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ORIGINAL PAPER

Biochemical profile of cholestasis and oxidative stress markers in chronic hepatic disorders

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Mihaela BASA[1], Natalia Rosoiu[2], Adrian Sever RUS[3]
[1] Clinical Laboratory, Emergency Military Hospital, Constanta, Romania
[2] "Ovidius" University Faculty of Medicine, Biochemistry Department, Constanta, Romania; Academy of Romanian Scientists 54 Splaiul Independentei 050094, Bucharest,
[3] Emergency Military Hospital, Constanţa, Romania

Abstract
Background: Due to the essential role in the metabolization of exo- and endogenous compounds, the liver is exposed to numerous oxidative stresses and, when the production of reactive species outruns the activity of purifying enzymes, hepatic lesions occur. Oxygen reactive species may affect reversibly or irreversibly the biochemical substances thus influencing the fluidity and the membranous function, the cellular metabolism and even the genes expression.
Methods: in this study were included 194 patients, from which: 64 patients with C-type hepatic disease (viral C hepatitis – 41, viral C cirrhosis - 23), 44 patients with B-type hepatic disease (viral B hepatitis – 27, viral B cirrhosis - 17), 71 patients with alcoholic etiology hepatic disease (chronic alcoholic hepatitis – 37, chronic alcoholic cirrhosis – 34) and 15 healthy patients as a control group. For these patients we determined the markers for the cholestasis syndrome and the antioxidant system (both enzymatic and nonenzymatic)
The objective of this study is to provide information regarding the estimation of the disease severity through appreciation methods of the cholestasis and oxidative stress.
Results: between the parameters means from hepatitis and cirrhosis with the same etiology there are statistical significant differences for the following analytes: total bilirubin, conjugated bilirubin, unconjugated bilirubin (not valid for the C-type viral diseases), gamma glutamyl transpeptidase (valid only for the B-type viral chronic hepatic diseases), alkaline phosphatase, total cholesterol, glutathione reductase (valid only for the C-type viral diseases), super oxide dismutase (valid only for male patients infected eith B-type viral chronic hepatic diseases), serum albumin.
Conclusions*:* When the aggression over the hepatic cell is caused only by the alcohol metabolites, the cholestasis extension, compared to the injury of the hepatocyte membrane, is smaller than in the cases with double determination (viral and alcoholic). The extension of the cholestasis syndrome and the permeability increase of the hepatocyte membrane lead to an accentuated imbalance between antioxidants and the reactive species of oxygen by the decrease of total antioxidant status and the increase of antioxidant enzymes activity (glutathione reductase, super oxide dismutase). The great efficiency of antioxidants is represented by their synergy in action, each of them acting on different levels of the free radicals evolution chain; this efficiency is demonstrated by the statistical significant correlations between them.

Key words: oxidative stress (SO), total antioxidant status (TAS), glutathione reductase (GR), super oxide dismutase (SOD), uric acid (AcU), total bilirubin (BT), conjugated bilirubin (BD), gamma glutamyl transpeptidase (GGT), alkaline phosphatase (ALP)

Introduction

Chronic hepatitis is a pluriethiological syndrome, which has in common the existence of some necroinflammatory lesions and of a variable degree of fibrosis, which progresses without improvement for 6 months minimum. These histological changes come together with clinical manifestations with variable intensity and expressiveness, and biochemical, immunological and imagistic anomalies [2].

The concept of hepatic cirrhosis does not define one single disease, but the advanced, generally irreversible stage of numerous chronic hepatic disorders, where an active inflammation of the liver occurs. Morphologically, the hepatic cirrhosis is characterized by:

- variable destruction of hepatocyte mass through extensive necroses;
- formation of conjunctive septum;
- nodule regeneration;
- disorganization of liver architecture;
- vascularization alteration [4].

Physiopathologically, **cholestasis** may be defined as a **disturbance in the process of bile formation and flow, and a decrease of biliary flow.** Morphologically, cholestasis is characterized by a visible accumulation of biliary pigments in hepatocytes and in biliary canaliculi in the presence of "biliary thrombus"; from the point of view of the clinic aspect, the great incidence of pruritus is very impressive [5].

The biochemical aspects reside in the blood retention of the bile components, mainly the biliary acids, and in the increase of the "cholestasis enzymes" activity, namely ALP, GGT, 5-nucleotidase (5-NT) and leucinaminopeptidase (LAP) [12].

The normal liver is a well equipped organ regarding the enzymatic and non enzymatic antioxidants. The oxidative stress is the result of the unbalanced ratio between the oxidant agents and antioxidant systems with reactive oxygen species (SRO) [13].

The functional hepatic exploration is complex, and includes functional-biochemical, hematological, immunological, ultrasonic, tomographic exploration.

The objectives of the biochemical exploration in the present study are as follows:

a. to estimate the severity of the disease;
b. to evaluate the functional reserves and prognosis;

c. to determine the level of oxidative stress;
d. to determine the cholestasis extension degree compared to the hepatocyte membrane injury;
e. to establish the existent correlations between cholestasis and oxidative stress markers.

Material and Methods

For all 194 patients included in the study we made serum determinations for the **cholestasis syndrome** markers: BT, BD, indirect/ unconjugated bilirubin (BI), GGT, ALP, and total cholesterol (CT), and for the **antioxidants:** SOD - determined from erythrocytic lysate, TAS, GR, albumin (ALB), and AcU. SOD was not determined for the patients with alcoholic chronic hepatitis (HC Alc.).

The determinations were performed with Beckman Coulter Synchron CX7 automatic analyzer. The patients included in the study were analyzed either when admitted in the department of Internal Disease of the Emergency Military Hospital, or in the polyclinic department of the same hospital.

All determinations were performed the same day the biological material was harvested, which was not frozen.

To determine the activity of the enzymes alanine aminotransferase (ALT) and aspartat aminotransferase (AST) we used kinetic methods (decreasing reaction), with reading at 340 nm (oxidation speed of NADH + H^+ is directly proportional with catalytic activity of the enzyme).

To determine the activity of the enzymes GGT and ALP kinetic, colorimetric methods were used (increasing reaction), with readings at 410 nm (read absorbance is directly proportional with the activity of the enzyme from the analyzed sample, and it is used to calculate and express its activity).

The concentrations of the analytes BT, BD, AcU, and ALB were determined with colorimetric time-end point methods, with readings taken at 520 nm (BT, AcU, CT), proportional with the concentration of the analyte from the analyzed sample.

Indirect bilirubin was determined with the following formula: BI = BT – BD

Oxidative stress was evaluated using reactants produced by RANDOX Laboratories, UK.

In time end point reactions the concentration of the analyte was determined by using a standardizing curve, obtained on the basis of standards or a calibrating device.

The reactions were controlled (with reading in VIS, UV) using control serums for low, medium, and high values; the reactions were calibrated and controlled in each series of determinations.

The age intervals for the patients included in our study are presented in table 1.

Table 1 Patients distribution according to age groups in chronic hepatic diseases

Age groups	HCVC	CHVC	HCVB	CHVB	HC Alc.	CH Alc.
20 –30 years	3 (7.3%)	-	2 (7.4%)	-	-	-
30 -40 years	8 (19.5%)	1 (4.3%)	6 (22.2%0	-	9 (24.3%)	1 (2.9%)
40 -50 years	16 (39.0%)	5 (21.7%)	9 (33.3%)	2 (11.8%)	10 (27.0%)	5 (14.7%)
50 -60 years	9 (22.0%)	5 (21.7%)	9 (33.3%)	8 (47.1%)	11 (29.7%)	13 (38.2%)
60 -70 years	5 (12.2%)	4 (17.4%)	1 (3.7%)	6 (35.3%)	5 (13.5%)	10 (29.4%)
70 -80 years	-	7 (30.4%)	-	1 (5.9%)	2 (5.4%)	5 (14.7%)
80 -90 years	-	1 (4.3%)	-	-	-	-
Total	41 (100%)	23 (100%)	27 (100%)	17 (100%)	37 (100%)	34 (100%)

The mean and the standard deviation were calculated for the determined analytes, as shown in table 2.

Table 2 Average values and standard deviations in chronic hepatic diseases

Variable	Values in HCVC	Values in CHVC	Values in HCVB	Values in CHVB	Values in HC Alc.	Values in CH Alc.	Values for control group
BT (mg/dl)	1.01 ± 0.38	1.89 ± 1.98	1.07 ± 0.34	2.83 ± 2.22	1.18 ± 0.49	2.98 ± 2.39	0.88 ± 0.21
BD (mg/dl)	0.12 ± 0.06	0.72 ± 1.55	0.13 ± 0.06	1.18 ± 1.59	0.16 ± 0.14	1.08 ± 1.22	-
BI (mg/dl)	0.88 ± 0.35	1.16 ± 0.64	0.93 ± 0.30	1.64 ± 0.85	1.02 ± 0.41	1.90 ± 1.26	-
GGT (UI/L)	76.58 ± 85.69	141.47 ± 149.58	40.37 ± 28.07	152.13 ± 151.61	211.39 ± 137.84	174.64 ± 164.12	19.33 ± 4.35
ALP (UI/L)	65.60 ± 19.74	105.82 ± 46.98	65.40 ±16.46	94.94 ± 28.09	70.87 ± 25.56	116.82 ± 61.10	
CT (mg/dl)	181.41 ± 37.50	140.52 ± 49.10	197.00 ± 55.83	129.17 ± 31.09	216.78 ± 40.98	146.76 ± 46.51	178.00 ± 17.26
TAS (mmol/L)	1.23 ± 0.11	1.21 ± 0.14	1.23 ± 0.11	1.16 ± 0.12	1.16 ± 0.13	1.22 ± 0.12	1.53 ± 0.08
GR (UI/L)	76.86 ± 12.49	81.87 ± 12.37	73.71 ± 9.48	84.28 ± 7.81	85.47 ± 11.38	77.98 ± 12.69	58.46 ±7.05
SOD (U/ml)	284.25 ± 51.63	287.53 ± 40.44	269.04 ± 39.55	303.80 ± 35.31	–	279.83 ± 38.42	200.87 ± 12.03
ALB (g/L)	42.26 ± 4.28	32.21 ± 5.30	42.96 ± 4.49	28.29 ± 5.28	40.64 ± 3.93	26.97 ± 6.58	42.26 ± 3.01
AcU (mg/dl)	5.00 ± 1.36	5.56 ± 1.98	5.22 ± 1.20	4.81 ± 1.66	6.10 ± 1.80	5.43 ± 1.97	4.62 ± 0.89

Using the software Statistical Package for Social Sciences (SPSS), we performed the statistical "Student" test on the independent research groups for all determined analytes for the patients with viral and alcoholic chronic hepatitis and hepatic cirrhosis (tables 3 and 4).

Also, the "Pearson" coefficient was calculated in order to establish the correlations with or without statistical significance between the determined analytes.

Table 3 Statistic significance for the average values of the analytes in comparative studies between viral and alcoholic hepatitis and cirrhosis

Analyte	HCVC - CHVC	HCVB - CHVB	HC Alc.- CH Alc.
BT (mg/dl)	$p < 0.05$	$p < 0.05$	$p < 0.05$
BD (mg/dl)	$p < 0.05$	$p < 0.05$	$p < 0.05$
BI (mg/dl)	$p > 0.05$	$p < 0.05$	$p < 0.05$
GGT (UI/L)	$p > 0.05$	$p < 0.05$	$p > 0.05$
ALP (UI/L)	$p < 0.05$	$p < 0.05$	$p < 0.05$
CT (mg/dl)	$p < 0.05$	$p < 0.05$	$p < 0.05$
TAS (mmol/L)	$p > 0.05$	$p > 0.05$	$p > 0.05$
GR (UI/L)	$p > 0.05$	$p < 0.05$	$p < 0.05$
SOD (U/ml)	$p > 0.05$	$p < 0.05$ (♂) $p > 0.05$ (♀)	-
AcU (mg/dl)	$p > 0.05$	$p > 0.05$	$p > 0.05$
ALB (g/L)	$p < 0.05$	$p < 0.05$	$p < 0.05$

* $p < 0.05$ = correlation with statistical significance
$p > 0.05$ = correlation without statistical significance
- = no determination

Table 4 Correlations between analytes from the same chronic hepatic disorder

Analytes correlation	HCVC	CHVC	HCVB	CHVB	HC Alc.	CH Alc.
BT- ALB	r = 0.403 p < 0.01	r = - 0.462 p < 0.05	r = - 0.186 p = 0.354	r = - 0.056 p = 0.831	r = 0.059 p = 0.730	r = - 0.386 p < 0.05
BT- GGT	r = 0.290 p = 0.066	r = 0.511 p < 0.05	r = 0.213 p = 0.287	r = 0.665 p < 0.01	r = 0.069 p = 0.687	r = 0.357 p < 0.05
BT – GR	r = 0.268 p = 0.090	r = 0.689 p < 0.01	r = - 0.208 p = 0.299	r = 0.761 p < 0.01	r = - 0.109 p = 0.523	r = 0.617 p < 0.01
BT –TAS	r = - 0.310 p < 0.05	r = - 0.625 p < 0.01	r = 0.214 p = 0.285	r = - 0.475 p = 0.054	r = 0.029 p = 0.866	r = - 0.285 p = 0.102
GGT –GR	r = 0.527 p < 0.01	r = 0.495 p < 0.05	r = 0.316 p = 0.108	r = 0.601 p < 0.05	r = 0.342 p < 0.05	r = 0.602 p < 0.01
GR –ALB	r = 0.030 p = 0.853	r = - 0.278 p = 0.200	r = - 0.283 p = 0.153	r = - 0.176 p = 0.499	r = - 0.120 p = 0.479	r = 0.162 p = 0.360
TAS –ALB	r = - 0.025 p = 0.874	r = 0.356 p = 0.096	r = 0.165 p = 0.411	r = 0.121 p = 0.644	r = 0.248 p = 0.139	r = 0.048 p = 0.786
TAS –CT	r = - 0.264 p = 0.096	r = - 0.406 p = 0.054	r = - 0.243 p = 0.223	r = 0.282 p = 0.272	r = 0.185 p = 0.273	r = 0.420 p < 0.05
TAS –GGT	r = - 0.403 p < 0.01	r = - 0.586 p < 0.01	r = - 0.262 p = 0.186	r = - 0.522 p < 0.05	r = - 0.441 p < 0.01	r = - 0.597 p < 0.01

TAS – GR	r = - 0.682 p < 0.01	r = - 0.737 p < 0.01	r = - 0.706 p < 0.01	r = - 0.652 p < 0.01	r = - 0.629 p < 0.01	r = - 0.530 p < 0.01
SOD – GGT	r = 0.405 p < 0.01	r = 0.433 p < 0.05	r = 0.280 p = 0.157	r = 0.650 p < 0.01	-	r = 0.668 p < 0.01
SOD –GR	r = 0.786 p < 0.01	r = 0.879 p < 0.01	r = 0.782 p < 0.01	r = 0.862 p < 0.01	-	r = 0.797 p < 0.01
SOD –AcU	r = 0.071 p = 0.659	r = - 0.281 p = 0.195	r = 0.158 p = 0.431	r = - 0.135 p = 0.605	-	r = - 0.273 p = 0.119
SOD - TAS	r = - 0.663 p < 0.01	r = - 0.648 p < 0.01	r = - 0.781 p < 0.01	r = - 0.508 p < 0.05	-	r = - 0.581 p < 0.01

* $p < 0.01$, $p < 0.05$ = correlation with statistical significance
$p > 0.05$ = correlation without statistical significance
- = no determination

The cholestasis extension was calculated depending on the extension of hepatocyte membrane injury, evaluated with GGT/ALT or GGT/AST ratio.

Results and discutions

Most patients suffering from chronic alcoholism and B- type viral chronic hepatic disorders are between 50 – 60 years of age, (actually for those with viral B hepatitis (HCVB) the number of patients aged between 50 – 60 years is equal to those belonging to 40 – 50 years range). Most patients with viral C hepatitis (HCVC) are 40 – 50 years of age, and those with viral C cirrhosis (CHVC) are between 70 -80 years of age.

Bilirubin is produced through the oxidative decomposition of the porphyrin ring of hemoglobin and other hemoproteins into the cells of the reticuloendothelial system in the spleen, liver (Kupffer cells), lymphatic ganglions and the macrophages from various tissues [1].

The enzymatic system, which catalyzes the formation of bilirubin is inducible, which means that its activity increases when the substratum it actions upon accumulates.

The appearance of jaundice in the evolution of a cirrhotic disorder mostly depends on the extension of the hepatocytic necrosis and the decrease of the functional remaining hepatocytic mass. Most likely, the jaundice suggests decompensated cirrhosis [8].

During cholestasis, not only ALP produced by the epithelium of biliary ducts increases, but also GGT, which is produced in the hepatocytic microsomes [12]; the increase cannot be seen as only a retention process; it looks like the result of a microsomial induction, i.e. an accelerated enzyme synthesis.

GGT levels are increased in regenerative areas of the cirrhotic liver [6]. The increase of the serum level of GGT is due to the lesions of the cellular membrane,

produced by toxic substances (alcohol included), ischemia, and injections or by the detachment of the enzyme from the cellular membrane, caused by the biliary acids [9].

On the other hand, it is considered that the GGT modifications are not caused by lesions of the cellular membrane, not occurring together with aminopherase modifications; it is the result of the microsomial enzyme induction. In other words, biliary stasis could stimulate enzyme synthesis in the liver, possibly increasing permeability of microsomial membrane.

The extension of cholestasis in comparison with membrane injury can be assessed by GGT/AST or GGT/ALT ratio.

In patients with jaundice, GGT activity in comparison with aminopherase, i.e. GGT/AST or GGT/ALT ratio, evaluates the extension of cholestasis as against the cellular membrane injury. The highest values are found in chronic patients with viral aggression and acute ethanol ingestion, the amplified increase of GGT is caused also by this toxic substance, added to cholestasis syndrome (in viral diseases (Tables 5, 6, 7, 8).

Table 5 Cholestasis syndrome in C-type viral chronic hepatic disorder

	Viral C hepatitis			**Viral C cirrhosis**		
	Values in percentage expression		**Absolute pathological value**	**Values in percentage expression**		**Absolute pathological value**
analyte	**VN**	**> LSN**	**maxim**	**VN**	**> LSN**	**maxim**
BT	83.0	17.0	2.4	39.1	60.9	10.1
BD	92.7	7.3	0.3	26.1	73.9	7.7
BI	73.2	26.8	2.2	52.2	47.8	2.8
GGT	65.8	34.2	276.0	39.1	60.9	540.0
ALP	92.7	7.3	131.0	39.1	60.9	238.0

* VN = normal values; LSN = normal superior limit

In HCVB, the only statistically meaningful correlations are those between antioxidants (AO), which implies that, the lower the total antioxidant capacity, the more intense the activity of enzyme GR and SOD in order to get balance between free radicals and antioxidants.

In patients up to 30 years of age, there are no modified markers (over the normal limits), neither in cholestasis syndrome, nor in antioxidant system; the same aspect can be found in one patient of 45 years of age; there can be said that these patients are in the compensated stage of the disease, its evolution depending on the aggressiveness of virus, and the patient's balanced way of living, as well.

Table 6 Cholestasis syndrome in B-type viral chronic hepatic disorder

	Viral B hepatitis			Viral B cirrhosis		
	Values in percentage expression		Absolute pathological value	Values in percentage expression		Absolute pathological value
analyte	VN	> LSN	maxim	VN	> LSN	maxim
BT	70.4	29.6	1.8	23.5	76.5	8.6
BD	96.3	3.7	0.4	35.3	64.7	5.7
BI	66.7	33.3	1.6	23.5	76.5	3.7
GGT	85.2	14.8	149.0	35.3	64.7	588.0
ALP	92.6	7.4	103.0	35.3	64.7	162.0

* VN = normal values; LSN = normal superior limit

Table 7 Cholestasis syndrome in chronic alcoholic hepatic disorder

	Chronic alcoholic hepatitis			Hepatic alcoholic cirrhosis		
	Values in percentage expression		Absolute pathological value	Values in percentage expression		Absolute pathological value
analyte	VN	>LSN	maxim	VN	> LSN	maxim
BT	62.20	37.80	2.50	17.70	82.30	13.00
BD	86.50	13.50	0.80	14.70	85.30	6.27
BI	56.80	43.20	2.10	26.50	73.50	6.73
GGT	8.10	91.90	566.00	35.30	64.70	558.00
ALP	83.80	16.20	148.00	47.10	52.90	309.00

* VN = normal values; LSN = normal superior limit

Table 8 Chlolestasis extension compared to hepatocyte membrane injury

	GGT/ALT			GGT/AST		
Disorder	average	minimum	maximum	average	minimum	maximum
HCVC	1.93	1.22	3.20	3.06	0.91	8.50
CHVC	3.90	0.45	19.28	2.35	0.30	9.46
HCVB	2.85	0.73	4.96	3.76	1.01	6.50
CHVB	3.94	0.60	9.80	2.29	0.52	7.70
HC Alc.	5.04	0.80	14.35	3.42	0.70	7.90
CH Alc.	5.85	0.55	12.00	2.90	0.50	8.12

In most patients, there can be noticed that TAS is much below the inferior limit of the disease (Table 9), this value also depending on the period of virus aggressiveness, determined mainly by the intensity of viral replication, which results in the increase of the antioxidant enzymes activity; thus, while the disease keeps active HC, without treatments of hepatic protection (with permanent cytolisis and necrosis aspects), the two antioxidant enzymes we identified intensify their activity, but the total antioxidant capacity decreases; in these patients, the cholestasis is absent; if present, there are no high values for either specific analytes.

Table 9 Antioxidants in B-type viral chronic hepatic disease

	Viral B hepatitis					**Viral B cirrhosis**				
	Values in percentage expression			**Absolute pathological value**		**Values in percentage expression**			**Absolute pathological value**	
analyte	**< LIN**	**VN**	**> LSN**	**Min.**	**Max.**	**< LIN**	**VN**	**>LSN**	**Min.**	**Max.**
TAS	70.4	29.6	-	1.04	-	94.1	5.9	-	0.81	-
GR	-	51.9	48.1	-	98.2	-	-	100.0	-	102.4
SOD	-	18.5	81.5	-	365.5	-	-	100.0	-	402.1
AcU	-	92.6	7.4	-	8.0	-	94.1	5.9	-	9.9
ALB	3.7	88.9	7.4	34.00	-	82.4	17.6	-	21.00	-
BT	-	70.4	29.6	-	1.8	-	23.5	76.5	-	8.6

* LIN = normal inferior limit; VN = normal values; LSN = normal superior limit

We also found very low TAS (due to deficiency of non enzymatic AO, vitamin A and E especially), and high CT, without other signs of cholestasis (Table 7); this stage of the disease coexists with the increase of lipid peroxidation. In viral C cirrhosis (CHVB) there are statistic correlations between antioxidants (as in HCVB), between analytes that indicate cholestasis, and between antioxidants and these analytes; **these correlations evidence the considerable proportion of SRO in the hepatic cell.** Thus, in cirrhosis with virus B, the cholestasis syndrome is much better expressed than in HC with virus B, the concentration of antioxidant enzymes being much higher in cirrhosis, the difference between means is statistically significant (in SOD only in males). The cholestasis enzymes, antioxidant enzymes and bilirubin increase, and TAS decreases; the lowest concentrations of the total antioxidant status are found in patients with highest values of bilirubin and even of GGT, which reflects hepatotoxicity (this fact is also encountered in alcoholic and viral C cirrhosis).

In this stage of the chronic hepatic disease (CHVB), cholestasis is not joined by total cholesterol, which does not exceed LSN; on the contrary, the concentrations of this analyte decrease, which is very important in maintaining the

integrity of the cellular membrane. The uric acid may even equal LIN in advanced stages of cirrhosis with intense cholestasis (Table 9).

In HCVC total cholesterol increases moderately and joins the cholestasis syndrome; CT increase is much better expressed in women than in men.

There are patients where cholestasis is not expressed by the increase of total cholesterol, or cases when there is no cholestasis syndrome, and the cholesterol increase indicates dislipidemia (DLP) - (Table 10).

Table 10 Total cholesterol behavior in chronic hepatic disease

Total cholesterol values percentage ratio (%) in chronic hepatitis and cirrhoses					
Disorder	**< LIN**	**VN**	**> LSN**	**Minimum**	**Maximum**
HCVC	14.63	56.10	29.26	102 mg/dl	264 mg/dl
CHVC	65.21	21.74	13.04	59 mg/dl	246 mg/dl
HCVB	18.50	37.00	44.50	124 mg/dl	330 mg/dl
CHVB	64.70	35.30	0	79 mg/dl	192 mg/dl
HC Alc.	5.40	24.30	70.30	112 mg/dl	297 mg/dl
CH Alc.	35.30	55.90	8.80	41 mg/dl	250 mg/dl

* LIN = normal inferior limit; VN = normal values; LSN = normal superior limit

An important role comes to the oxidative stress, which has a direct cytopathic effect. The reactive oxygen species, produced by the activated macrophage cells and the reactive aldehydes resulted from the lipidic peroxidation activate upon stellate hepatic cells, transforming them in miofibroblasts, and encouraging the synthesis of extracellular matrix, fibrosis, and cirrhosis [7,10]. .

High SOD implies high concentrations of aggressive oxygen compounds, which are to be transformed into inactive compounds. The systems that generate superoxide radicals are in the plasmatic membrane, in peroxisomes, and in cytosol, as well. Radical anion superoxide is extremely reactive, being able to interact with proteins, nucleic acids, lipids, etc. On the other hand, anion superoxide generates other free radicals and molecules, which are highly reactive, too. Very important are hydroxile radical and hydrogen peroxide.

SOD is between the normal limits in 6 patients with HCVC (table 11); in these patients and the others, AO determined are normal and cholestasis syndrome is not expressed. Out of the 6 patients, 2 of them had finished the treatment with interferon 1-2 years before, but the antibodies anti HCV were still present; in these patients the viral threat was probably small, and the treatment and way of living were proper.

Tabel nr.11 Antioxidants in C-type viral chronic hepatic disease

	Viral C hepatitis					Viral C cirrhosis				
	Values in percentage expression			Absolute pathological value		Values in percentage expression			Absolute pathological value	
	< LIN	VN	>LSN	Min.	Max.	< LIN	VN	>LSN	Min.	Max.
TAS	78.00	22.0	—	0.88	—	74.0	26.00	—	0.80	—
GR	—	34.1	65.85	—	106.31	—	21.74	78.26	—	118.40
SOD	—	14.6	85.40	—	389.65	—	8.70	91.30	—	366.20
AcU	2.44	87.8	9.76	—	9.50	—	69.60	30.40	—	9.90
ALB	12.20	85.4	2.40	33.00	51.00	78.3	21.70	—	20.00	—

* LIN = normal inferior limit; VN = normal values; LSN = normal superior limit

The statistically significant correlations between antioxidants (TAS, GR, SOD) and in HCVC and CHVC indicate that these markers work together in each of the two stages of the chronic disease.

The uric acid is another antioxidant, which inhibits AGPN peroxidation, extinguishes $^1O_2$, protects the nucleic acids in vitro, prevents oxidation of vitamin C, removes oxygen metabolites soluble in water, and purges anion superoxid and hydrogen peroxide [11].

The mean of AcU in HCVC and CHVC is not statistically different, but, in CHVC, AcU exceeds LSN in 30.4% of patients, while in HCVC, in only 9.76% patients (Table 9). Thus, it can be considered that XOD, the enzyme which catalyzes the synthesis reaction of AcU has an intensified activity, and the resulted proportion of ion superoxide is higher in CHVC than in HCVC (same situation in HCVB and CHVB).

In CHVC, the cholestasis syndrome is much better expressed than in HCVC (from the frequency and value point of view).

The difference between the means of ALP values in each of the tested performed, i.e. between hepatitis and cirrhoses explains on one hand cholestasis much more intense in CH than in HC, and the increase of the mesenchimal activity reflected by the increase of ALP activity, on the other hand.

The means of AO values (TAS, GR, SOD, AcU) in CHVC are not statistically different from AO values in HCVC.

The 2 normal SOD determined values in CHVC are found in patients under maintenance treatment. The treatment aimed to reestablish the normal metabolism of hepatocyte and the functional integrity of the plasmatic membrane, in order to recuperate from the most severe forms of the disease (decompensated cirrhosis,

encephalopathy) to a satisfactory clinical status; the results prove therapeutic efficiency.

In chronic alcoholic disease, ethanol oxidation leads to formation of free radicals in hepatocytes, including hydroxiethyl radical, anion superoxide, and hydroxyl radical. These free radicals induced by ethanol determine oxidative alterations of the intracellular components [3]. .

In CH Alc. there is established a statistically significant correlation between GGT and BT in the excreto-biliary syndrome; this correlation does not occur in alcoholic hepatitis, the cholestasis syndrome being much better expressed in cirrhosis; the differences between the means of the cholestasis marker values being statistically significant, too (Table 3). GGT is statistically correlated with TAS, SOD, and GR, which emphasizes the high level of oxidative stress, as the cholestasis markers increase (Table 4). CT is statistically correlated with TAS, both analytes evolve in the same direction; the decrease of CT goes together with the decrease of TAS level; as a result, the hepatic insufficiency expressed by the decrease of CT synthesis is joined by the decrease of the AO concentration (especially non enzymatic) [3].

SOD is the main enzyme against oxidative aggressiveness; SOD does not only decompose anion super oxide, it can "neutralize" the action of singlet oxygen and AGPN peroxidation, indirectly. The great quantities of H_2O_2 could diminish GSH and nicotinamide nucleotides reduced when H_2O_2 is reduced in the reaction catalyzed by glutathion peroxidase [3]. The intracellular accumulation of GSSG is a toxic manifestation of oxidative stress. GSH is a important part of the anti stress mechanism.

The increased activity of GR demonstrates a deficient reduced glutathion and a high quantity of oxidated glutathion [13].

Through its values, the total antioxidant status demonstrates a decrease of antioxidants (non enzymatic, especially; glutathione, for example)

As for the total antioxidant status, we found that it could have values between limits of normal even in an advanced stage of cirrhotic disease with vascular and parenchymatous decompensation. It is the case of 5 patients with CH Alc., where the analytes were not determined immediately after admission in the hospital, but in the 5^{th} day from hospitalization. In one of these patients, TAS has the highest value, at the superior limit of normal; the treatment helped him to improve his biochemical picture, including the antioxidants, but the diagnosis at hospitalization included also the acute ethanol ingestion, which was also found biochemically, by GGT dosage. In cases of advanced cirrhosis, ethanol ingestion threats life, the evolution of the disease being often unfavorable.

The SOD value is between the normal limits in cirrhotic patients with normal TAS (obtained from the patients under treatment while hospitalized). The normalization of the enzyme, with a tendency to decrease, though, can be ascribed

to the reduction of its synthesis (the stage of advanced cirrhosis, when the patient can be saved only by hepatic transplant, and the reserve of functional parenchyma is reduced); a high synthesis is ascribed to enzyme inductibility, which helps the organism, because biosynthesis is greater at higher concentrations of molecular oxygen, and anion superoxide.

In HC Alc., cholestasis markers increase lesser than in the cirrhotic stage, cholestasis is joined by increased CT, and in the rest of cases without cholestasis, the increased CT value indicates dislipidemia, directly connected to hepatic steatosis (alcoholic). Cholestasis syndrome is correlated to antioxidants only through GGT; total antioxidant status decreases as GGT increases (rather after alcohol ingestion, than in cholestasis context); in this context, also, the necessary of reduced glutathion

The GR activity intensifies more in order to reestablish the balance between SRO and AO.

AcU exceeds LSN rather significantly in patients with hepatitis (29.7%), and cirrhosis (23.4%) - (Table 12).

Table 12 Antioxidants in chronic alcoholic disorder

	Chronic alcoholic hepatitis					**Hepatic alcoholic cirrhosis**				
	Values in percentage expression			**Absolute pathological value**		**Values in percentage expression**			**Absolute pathological value**	
Analyte	**<LIN**	**VN**	**>LSN**	**Minim**	**maxim**	**<LIN**	**VN**	**>LSN**	**minim**	**maxim**
TAS	89.2	10.8	—	0.81	—	85.30	14.70	—	0.88	—
GR	—	8.1	91.9	—	117.26	—	14.70	85.30	—	115.74
SOD	—	—	—	—	—	—	17.70	82.30	—	396.15
AcU	—	70.3	29.7	—	9.70	5.90	70.60	23.50	1.70	9.80
ALB	13.5	86.5	—	32.00	—	88.20	11.80	—	14.00	—
BT	—	62.2	37.8	—	2.50	—	17.70	82.30	—	9.90

* LIN = normal inferior limit; VN = normal values; LSN = normal superior limit

Albumin is considered a "sacrifice" antioxidant (it stops $^1O_2$, it is a metallic chelating for Fe, Cu, Zn, Se, transports bilirubin, and removes HOCl) [7].

The means of bilirubin statistic values differs significantly between chronic hepatitis and cirrhoses (regardless the etiology); when in normal concentrations, it can function successfully as antioxidant. In cirrhotic severe hepatic insufficiency, the plasmatic concentration of albumin may decrease to very low values (14 g/L from alcoholic CH, in our study); albumin does not statistically correlate to AO, TAS, GR either in HC, where synthesis is between limits of normal, nor in CH where its synthesis is reduced; this is perhaps because their different place of action as AO (ALB is a plasmatic AO); this can be also explained through the much different action levels of albumin and of other AO the correlation was

checked (the main antioxidant role being played by the antioxidant enzymes rather than the albumin, and their actions have different intensities). Further studies must verify this aspect.

Conclusions

1. BT statistically correlates to GGT only in cirrhosis (viral, alcoholic) when the cholestasis syndrome is much more expressed, but not in hepatitis, when total bilirubin rarely overpasses 2 – 3 mg/dl.
2. In cirrhosis, the correlation between BT and GR indicates that, as hyperbilirubinemia in cholestasis accentuates, GR level increases, the latter being statistically significantly correlated to GGT, as well, which increases its activity of transpeptidase, reflecting also the hepatotoxicity exercised by viral particules and alcohol metabolites;
3. Intense cholestasis leads to massive oxidative stress in hepatobiliary tissue; as the cholestasis markers increase, the oxidative stress develops, this fact being indicated by the increase of the enzyme from the first line of defense (SOD), and of the secondary enzyme (GR), as well. TAS decreases, as jaundice intensifies;
4. The difference between the mean of ALP values between hepatitis and cirrhoses with the three ethiologies explains the increase of the mesenchymal activity reflected by the increase of ALP activity in cirrhosis, which demonstrates an intensification of the hepatic regeneration, with supply of substrata and precursors for the hepatocytic proteinic synthesis, thus sustaining the anabolic functions of the cell.
5. Patients with VN are probably infected with less aggressive genotypes and low viral load, both for cholestasis indexes, and antioxidants, or the maintenance treatment (hepatoprotective, trophic, anticholestatic, of immune reaction modulation) has a favorable effect.
6. In HC, the high CT values can join cholestasis or are the causes of DLP; in decompensated CH, as the gravity of illness develops, a progressive CT decrease can be observed, being one of the severe signs of hepatic insufficiency;
7. A high GR indicates a high level of oxidated glutathione, which, under the action of dependent $NADPH^{+}$ enzyme, regenerates reduced glutathione (the main non enzymatic antioxidant).
8. TAS parameter, as cumulative indicator of all AO molecules in the plasma demonstrates the exceed of the "fighting" capacity of the organism against toxic RL in excess; the precarious AO defending conditions help the development of the following processes: necrosis, apoptosis, fibrosis, proliferation, regeneration, statistically significant correlations between BT and TAS in HCVC

and CHVC; these processes indicate that, as BT increases, total antioxidant status decreases.

9. SOD activity intensifies, as the histological severity of the disease increases, and implicitly the pathological domain of the biochemical parameters, which measure the functional capacity of the liver, especially those which record synthesis deficiencies, and also the other biochemical tests which certify chronic hepatitis or indicate acute phases.

10. the correlations between determined antioxidants lead us to the conclusion that, although these are located in different places and activate through different mechanisms, their synergic action gives them a higher antioxidant efficiency.

11. We consider the AcU values which overpass LSN (more in viral, alcoholic cirrhosis, and in HC Alc) have a correspondent in the high level of ion superoxides, which result from the action of XOD.

12. Peroxidative degeneration of lipids in the structure of the cellular membranes alters their function and normal structure, often with dramatic consequences.

13. When the aggression upon the hepatic cell is caused only by the alcohol metabolites, the extension of cholestasis as against hepatocytic membrane lesion is smaller than in the cases with double determination (viral and alcoholic). The extension of the cholestasis syndrome and the increase of the permeability of the hepatocytic membrane result in a more emphasized lack of balance between AO and SRO, manifested through the decrease of the total antioxidant status and the increase of the antioxidant enzymes activity. (GR, SOD)

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