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ORIGINAL ARTICLE

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

Jarosław Jerzy Sak[1,2,A,C-F], Andrzej Prystupa[3,A-C,E-F], Hanna Bis-Wencel[4,C-E], Paweł Kiciński[5,B-D], Dorota Luchowska-Kocot[6,B-D], Henryk Krukowski[4,D-E], Grzegorz Józef Nowicki[7,B-C], Lech Panasiuk[8,E-F]

[1] *Chair and Department of Humanities and Social Medicine, Medical University, Lublin, Poland*
[2] *BioMolecular Resources Research Infrastructure (BBMRI.pl), Poland*
[3] *Department of Internal Medicine, Medical University, Lublin, Poland*
[4] *Department of Microbiology and Reproductive Biology, University of Life Science, Lublin, Poland*
[5] *Department of Experimental Haematooncology, Medical University, Lublin, Poland*
[6] *Department of Medical Chemistry, Medical University, Lublin, Poland*
[7] *Department of Family Medicine and Community Nursing, Medical University, Lublin, Poland*
[8] *Institute of Rural Health, Lublin, Poland*
A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of the article

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Abstract

Introduction and Objective. Human oxidative stress-induced growth inhibitor 1 (OSGIN1) is a protein identified in 2001 which belongs 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

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 protein has several isoforms, the dominant one (HuOKL38–1a/2a) with 477 amino acids (52 kDa molecular weight), two longer isoforms with 560 amino acids each (HuOKL38–2c – 59 kDa and HuOKL38–2b – 61 kDa), and a shorter isoform with 317 amino acids (34 kDa). OSGIN, identified in 2001, is located on chromosome 16 in the region of q23.3 [1].

The OSGIN1/OKL38 regulates cells death [2]. The loss of the OSGIN1 protein disrupts the balance between cells' growth, differentiation, and death within the tissues resulting in their uncontrolled growth and the formation of tumours. OSGIN1/OKL38 seems to be important both for the course of the disease as well as for the response to unfavourable environmental factors, such as dust or tobacco smoke. Tsai et al. showed that OSGIN1 protein determines the effects of docosahexaenoic acid (DHA) in MCF-7 breast cancer cells, i.e.: elevates the generation of reactive oxygen species (ROS) in mitochondria and promotes autophagosome formation [3]. In their latest report, Liu et al. indicated that persons with the OSGIN1 1494A variant had the shortest mean survival time among hepatocellular carcinoma patients [4]. Yuan et al. showed that a methyltransferase-like 3 mediated particulate matter 2.5 (PM2.5)-induced cell injury by targeting OSGIN1 in human airway epithelial cells [5]. What is more, Wang et al. indicated that OSGIN1 plays an important role in an enhanced-autophagy in the human airway epithelium as a reaction to smoking-induced stress [6].

Address for correspondence: Hanna Bis-Wencel, Department of Microbiology and Reproductive Biology, University of Life Science, Lublin, Poland
E-mail: hanna.biswencel@up.lublin.pl

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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 determined using Sandwich enzyme immunoassay kits (Cloud Clone Corp., Katy, TX, USA). Depending on the recommendations of the manufacturers' protocol, prior to the assay, serum samples were used without dilution. The research was carried out in accordance with the typical standard applicable for enzyme-linked immunoassays: samples, standards and blanks were applied on pre-coted plate with an antibody specific for the determined factors. Subsequently, horseradish peroxidase conjugated avidin was added to each well, and the plate was incubated for one hour at 37 °C. Next, TMB substrate was added, and in wells with a determined parameter, biotin-conjugated antibody and enzyme-conjugated avidin the colour change was observed. The enzyme-substrate reaction was terminated by adding acidic solution and the absorbance of formed complex was measured at the wavelength of 450 nm. The concentrations of previously established parameters were determined using the standard curve.

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

	Control group (n=18)	Study group (liver cirrhosis)		p
		Pugh-Child A+B (n=30)	Pugh-Child C (n=30)	
Age (years)	44.3±15.2	57.3±13.1	56.9±11.7	0.71
Percentage of males (%)	66.7%	78.6%	82.35%	0.48
Body weight (kg)	69.2±9,1	79.5±11.6	78.2±12.2	0.82
Height (cm)	172±6.5	175.8±6.7	171.5±8.1	0.39
Duration of alcohol abuse (years)	-	22.2±11.2	23.7±8.5	0.99
Oesophageal varices (%)	-	50.1%	94.1%	<0.0001
Encephalopathy (%)	-	28.6%	82.4%	<0.0001
Ascites (%)	-	42.8%	88.2%	<0.0001

Table 2. Patients' clinical characteristics

	Control group	Study group (Liver cirrhosis)		p
		Pugh-Child A+B	Pugh-Child C	
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 χ^2 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		p
		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	
	r	p
FGF1 & OSGIN1	0.39	0.004
FGF21 & OSGIN1	0.53	<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
Constant			0.001	0.0174	0.9402
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

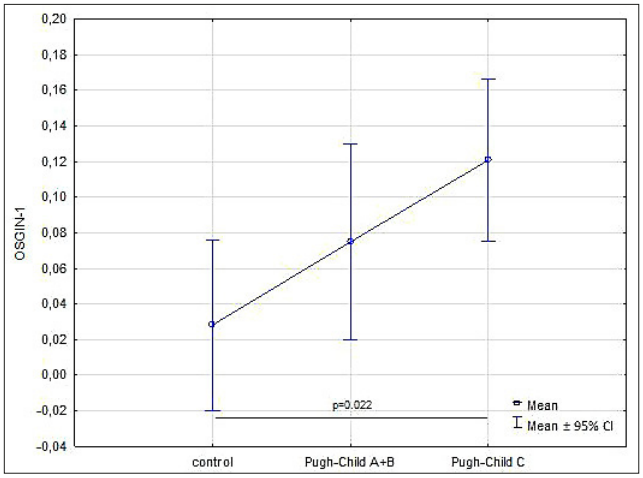

Figure 1. Concentration of oxidative stress induced growth inhabitor 1 (OSGIN-1) associated with/linked to the stage of alcoholic liver cirrhosis

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).

Significant 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 R2=0.51).

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 steatoheaptitis (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 and FGF21. All these observations suggest the possibility of using OSGIN-1 as a potential marker of ALD severity. A greater sensitivity of OSGIN1 than FGF-21, which has been tested in this aspect, should be suggested.

The dependence of OSGIN-1 on the degree of liver damage has not yet been evaluated. There are no scientific reports on the relationship between the OSGIN-1 expression and the clinical

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 OSGIN1/OKL38 may be used in diagnosis, prognosis, and treatment of kidney cancer [23]. It was showed that the absence of OSGIN1/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 OSGIN1/OKL38 may play an important role in tumurigenesis. These authors also showed that OSGIN1/OKL38 is essential in the growth regulation and differentiation of breast epithelial cells during pregnancy [25]. Taking these reports into account, it is worth assessing the usefulness of determining OSGIN1 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 OSGIN1 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 OSGIN1–61 kDa isoform (HuOKL38–2b) [26]. In a recent report, Watanabe et al. showed that statins can reduce OSGIN1 levels in a mouse model, mainly visible among male mice [27].

However, in order to confirm the usefulness of OSGIN1 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 OSGIN1 increased significantly in the Pugh-Child C stage of alcohol-induced liver cirrhosis. It is possible that OSGIN1 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 OSGIN1 levels in future studies.

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