver damage. Additionally, Brennan et al. showed that the bioactive metabolite of dimethyl fumarate – monomethyl fumarate (MMF) can induce antioxidant gene expression. These authors identified the mechanism of MMF-mediated cytoprotection in human astrocytes via upregulation of the OSGN1–61 kDa isoform (HuOKL38–2b) [26]. In a recent report, Watanabe et al. showed that statins can reduce OSGN1 levels in a mouse model, mainly visible among male mice [27].

However, in order to confirm the usefulness of OSGN1 determinations in ALD, further research conducted on larger groups of patients diagnosed with alcoholic cirrhosis are necessary. In the scientific literature, a similar postulate is reported in relation to the alpha-klotho protein [28–30]. One of the limitations of the current study is the small number of research subjects. It would also be advisable to use the MELD score in future studies.

CONCLUSIONS

The presented study revealed that the level of OSGN1 increased significantly in the Pugh–Child C stage of alcohol-induced liver cirrhosis. It is possible that OSGN1 may be used for the non-invasive diagnosis of ALD, but its role in alcohol-induced liver cirrhosis is not clear and its possible diagnostic value is still very uncertain. Further studies in larger populations are needed to confirm the preliminary results. The authors also suggest evaluating the possibility of ALD therapy by modifying OSGN1 levels in future studies.

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