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Erythrocyte antioxidative enzymes activities in patients with chronic hepatitis C treated with pegylated interferon alpha-2a and ribavirin

Aktivnost antioksidativnih enzima eritrocita kod obolelih od hroničnog hepatitisa C, lečenih pegilovanim interferonom alfa-2a i ribavirinom

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Abstract

Background/Aim. In hepatitis C virus infection, oxidative stress and antioxidant imbalance are major triggers for the disease occurrence and its progression. The aim of the research was to determine the erythrocyte antioxidative enzymes activities, superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), before and after therapy with pegylated interferon alpha-2a and ribavirin and to evaluate their clinical significance as potential diagnostic markers of sustained virological response (SVR). **Methods.** The study included 53 patients with chronic hepatitis C (CHC) and 56 healthy controls. SOD, GPx, CAT, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured in patients both before and after the treatment. **Results.** SOD, GPx and CAT activities prior to the treatment were significantly lower in CHC patients compared to the controls ($p < 0.001$), and they were significantly higher after the treatment ($p < 0.001$). A significant positive correlation existed between SOD, GPx, and CAT activites, before and after the treatment ($p < 0.001$) and with those of aminotransferases prior to the treatment ($p < 0.001$). After the treatment, only GPx activity showed significant negative correlation with that of aminotransferases ($p < 0.001$). Receiver operating characteristic curve analysis for SOD, GPx and CAT showed following values: area under the curve of 0.975, 0.988, and 0.817 respectively; sensitivity of 93.5%, 71.7%, 100% respectively and specificity of 100% for all, respectively. Forty six SVR achievers had significant increase of SOD, GPx and CAT activities ($p < 0.001$ for all), unlike 7 SVR non-achievers ($p = 0.31$, $p = 0.717$, $p = 0.85$, respectively). **Conclusion.** Oxidative stress is the initiator of onset and progression of CHC. The combined antiviral therapy leads to the restoration of antioxidant balance. GPx, SOD and CAT may be diagnostic markers of CHC treatment outcome.

Key words:
hepatitis c; erythrocytes; oxidative stress; antioxidants; superoxide dismutase; glutathione peroxidase; catalase; interferon-alpha; ribavirin.

Apstrakt

Uvod/Cilj. Oksidativni stres i narušena antioksidantna ravnoteža su važni pokretači nastanka i progresije hepatitis C virusne infekcije. Cilj ovog istraživanja bio je određivanje aktivnosti antioksidativnih enzima, superoksid dizmutaze (SOD), glutation peroksidaze (GPx) i katalaze (CAT), pre i posle terapije pegilovanim interferonom alfa-2a i ribavirinom i evaluacija njihovog kliničkog značaja kao potencijalnih dijagnostičkih markera stabilnog virusološkog odgovora (SVR). **Metode.** Istraživanjem je bilo obuhvaćeno 53 bolesnika sa hroničnim hepatitisom C (HHC) i 56 zdravih ispitanika. Aktivnost SOD, GPx, CAT, aspartat aminotransferaze (AST) i alanin aminotransferaze (ALT) određivane su kod bolesnika pre i posle terapije. **Rezultati.** Preterapijske aktivnosti SOD, GPx i CAT bile su statistički značajno niže kod bolesnika sa HHC u poređenju sa zdravim ispitanicima ($p < 0,001$), a posle terapije bile su statistički značajno više ($p < 0,001$). Utvrđena je statistički značajna pozitivna korelacija između aktivnosti SOD, GPx, CAT, pre i posle terapije ($p < 0,001$) i sa aktivnošću aminotransferaza posle terapije ($p < 0,001$). Posle terapije, jedino je aktivnost GPx statistički značajno negativno korelirala sa aktivnošću aminotransferaza ($p < 0,001$). Kriva operativnih karakteristika primaoca za SOD, GPx i CAT prikazala je sledeće vrednosti: površinu ispod krive redom

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od 0,975; 0,988; 0,817; senzitivnost redom 93,5%, 71,7%, 100% i specifičnost 100% za sva tri enzima. Četrdeset šestoro bolesnika sa postignutim SVR imali su značajno povećanje aktivnosti SOD, GPx i CAT ($p < 0.001$ za sva tri enzima) za razliku od sedam bolesnika bez postignutog SVR ($p = 0.31$, $p = 0.717$, $p = 0.85$ redom). **Zaključak.** Oksidativni stres je inicijator nastanka i progresije HHC. Kombinovanom antivirusnom terapijom postiže se uspostavljanje antioksidantne ravnoteže. Enzimi GPx, SOD i CAT mogu biti dijagnostički markeri ishoda terapije, kod bolesnika sa HHC.

Ključne reči:
hepatitis c; eritrociti; stres, oksidativni; antioksidansi; peroksid dismutaza; glutation peroksidaze; katalaza; interferon-alfa; ribavirin.

Introduction

In addition to the immune liver damage and direct cytotoxic damage mediated by a variety of the viral products, oxidative stress is also directly associated with the pathogenesis of chronic hepatitis C (CHC) [1, 2]. It is believed that antioxidant barrier impairment by viral hepatitis A and viral hepatitis B represents a consequential condition. On the contrary, oxidative stress present in hepatitis C virus (HCV) infection is the main trigger for the disease occurrence and progression [3–5]. The occurrence of oxidative stress in HCV infection is explained by chronic inflammation, lipid peroxidation, iron deposition in the liver parenchyma, glutathione (GSH) level alteration, and the effect of non-structural and structural proteins of HCV (core protein, NS3, NS5A) on enzyme activity, mitochondria and hepatocyte genes. Continuous accumulation of reactive oxygen species (ROS) occurs due to nicotinamide adenine dinucleotide phosphate [NAD(P)H] oxidase activity of the Kupffer cells and polymorphonuclear cells of the liver [6, 7]. Protein NS3 activates NAD(P)H oxidase and Nox 2 protein of the phagocytes, thus increasing the generation of ROS [7–9]. Deposition of excess iron causes the formation of free radicals. Expression of HCV core gene is associated with ROS increasing and reduction of intracellular and / or the mitochondrial content of GSH [6]. It is thought that the antioxidative enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), play a decisive role in the development and progression of CHC [10].

Oxidative damage in CHC is characterized by decreased activity of antioxidant enzymes such as CAT, GPx, and SOD. It is believed that combined treatment with pegylated interferon-alpha (PEG-IFN-α) and ribavirin (RBV) may be responsible for establishing antioxidant response in patients with CHC [11].

Pegylated interferon-alpha has a potent anti-viral, anti-inflammatory and immunomodulatory effect on HCV. Its antiviral activity is the result of the ability to induce interferon stimulated genes, responsible for the inhibition of various stages of viral replication. Pegylated interferon-alpha, also expresses an immunomodulatory effect which initiates the differentiation of T-helper cells referring to the Th2 cells consequently leading to the increased production of interleukin-2 and interferon gamma. Its anti-inflammatory effect is presented through inhibition of various cytokine syntheses such as tumor necrosis factor and interleukin-1 [12]. The effect of therapy is significantly higher when IFN is combined with RBV in chronic HCV infection. Hemolysis, which universally occurs with RBV therapy and which is considered a limiting side effect, is precisely the mechanism by which the anti-HCV effect is exerted. Passive hemolysis results in anti-inflammatory/antiviral actions within the liver that disrupt the innate immune tolerance, leading to the synergy of RBV with IFN-α [13].

The aim of this study was to determine activities of erythrocyte antioxidative enzymes in patients with CHC before and after PEG-IFN-α-2a and RBV therapy. Evaluation of the clinical significance of SOD, CAT, and GPx as potential diagnostic markers of treatment success was set up as another goal of the research.

Methods

The prospective study included 53 patients with CHC and 56 healthy subjects. The selection of participants who were included in the study was performed based on the criteria for implementation of standard PEG-IFN-α-2a and RBV therapy, and additional criteria in line with current research: they were stringently matched for age (average 38.2 ± 9.8 years), sex and disease duration. Additional, wider criteria for the involvement, designed in line with the topic of the research, include absence of all acute or chronic medical conditions that would further disturb the oxidative-antioxidative status, in addition to HCV infection, (patients with CHC on dialysis, hemophiliacs, former opiate addicts on withdrawal pharmacotherapy, as well as vitamin supplemented patients were not included in the study). The control group consisted of healthy volunteers of both sexes, matched for age (average 41.2 ± 11.9 years), who were also not supplemented by vitamins in near past. They were taken only blood samples so as to determine the activity of antioxidant enzymes. Written consent to participate in the research and implementation of treatment was obtained from all the patients and volunteers. Prior to the start of the treatment, blood samples were taken from the patients in order to determine antioxidant enzymes basal activities, as well as other parameters of routine hematological and biochemical analyses within the standard treatment procedures. According to the already established guidelines for the treatment, patients with HCV genotype 2 and 3 received PEG-IFN-α-2a by once a week, by subcutaneous injection, and RBV orally, on daily basis for 24 weeks of therapy. Patients with HCV genotype 1 and 4 received PEG-IFN-α-2a subcutaneous injection once a week, and RBV *per os*, daily, for 48 weeks of treatment. Treatment dosage was the same in both groups of genotypes – PEG-IFN-α-2a in a dose of 180 mg weekly, RBV 1,000 to 1,200 mg *per* day. According to the guidelines for the

evaluation of treatment of PEG-IFN-α-2a and RBV, blood was also taken for the analysis 24 weeks after the end of the treatment, in order to determine the achievement of the so-called curing, i.e., a stable virological response (SVR), and determining the activity of antioxidant enzymes. Accordingly, we set another category of patients to compare, those who achieved and those who did not achieve SVR. In addition to "after treatment evaluation", we retrospectively analyzed their baseline antioxidant enzyme activities.

Liver function test parameters, as well as determination of the activities of antioxidative enzymes – SOD, CAT, and GPx, were conducted at the Center for Medical Biochemistry, Clinical Center Niš.

Standard biochemical parameters including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by the Olympus analyzer (Olympus System Tokyo, Japan). Enzyme activity in plasma was expressed in units *per* liter (U/L).

Determination of the enzyme activities was performed in red blood cells haemolysate that was made from the red blood cells suspension of the heparinized blood by adding cold redestillated water and stored at -20°C until determination of the enzyme activity. CAT activity was determined by a kinetic method of Beutler [14]. Measurement of CAT activity was carried out on a Beckman spectrophotometer DU 650 (Beckman, USA). The enzyme activity was expressed in units *per* gram of hemoglobin $\times 10^4$ (U/gHb $\times 10^4$). SOD activity was determined by a commercial Randox Ransel assey, (Randox Laboratories Ltd, Crumlin, Co.Autrium, UK) on the automatic analyzer Olympus AU 400 (Olympus, Tokyo, Japan). SOD activity was expressed in international units *per* gram of hemoglobin (U/gHb), and a reference interval was from 1,092 to 1,817 U/gHb. GPx activity was determined by a commercial Randox Ransel assay (Randox Laboratories Ltd, Crumlin, Co.Autrium, UK). Enzyme activity was expressed in units *per* gram of hemoglobin (U/gHb). Reference values were from 29.6 to 82.9 U/gHb.

Determination of HCV ribonucleic acid (RNA) was carried out in the reference laboratory of the Institute for Infectious and Tropical Diseases in Belgrade, Serbia. We performed a qualitative Polymerase Chain Reaction (PCR) determination of HCV RNA by the COBAS Amplicor Hepatitis C Virus Test version 2.0, the sensitivity of the test is 50 IU/1 mL of plasma. Quantitative real-time PCR determination of HCV RNA was carried out using the Cobas TaqMan HCV Test, the sensitivity of the test is 15 IU/mL.

All statistical analyses were performed with SPSS statistical analysis software, version 10.0 (SPSS, Chicago, IL, United States), a significance level was set at $p < 0.001$. Characteristics of the study group were expressed as the mean ± standard deviation (SD) for normal distribution or median (interquartile range) for non-normal distribution, or with frequency and percentage for categorical data. Clinical and biochemical data of the CHC patients and the control group were compared by using Student *t*-test for normally distributed data and Mann-Whitney U test for data that were not normally distributed. The relationship between two variables was determined by Pearson's correlation coefficient (r). Receiver operating characteristic (ROC) curves were constructed to establish a sensitivity–specificity relationship. Cut-off values that provided the best combination of sensitivity and specificity were determined by ROC curve analysis.

Results

Comparing the values of the studied parameters in patients with hepatitis C and the results in the control group, it was found that there was a statistically significant difference in SOD activity between these two groups before treatment (913.41 ± 322.02 U/gHb *vs* 1,783.90 ± 189.69 U/gHb; $p < 0.001$), as it is shown in Figure 1.

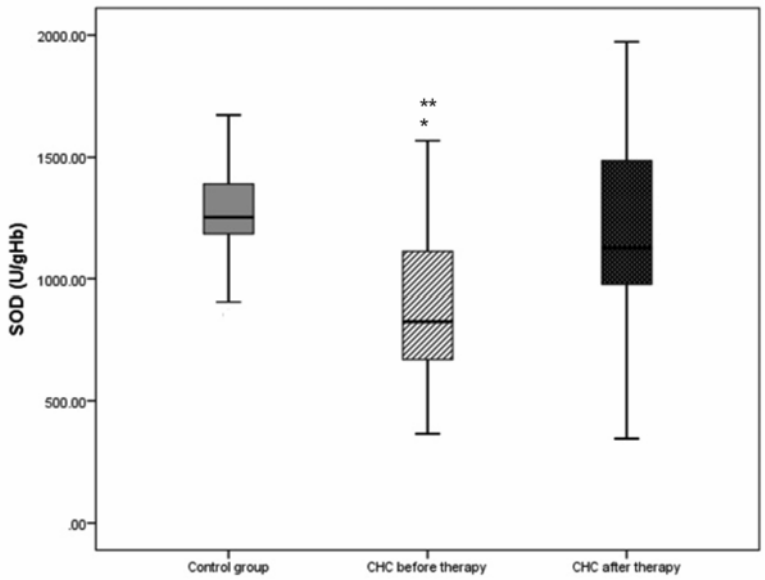

Fig. 1 – SOD activity of the control group and CHC patients before and after the treatment.
***p* < 0.001 – compared to the control group, ****p* < 0.001 – compared to the CHC patients after therapy;**
SOD – superoxide dismutase; CHC – chronic hepatitis C.

GPx activity in CHC patients before treatment was statistically significantly lower compared to healthy controls (22.49 ± 4.88 U/gHb *vs* 45.46 ± 7.77 U/gHb; $p < 0.001$) (Figure 2). CAT activity was also significantly lower in CHC patients before treatment compared to healthy subjects (5.12 ± 0.83 U/gHb × 10^4 *vs* 13.22 ± 2.44 U/gHb × 10^4; $p < 0.001$) (Figure 3). AST activity in patients with CHC before treatment was significantly higher compared to the activity of this enzyme in healthy subjects ($p < 0.001$) and compared to values in patients with CHC after the treatment ($p < 0.001$). ALT activity was also statistically significantly higher in patients compared to healthy subjects ($p < 0.001$) and compared to values in patients with CHC after the treatment ($p < 0.001$) (Table 1).

AST activity was statistically significantly reduced during the treatment ($p < 0.001$). ALT activity was also statistically significantly reduced during the application of therapy ($p < 0.001$) (Table 1).

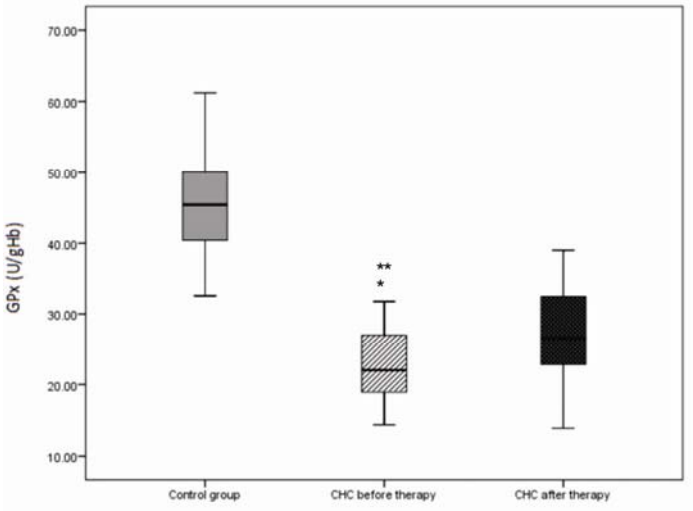

Fig. 2 – GPx activity of the control group and CHC patients before and after the treatment. *$p < 0.001$ – compared to the control group; **$p < 0.001$ – compared to the CHC patients after therapy; GPx – glutathione peroxidase; CHC – chronic hepatitis C.

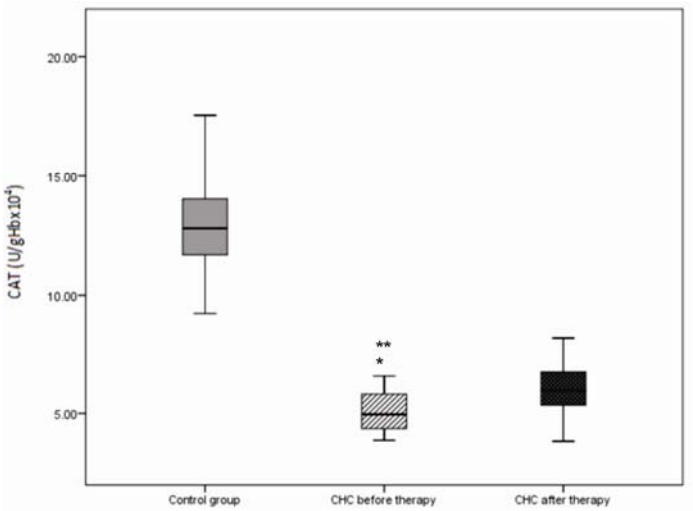

Fig. 3 – CAT activity of the control group and CHC patients before and after the treatment. *$p < 0.001$ – compared to the control group; **$p < 0.001$ – compared to the CHC patients after therapy; CAT – catalase; CHC – chronic hepatitis C.

Table 1

Aminotransferases activity of the control group and chronic hepatitis C (CHC) patients before and after the treatment

Aminotransferases	Control group (n = 56) $\bar{x} \pm SD$	CHC patients (n = 53) $\bar{x} \pm SD$	
		before therapy	after therapy
AST (U/L)	22.52 ± 7.43	66.58 ± 44.30 *, **	28.64 ± 40.76
ALT (U/L)	27.80 ± 19.35	85.72 ± 41.74 *,**	32.45 ± 37.82

$\bar{x} \pm SD$ – arithmetic mean ± standard deviation; p – significance of difference between groups; *$p < 0.001$ – compared to the control group, **$p < 0.001$ – compared to the CHC patients after therapy; AST – aspartate aminotransferase; ALT – alanine aminotransferase.

There was a statistically significant difference in SOD activity before compared to the after the treatment (913.41 ± 322.02 U/gHb *vs* 1172.83 ± 415.67 U/gHb; $p < 0.001$) (Figure 1).

Correlation analysis showed that there was a statistically significant correlation among all the parameters examined before the treatment. Statistically significant positive correlation ($p < 0.001$ for all) existed between SOD and GPx, SOD and CAT, GPx and CAT, AST and ALT. Statistically significant negative correlation ($p < 0.001$ for all) existed between the following parameters: SOD and AST, SOD and ALT, GPx and AST, GPx and ALT, CAT and AST, CAT and ALT (Table 2).

There was a statistically significant positive correlation ($p < 0.001$ for all) between activities of SOD and GPx, SOD and CAT, GPx and CAT, AST and ALT and ALT after the therapy, and statistically significant negative correlation ($p < 0.05$) between activities of GPx and aminotransferases (Table 2).

ROC curve analysis showed that GPx was the best diagnostic marker in monitoring the success of therapy. SOD had slightly lower values, while CAT had the weakest discriminative ability. The cut-off value, specificity and sensitivity of the studied parameters were also determined by this statistical methodology (Table 3). SOD and GPx have high specificity and sensitivity, and CAT had a slightly lower sensitivity (Figure 4).

Table 2

Correlation analysis between SOD, GPx, CAT and aminotransferases before and after the therapy

Enzyme	GPx	CAT	AST	ALT
SOD				
before therapy	0.722*	0.740*	-0.317*	-0.314*
after therapy	0.507*	0.429*	-0.169	-0.174
GPx				
before therapy	-	0.873*	-0.504	-0.603
after therapy		0.823*	-0.193†	-0.215†
CAT				
before therapy		-	-0.530*	-0.610*
after therapy		-	-0.144	-0.129
AST				
before therapy			-	0.901*
after therapy			-	0.912*

***p < 0.001; †p < 0.005; SOD – superoxid dismutase; GPx – glutathione peroxidase; CAT – catalase; AST – aspartate aminotransferase; ALT – alanine aminotransferase. The numerical values represent Pearson's correlation coefficient (r).**

Table 3

Analysis of the ROC curve of SOD, GPx and CAT in relation to the outcome of the therapy

Enzymes	Cut-off value	Sensitivity (%)	Specificity (%)	AUC	95% CI	*p*
SOD	627.58	93.5	100	0.975	0.938–1.013	< 0.001
GPx	17.10	97.8	100	0.988	0.961–1.014	< 0.001
CAT	4.66	71.7	100	0.817	0.706–0.928	0.007

***p* – significance of difference between groups; SOD – superoxid dismutase; GPx – glutathione peroxidase; CAT – catalase; ROC – receiver operating characteristics; AUC – area under the curve; CI – confidence interval.**

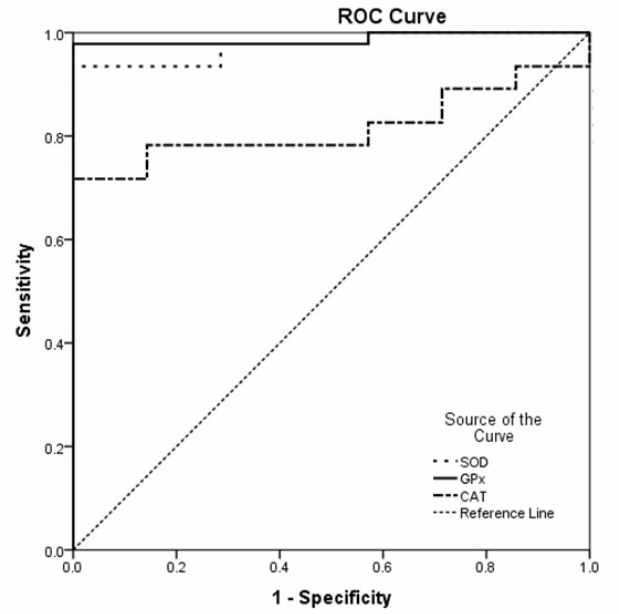

Fig. 4 – ROC curve for the activities of SOD, GPx and CAT in relation to the outcome of the treatment. SOD – superoxid dismutase; GPx – glutathione peroxidase; CAT – catalase; ROC – receiver operating characteristics.

In Table 4 we reported that examined patients who did not achieve SVR had significantly lower values of SOD, GPx and CAT measured before therapy initiation compared to subjects who achieved SVR ($p < 0.001$ for all). Also, it was shown significantly lower values of SOD, CAT and GPx after the therapy in patients who did not achieve SVR compared to subjects who achieved SVR ($p < 0.001$ for all three enzymes). No significant changes in activities of SOD, GPx and CAT were recorded in the group of patients who did not achieve SVR after completion of the therapy ($p = 0.310$, $p = 0.717$, $p = 0.850$, respectively), in contrast to significant changes of SOD, GPx and CAT activities after the treatment in patients who achieved SVR ($p < 0.001$ for all) (Table 4).

Analysis of the genotypes with respect to achievement of SVR is demonstrated in Table 5. Genotypes 1, 2, 3 and 4 consisted of 27, 2, 20 and 4 patients, respectively. The number, as well as the percentage of patients who achieved SVR with genotypes 1, 2, 3 and 4, was 22 (81.5%), 2 (100%), 20 (100%), 4 (50%), respectively. On the basis of the genotypes, patients were divided into two groups, according to the duration of the therapy and the presumed response to the therapy. The first group consisted of genotypes 1 and 4, while the second group consisted of genotypes 2 and 3. Insufficient patient numbers for statistical analysis within genotypes 2 and 4 also favored this mode of division.

Discussion

Results of researches related to the antioxidant status of patients with CHC in general, and the ability to predict the outcome of therapy are very contradictory. There is little data in the scientific literature concerning the levels of activity of all three antioxidative enzymes (SOD, CAT, GPx) in patients with CHC. The results of measurements of these enzymes

Table 4

Antioxidant enzymes activities in regard to the chronic hepatitis C (CHC) therapy outcome

Enzyme	Therapy outcome HCV positivity (n = 7) (SVR not achieved) $\bar{x} \pm SD$	p*	HCV negativity (n = 46) (SVR achieved) $\bar{x} \pm SD$	p*	$p^{\dagger}$
SOD (U/gHb)					
baseline	503.89 ± 87.10	0.310	975.73 ± 297.92	< 0.001	< 0.001
after therapy	475.90 ± 99.33		1.288 ± 337.91		< 0.001
GPx (U/gHb)					
baseline	15.44 ± 0.89	2.717	23.56 ± 4.31	< 0.001	< 0.001
after therapy	15.52 ± 1.32		28.72 ± 5.45		< 0.001
CAT (U/gHbx10^4)					
baseline	4.34 ± 0.21	0.850	5.24 ± 0.82	< 0.001	< 0.001
after therapy	4.36 ± 0.29		6.27 ± 0.81		< 0.001

$\bar{x} \pm SD$ – arithmetic mean ± standard deviation.
***p – significance of difference within the group (baseline *vs* after therapy); $^{\dagger}p$ – significance of difference between groups (SVR not acthived *vs* achived); HCV – hepatitis C virus; SVR – sustained virological response; SOD – superoxide dismutase; GPx – glutathione peroxidase; CAT – catalase.**

Table 5

Genotypes and therapy outcome

Genotypes	Before therapy, ($\bar{x} \pm SD$)		After therapy, ($\bar{x} \pm SD$)	
	SVR achieved	SVR not achieved	SVR achieved	SVR not achieved
1 and 4	n = 24	n = 7	n = 24	n = 7
SOD (U/gHb)	887.96 ± 250.47*,**	503.89 ± 87.10	1,212.80 ± 326.39	608.76 ± 140.17
GPx (U/gHb)	22.51 ± 4.15*,**	15.44 ± 0.89	27.31 ± 5.15	15.52 ± 1.32
CAT (U/gHbx10^4)	5.13 ± 0.79*,**	4.34 ± 0.21	6.11 ± 0.75	4.36 ± 0.30
2 and 3	n = 22	n = 0	n = 22	n = 0
SOD (U/gHb)	1,071.49 ± 321.06**,***		1,393.20 ± 340.83****	
GPx (U/gHb)	24.72 ± 4.27**,***		30.06 ± 5.30****	
CAT (U/gHbx104)	5.37 ± 0.85**		6.40 ± 0.86	

$\bar{x} \pm SD$ – arithmetic mean ± standard deviation; $^{*}p < 0.05$ – difference between SVR achievers and SVR non-achievers before therapy among one group of genotypes; $^{**}p < 0.05$ – difference between SVR achievers before and after therapy among one group of genotypes; $^{***}p < 0.05$ – difference between SVR achievers before therapy comparing two groups of genotypes; $^{****}p < 0.05$ – difference between SVR achievers after therapy comparing two groups of genotypes; SVR – sustained virological response; SOD – superoxide dismutase; GPx – glutathione peroxidase; CAT - catalase.

activities before and after the treatment with PEG-IFN α-2a and RBV are even scarcer. There could be found extremely different research results, some indicating reduced and some revealing increased activities, with different explanations [15–17]. Patients with CHC have lower activities of antioxidative defense enzymes such as SOD, GPx, and CAT in erythrocytes and peripheral mononuclear cells in CHC patients [17–23], although increased activities of these enzymes are also described [24]. Some studies proved that HCV may impair antioxidant body systems and reduce the activities of antioxidative enzymes along with an observation that manganese superoxide dismutase (MnSOD) is one of the first therapy candidates for the reversion of fibrosis process. It is considered that this role is performed through the activation of nuclear factor kappa-B [25, 26].

According to some authors, during the replication of HCV, the amounts of MnSOD, heme oxygenase 1, CAT and GSH are increasing as an adaptive response to non-structural proteins of HCV. HCV multifunctional non-structural protein NS5A, essential for replication of HCV, induces an increase of MnSOD activity, which is mediated by the activation of activating protein-1 transcription factor by p38 mitogen-activated protein kinase and Jun N-terminal kinase signaling pathways [27, 28]. In contrast, there are researchers who claim that intracellular GSH status does not change during replication, and that the level of ROS is not high enough to initiate an adaptive increase in GSH [4]. However, oxidative damage is characterized by reduced levels of GSH, and upregulation of antioxidative enzymes such as CAT, GPx and SOD, according to most authors [11].

Direct measurement of the liver tissue revealed increased concentrations of ROS, and two- to five times decrease in the activity of antioxidative enzymes. The significant increase is also described in lymphocytes of patients with CHC [29]. It is important to note that the intensity of decrease of antioxidative enzymes activities in the liver tissue and leukocytes (above- mentioned study) and erythrocytes (our results) is equal. It is considered that treatment with PEG-IFN α-2a and RBV is directly responsible for the establishment of antioxidant response in patients with CHC [11]. It is considered that change in the oxidative-antioxidative balance plays a crucial role in the progression of liver injury, and interferon-alpha can be effective in the treatment of liver damage and improvement of oxidative system [17]. During *in vivo* studies on rats exposed to oxidative stress, Japanese scientists found that the IFN-α dose-dependently increases the levels of SOD and GPx and reduces levels of lipid peroxidation products in the liver of experimental animals [30].

A small number of researches that focus on the pathogenesis of CHC and oxidative stress show the results which directly indicate that disturbed oxidative balance initiates liver damage, and not *vice versa* [3]. Correlation analysis in our study showed that there was a mutual significant negative correlation between all three antioxidant enzyme activities to aminotransferases levels before the therapy where one can not conclude what the primary trigger of the disease is, and what is the consequence and intermediary progression element. Correlation analysis of the above- mentioned parameters after the treatment showed that there was no significant negative correlation between antioxidative enzymes and AST and ALT except GPx.

It speaks to the fact that disturbed antioxidant status may be representative of oxidative stress as the initiator of the pathogenesis CHC, and not the result of liver damage. Only GPx significantly negatively correlated with AST and ALT after the treatment ($p < 0.05$). On the other hand, the analysis of ROC curves singled this enzyme out as the most sensitive diagnostic marker (97.8%) in monitoring the success of therapy, perhaps because its reduced activity is a consequence of liver damage. As well, SOD and CAT could be considered as good diagnostic markers, albeit with lower specificity (93.5% and 71.7%, respectively).

Our findings also point to a positive relationship between the SVR and antioxidant status. Antioxidant enzyme activities were not significantly changed in patients who did not achieve SVR. For the time being, there are no published results of other studies to show whether there are differences in SOD, CAT and GPx levels in patients who did and who did not achieve SVR. However, there are results that did not find any change in the oxidative stress level among the mentioned groups of patients, though, antioxidant status was determined by the total antioxidant capacity rather than enzyme activities [31]. Our study participants who achieved SVR showed a statistically significant increase in GPx and SOD, CAT (activities $p < 0.001$). This fact shows that the baseline antioxidant status in patients with CHC could serve as a predictor of success or failure of combination antiviral therapy [32]. However, the difference in enzyme activities before and after the treatment showed a statistically significant increase between the patients who achieved SVR and the ones who did not achieve SVR. Since we singled out a group of patients who did not achieve SVR, we compared the activity of the antioxidant enzymes. It could be observed that SOD, CAT, and GPx activities were not significantly changed. In contrast, patients who achieved SVR, presented with a statistically significant change in the activity of antioxidant enzymes i.e. normalization of the activities. This speaks in favor of basic antioxidant enzyme activities as a possible factor predicting SVR [33, 34].

The most important prognostic factor for SVR achievement prior to the start of the therapy was the genotype of the virus. Genotypes 1 and 4 exhibited much lower SVR rates compared to genotypes 2 and 3. Examination of the relation between oxidative stress and HCV genotypes showed that more serious disease in HCV genetic subtype 1a/1b might be associated with more severe oxidative stress. Milder damage in subtypes 4, 2a/c, 2b and 3a could be related to lower oxidative response, respectively [35]. In our study, patients with genotypes 1 and 4 also had lower rates of SVR. Group of patients with genotypes 2 and 3 showed a statistically significant increase in both "baseline" and "after the therapy" SOD and GPx activities comparing to genotypes 1 and 4, as was expected ($p < 0.05$). Unexpectedly, there was no statistically significant difference in CAT activities between aforementioned groups, neither before nor after the treatment.

Conclusion

Oxidative stress present in patients with CHC is manifested by reduced activities of antioxidative enzymes. The combined antiviral therapy, which is lately considered to have antioxidant potential, leads to the restoration of antioxidant balance. Oxidative stress is the cause and not the consequence of the occurrence and development of CHC. Antioxidative enzymes (SOD, CAT, GPx) are good diagnostic markers of CHC treatment success. The results of this study, simultaneously considered together with the results of other authors may be the basis for the proposal of adding the antioxidative enzymes to the antiviral therapy, in order to achieve a better therapeutic response.

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