Academy of Romanian Scientists *Volume 1, No. 2, 2012, pp. 105 -115*
Annals Series on Biological Sciences Online Edition ISSN 2285 - 4177

ORIGINAL PAPER

The behavior of total antioxidant status and other biochemical parameters in serum and ascitic fluid from cirrhotic patients

Received for publication, october, 1, 2012.
Accepted, November, 15, 2012

Mihaela BASA[1], Radu Dumitru ROSOIU[2], Natalia ROSOIU[3], Adrian Sever RUS[4]
[1] Emergency Military Hospital Constanta, Clinical Laboratory, Romania, e-mail: mihaela_basa@yahoo.com
[2"]Ovidius" University, the Center for Educational and Professional Counseling, Constanta, Romania
[3"]Ovidius" University, Faculty of Medicine, Department of Biochemistry, Constanta, Romania; Academy of Romanian Scientists 54 Splaiul Independentei 050094, Bucharest, Romania
4 Emergency Military Hospital Constanta, Romania

Abstract:
We have included in the present study 15 patients diagnosed with decompensated CH, with ages ranging from 55 to 83 years age old.
For these patients we evaluated the level of antioxidants (TAS), of the hepatocytolytic syndrome (AST, ALT, AST/ALT, Fe), of the excreto-biliary syndrome (BT, BD, BI, ALP, GGT), of the mesenchymal inflammation (A/G), of the hepatoprive syndrome (CHE, ALB), of the lipid profile (CT, TG) and of the renal function (urea, creatinine, uric acid).
The sensitivity to the oxidative stress increases as the hepatoprive and the excreto-biliary syndromes are more clearly expressed.

Abbreviations: ALB - albumin A/G-albumin-globulin ratio, AcU-uric acid, ALB-albumin, ALP-alkaline phosphatase, ALT-alanine amino transferase, AST/ALT-de Ritis ratio, AST-Aspartate Amino Transferase, AT-aminotransferase, BD-conjugated bilirubin, BI - nonconjugated bilirubin, BT-total bilirubin, CH-cirrhotic hepatitis, CHE-cholinesterase, Crea-creatinine, CT-total cholesterol, ELFO-protein electrophoresis, Fe-iron, GGT-gamma glutamiltranspeptidase, LA-ascitic fluid, TG-triglycerides.

Key words*:* decompensate cirrhosis, LA, TAS, AST/ALT, A/G, CT, Urea, Cr, AcU.

Introduction

Chronic hepatic disorders, which cause cirrhosis, have cellular death as common and compulsory starting point.

Cirrhosis is the final stage of evolution for all the inflammatory or degenerative chronic hepatic diseases and morphologically is characterized by:

- variable destruction of hepatocyte mass through extensive necroses;
- formation of conjunctive septum;
- nodule regeneration;

- disorganization of liver architecture;
- vascularization alternation.

The appearance of ascites marks the start of "decompensation", which is the stage of liver cirrhosis in which the compensatory mechanisms are exceeded and the fluid goes beyond the vascular space (vascular decompensation) as discussed by Henriksen (Henriksen, 1995).

The objective of this study was to examine, biochemically, the serum and ascitic fluid from the patients with decompensated hepatic cirrhosis and to establish the importance of the determination of some biochemical analytes done in parallel or only using ascitic fluid for the confirmation of the diagnosis and even for the differential diagnosis.

Materials and Methods

The study included 15 patients (10 male patients=66.7%, and 5 female patients = 33.3%), with ages between 55 and 83 (Table 1). In these patients the etiological factors of cirrhosis are the infections with hepatic viruses B and C and alcohol consumption.

Table 1 - Descriptive age/gender statistics regarding the patients with ascitic crrhosis included in the study

Age	**Total**	Male	Female
50 – 60 years	2 (13.3%)	2 (20%)	-
60 – 70 years	9 (60.0%)	5 (50%)	4 (80%)
70 – 80 years	3 (20.0%)	2 (20%)	1 (20%)
80 – 90 years	1 (6.7%)	1 (10%)	-

For all 15 patients hospitalized in the Internal Diseases department of the Emergency Military Hospital, Constanta there were performed determinations from serum and ascitic fluid for the analytes which characterize: the level of non-enzymatic antioxidants (TAS), the ***hepatocytolytic syndrome*** (ALT, AST, AST/ALT, Fe), the ***excreto-biliary syndrome*** (BT, BD, BI, GGT, ALP), the ***hepatoprive syndrome*** (CHE, ALB), the ***mesenchymal inflammation*** **(**A/G from protein electrophoresis), ***the lipid profile*** (CT, TG) ***and the renal function*** **(**urea, creatinine (Cr), uric acid (AcU)**.**

The determinations were performed with the Beckman Coulter Synchron CX7 Clinical System automatic analyzer, Concept 2000 analyzer for electrophoresis of serum proteins.

For the determination of the concentrations for the analyzed analytes we used the following methods:

- kinetic methods (decreased), reading at 340 nm (ALT, AST, Urea);
- kinetic, colorimetric methods (increased), reading at 410 nm (GGT, ALP, CHE);
- colorimetric methods with time end point and enzyme-colorimetric methods with time end point (BT, BD, ALB, Crea Fe, AcU, CT, TG, TAS) reading the extensions at 520, 560 and 600 nm.

For all the determined analytes we calculated the mean, the standard deviation, minimum and maximum; we performed the statistical "Student" test in order to evaluate if the difference between the mean of an analyte from the two biological materials (serum, ascitic fluid) has statistical significance.

Results and Discussions

Hepatocytolisis is one of the ways the liver responds to different brutal assaults (infectious, toxic and anoxic) which lead to cytolysis; the various structures of the liver react differently to aggressions, the hepatocyte sector being the most fragile and the first to respond to aggressions.

Morphological changes of the hepatocyte are preceded by a biochemical lesion stage characterized by infrastructural and metabolic changes of the hepatocyte. In this stage the lesions are reversible.

Table 2 - Means, standard deviations, minimum and maximum values for the analytes determined in serum and ascitic fluid in cirrhotic patients (*hepatocytolytic syndrome*)

	Serum			**Ascitic fluid**		
Analyte	**M ± DS**	minim	maxim	**M ± DS**	mini m	maxim
ALT (UI/L)	30.52 ±17.07	11	68	7.53 ±1.64	5	10
AST(UI/L)	56.68 ± 29.18	25	127	16.92 ± 7.12	8	31
AST/ALT	2.03±0.76	0.96	3.50	2.27 ± 0.86	1.12	4.28
Fe (µg/dl)	112.0 ± 55.83	41	227	28.0 ± 7.29	18	42

Aminotranserases (ALT and AST) from the ascitic fluid (LA) have values which are inside the reference interval (reference interval valid for serum enzymes) even for those patients whose serum values for the same enzymes are higher than LSN. In serum are predominant the normal values for ALT (86,6%) and the pathological values for AST (60%).

The highest ALT value is 68 UI/L (mild cytolysis) and for AST, 127 UI/L (average cytolysis) (table 2).

The de Ritis ratio shows us that at the hepatocyte level necrosis occurs as a result of viral and/or alcoholic aggression (Figure 1).

The AST/ALT ratio greater than 2 is a result of the decrease of the hepatic content of ALT through a lower quantity of pyridoxale phosphate; it occurs in alcoholic liver more frequently than in other hepatic lesions Başa et al., 2010). It also signifies the mitochondrial ailment caused by alcohol.

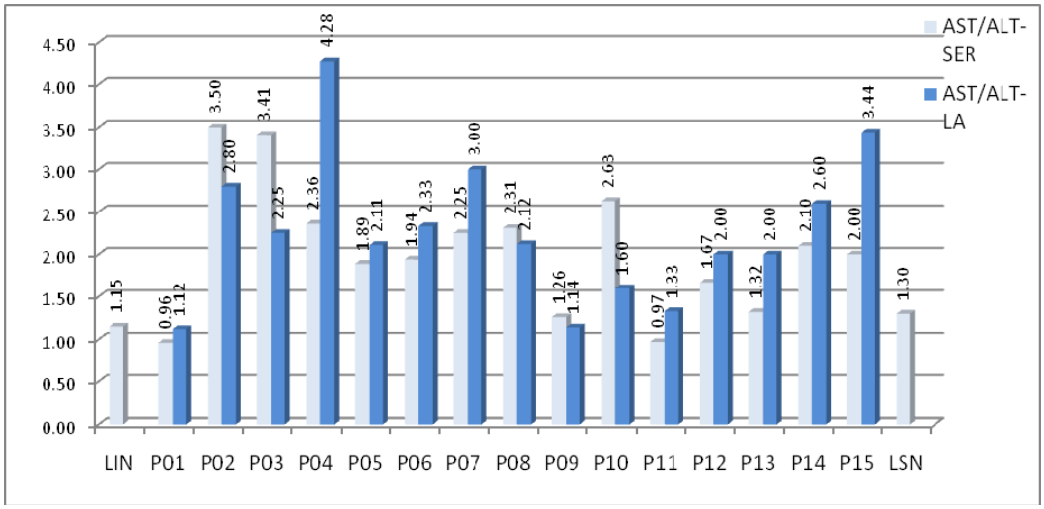

Fig. 1 - The variation of the de Ritis ratio in serum (AST/ALT-SER) and ascitic fluid (AST/ALT-LA) in cirrothics

The values of the de Ritis ratio in LA are similar to those in serum; between them there is no significant statistical difference (Table 3).

Table 3 - The Student test performed on the analytes determined in serum and LA in cirrhotic patients (*hepatocytolytic syndrome*)

Analyte in serum and ascitic fluid	Statistical significance
ALT	$p < 0.05$
AST	$p < 0.05$
AST/ALT	$p > 0.05$
Fe	$p < 0.05$

In acute viral hepatitis the iron is freed from hepatocytes through cytolysis, but it also increases in the progressing stages of chronic hepatitis and in hepatic cirrhosis, thus this could be a useful test when evaluating the disease progress.

In the hepatocyte, when the siderosomes membrane ruptures, where the iron is stored as hemosiderin, it will exercise its harmful action over the hepatocyte:

- uncoupling of oxidizing phosphorylation;
- decrease of ATP and enzymes content;
- lactate accumulation;
- decrease of NADH2 oxidation.

The iron in ascetic fluid has values between 18 and 42 μg/dl (figure 2 and figure 3), but in serum it exceeds LSN in 26.6% of the patients, the highest value being 197 μg/dl, the respective patient presenting increased activity for the other cytolysis enzymes. In 20% of patients, the serum iron was determined to be lower than the inferior normal limit; these patients are in an advanced stage of cirrhosis when anemia may also occur.

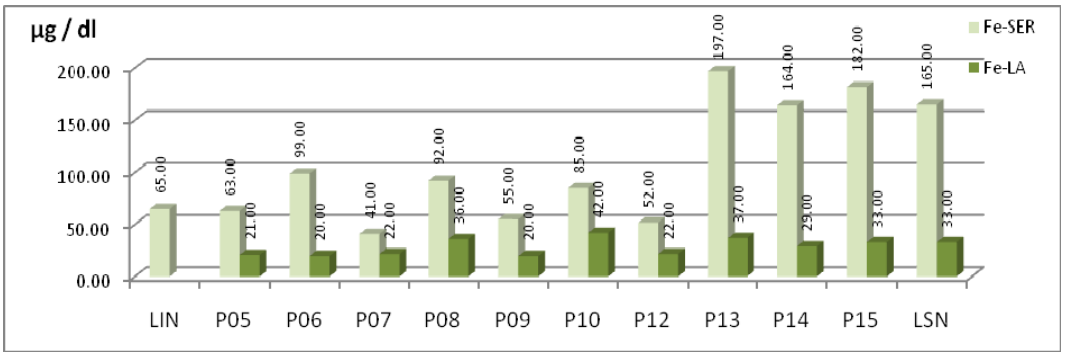

Fig. 2 - The variation of iron in serum (Fe–SER)
and ascitic fluid (Fe–LA) in cirrhotics - males

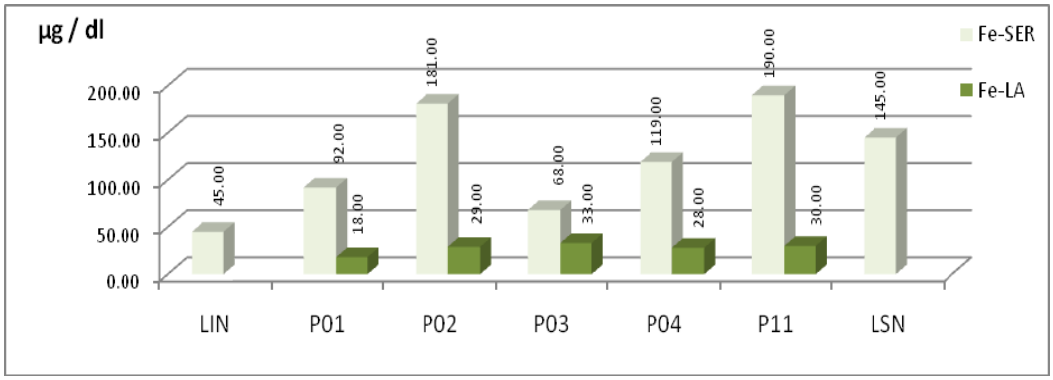

Fig. 3 - The variation of iron in serum (Fe–SER)
and ascitic fluid (Fe–LA) in cirrhotics – females

Normally, the level of serum bilirubin is a balance between the hepatic input and output of the pigment; it reflects the intensity of jaundice and the total quantity of biliary pigment in the organism (Mody et al., 2000).

The appearance of jaundice in the progress of cirrhosis depends most frequently upon the extension of the hepatocytic necroses and the decrease of the functional remaining hepatocytic mass (Grigorescu, 2004); when this happens, the jaundice mechanism is explained through abnormalities in collection, transport

and metabolize the bilirubin, lack of UDP – glycuroniltransferase and canalicular obstructions (Roşoiu, 2002).

BT values in serum exceed LSN in 80% of the patients, the highest value being 6,30 mg/dl; in LA, the BT values do not exceed those in serum.

More than 5 mg/dl of bilirubin means poor prognosis.

GGT in LA has values between 6 – 61 UI/L (table 4); 61 UI/L is found in the patient with the highest value of GGT in serum (303 UI/L, which exceeds LSN 3.4x times). GGT in serum has values over LSN in 60% of the patients.

ALP in LA is between 7 – 45 UI/L (it has values lower than LIN in serum and does not exceed LSN in serum), while in serum it exceeds LSN in 66.6% of the patients (table 4).

Table 4 - Means, standard deviations, minimum and maximum values for the analytes determined in serum and ascitic fluid in cirrhotic patients (the *excreto-biliary syndrome*)

	Serum			**Ascitic fluid**			**t. Student**
Analyte	**M ± DS**	Min.	Max.	**M ± DS**	Min.	Max.	**Value of "p"**
BT (mg/dl)	2.8 ± 1.5	0.60	6.30	0.87 ± 0.47	0.20	2.0	$p < 0.05$
BD (mg/dl)	0.98 ± 0.68	0.10	2.40	0.23 ±0.13	0.10	0.6	$p < 0.05$
BI (mg/dl)	1.81 ±0.91	0.50	3.90	0.66 ±0.34	0.10	1.4	$p < 0.05$
GGT(UI/L)	132.52 ±95.49	36	303.3	29.82 ± 17.61	10	61	$p < 0.05$
ALP(UI/L)	104.4 ± 36.68	56	180	22.74 ± 9.41	13.3	45	$p < 0.05$

The hepatoprive syndrome appears in the advanced stages of the disease, having predictive value with regard to the survival.

The serum albumin concentration reflects the functional status of the hepatocytes and is useful in tracking the progression of liver disease (Roşoiu and Verman, 2008).

The albumin decrease is accentuating when the ascites occurs due to the increase of the venous pressure in the portal system over VN 5- 10 mmHg or 10-14 cm H_2O manometric measured column.

The ratio between the ascitic fluid and serum albumins is less than 0.41, which excludes the malignant ascites and confirms the cirrhotic disease.

In all the cirrhosis cases that we studied, serum **CHE** is below LIN (table 5), in LA, the values are between 105 – 637 UI/L.

The progressive decrease of pseudocholinesterasis, parallel with the decrease of albumin, signifies the formation of advanced and irreversible lesions, the respective analytes being significant for the prognostic (Zălaru et al., 2001).

The level of CHE decrease is directly proportional to the intensity of hepatic lesions and helps the evaluation of the remaining functional hepatocytic mass.

Table 5 - Means, standard deviations, minimum and maximum values for the analytes determined in serum and ascitic fluid in cirrhotic patients (*the hepatoprive syndrome*)

	Serum			**Ascitic fluid**			**Student Test**
Analyte	**M ± DS**	Min.	Max.	**M ± DS**	Min.	Max.	Value of " p"
ALB (g/L)	26.6 ± 5.57	17	36	6.54 ± 2.19	1.20	10	$p < 0.05$
CHE (UI/L)	2708.8 ± 767.7	1830	4451	393.2 ± 157.85	105	637	$p < 0.05$

The normal liver is a well equipped organ in regard of enzymatic and non enzymatic antioxidants ; the protective and plasma purification functions are executed by the hepatic parenchymal cells (65% of the structure) and by the non-parenchymal cells of the reticuloendothelial system (Kupffer cells, which represent 35% of the structure) (Grigorescu et al., 2004).

TAS is an indicator of the non enzymatic antioxidant molecules on plasmic and hepatic level (alpha tocopherol, considering the composition of the standard which served to establish the calibration curve).

For seric TAS we obtained an average value of 1.16± 0.12, and for TAS is LA the average value is 1.53± 0.08 (neither in serum nor in LA we did not obtained values higher than the superior limit of the reference range).

Persistent low values of TAS demonstrate the overcoming of the body "fighting" capacity compared to the excess of harmful free radicals, poor antioxidant defensive conditions favoring the progression of necrosis, apoptosis, fibrosis, proliferation-regeneration processes thus creating the preliminary state for the advanced stages of the cirrhotic disease.

The first indication of an increased immunological reactivity in chronic hepatic disease is given by the electrophoresis of plasmatic proteins, which underlines the higher level of globulins, γ – globulins, especially.

The A/G ratio is in the pathological area characteristic to cirrhosis, both for the values in serum, and those in LA (figure 4).

When the albumin synthesis decreases, the A/G index decreases.

The serum level of globulins indicates the presence of inflammation (Buligescu et al., 1999); the increase of gamma-globulins expresses quite accurately the activation of the hepatic mesenchyme, this increase being directly proportional with the inflammation degree of the mesenchyme.

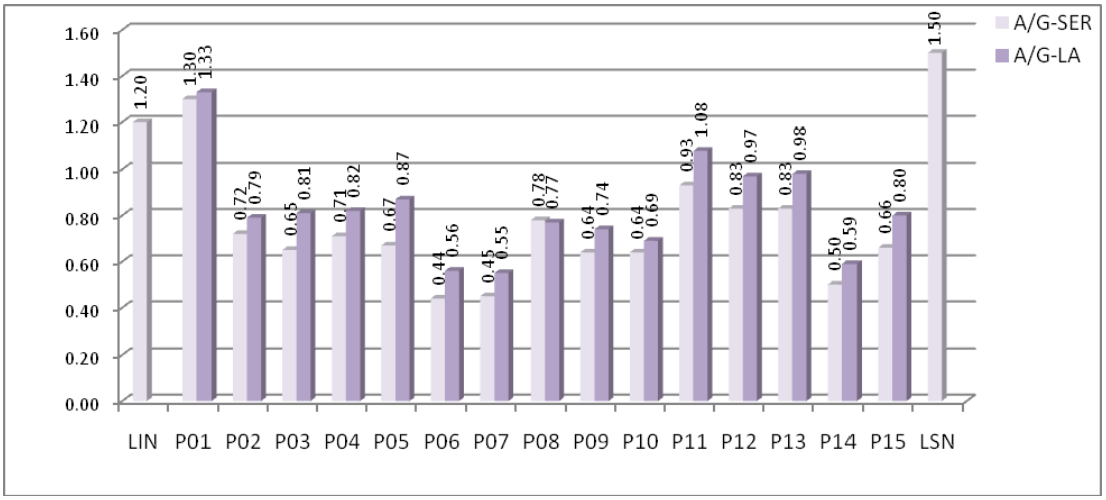

Fig. 4 - A/G ratio variation in serum (A/G-SER) and ascitic fluid (A/G-LA) in cirrhotic patients

In cases of cirrhosis, an adequate concentration of the osmotic pressure can not be maintained (through lack of albumin synthesis), which causes ascites (Friedman et al., 2003).

Using the threshold of 45 – 48 mg/dl, which differentiates the cirrhotic ascites (with hepatic cause) from the malignant ascites (with peritoneal cause), we observe that all the obtained values for cholesterol from LA correspond to the cirrhotic ascites (figure 5).

60% of the patients present serum CT below LIN, and the value higher than LSN (6.7%) is found in a female patient with ethylic cirrhosis.

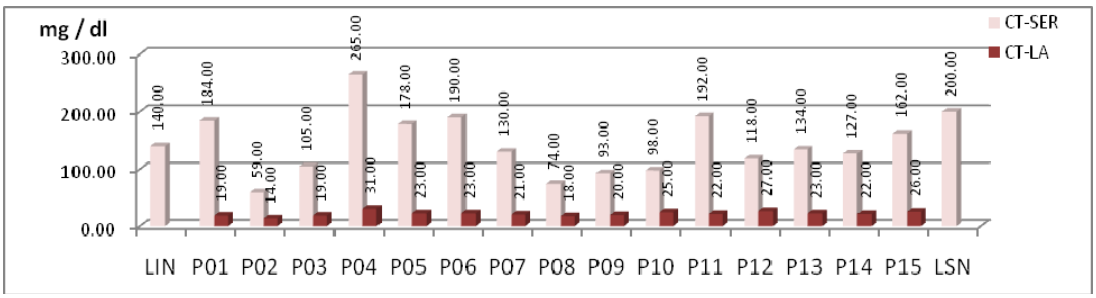

Fig. 5 - Total cholesterol variation in serum (CT-SER) and ascitic fluid (CT-LA) in cirrhotics

Triglycerides from ascitic fluid have lower values than the serum ones (figure 6 and figure 7), a fact that excludes the chylous ascites which is caused by lymphatic obstruction (Ciurea et al., 2004), as well as the malignant ascites (the TG values are increasing)

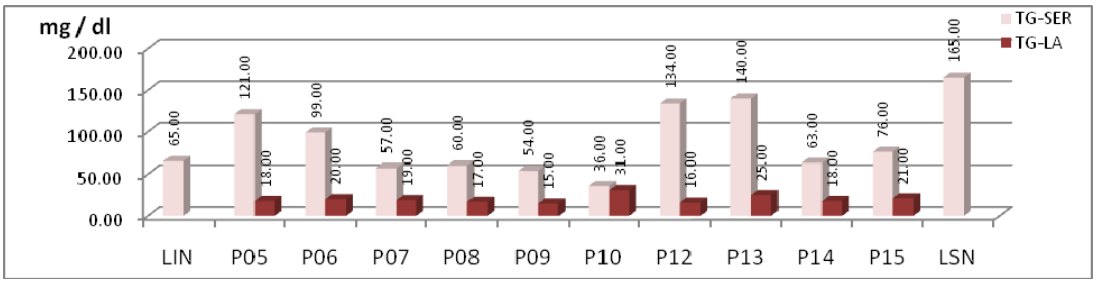

Fig. 6 - Triglycerides variation in serum (TG-SER) and ascitic fluid (TG-LA) in cirrhotics -males

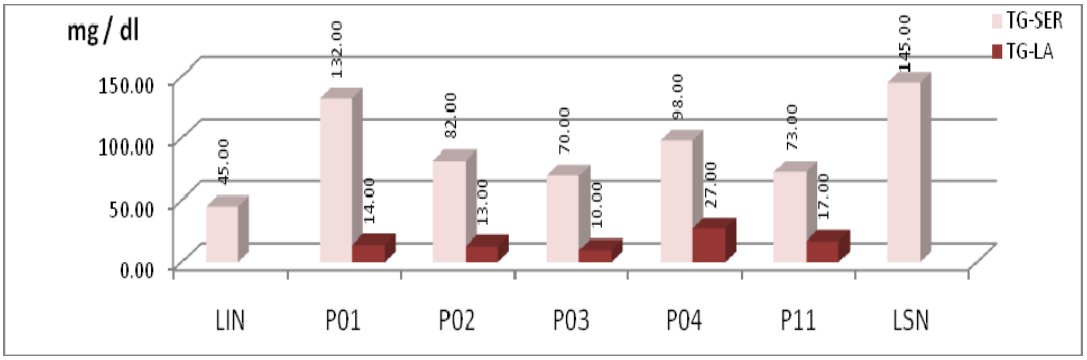

Fig. 7 - Triglycerides variation in serum (TG-SER) and ascitic fluid (TG-LA) in cirrhotics -females

The values of urea, creatinine and uric acid in ascitic fluid are similar to those determined in serum; between the means of these analytes there is no statistical significant difference (table 6) (their passing into the ascitic fluid is probably done through the sinusoidal trancapilar diffusion process).

The AcU level is predominantly high both in serum and ascetic fluid, which determines us to believe that the xanthine oxidase is highly active and that the superoxide radicals resulted under the enzyme action are in a higher proportion.

Table 6 - Means, standard deviations, minimum and maximum values for the analytes determined in serum and ascitic fluid in cirrhotic patients (*the renal function*)

	Hepatic cirrhosis - serum			**Value of "p"**	**Hepatic cirrhosis – ascetic fluid**		
Analyte	**M ± DS**	**Min.**	**Max.**	**Serum – ascetic fluid**	**M ± DS**	**Min.**	**Max.**
Urea (mg/dl)	45,0 ±24,67	17,0	96,0	**p > 0,05**	46,73 ± 28,96	20,0	103,0
♂	38,70 ±20,58	17,0	90,0	p > 0,05	40,40 ± 23,66	20,0	101,0
♀	57,60 ± 29,65	26,0	96,0	p > 0,05	59,40 ± 37,07	24,0	103,0
Creatinine (mg/dl)	1,09 ± 0,33	0,6	1,7	**p > 0,05**	0,96 ± 0,28	0,6	1,7
♂	1,10 ± 0,35	0,6	1,7	p > 0,05	0,96 ± 0,22	0,6	1,3
♀	1,08 ± 0,31	0,6	1,4	p > 0,05	0,98 ± 0,42	0,6	1,7
Uric Acid (mg/dl)	6,2 ± 1,67	4,1	10,0	**p > 0,05**	5,55 ± 1,33	3,6	9,0
♂	6,11 ± 1,81	4,1	10,0	p > 0,05	5,82 ± 1,41	4,3	9,0
♀	6,38 ± 1,54	5,0	8,9	p > 0,05	5,02 ± 1,09	3,6	6,4

Conclusions

1. The increase of AT is lightly correlated to the degree of extension of hepatocellular necrosis, and have a low prognosis value. The normal values of AT do not exclude the hepatic disorder. Because the absolute values of AT do not correlate exactly with the severity of the hepatic tissue lesion and with the prognosis, we consider that the dynamic seriate AT determinations are the most useful when evaluating the structural and functional integrity of the hepatocyte.

2. Normal valued ALT is more numerous in cirrhotic patients than normal valued AST, which is a bilocular enzyme, and in cirrhosis the mitochondria are subject to structural alterations, which cause the decrease of their functions.

3. The de Ritis ration is ascetic fluid reveals the presence of hepatocyte necrosis, although the determined values of AT in ascetic fluid are normal.

4. The liver failure in cirrhotics is also expressed through the low values of serum cholesterol, and the concentration of cholesterol in ascitic fluid confirms or infirms the hepatic cirrhosis.

5. The values of gamma globulins in serum and ascitic fluid do not differ significantly differ from a statistical point of view, their level indicating the advanced grade of mesenchymal inflammation, which is characteristic to hepatic cirrhosis.

6. The A/G ratio is higher for the values obtained in protein electrophoresis in ascitic fluid than for those in serum, but the mean of the A/G values does not significantly differ statistically (the graphic has approximately the same course); the protein electrophoresis in ascitic fluid may substitute the serum one in establishing the diagnosis and prognosis.

7. The renal function in cirrhotic patients can be investigated by determining the urea, creatinine and uric acid only in ascitic fluid but not in serum.

8. The sensitivity to the oxidative stress increases as the hepatoprive and the excreto-biliary syndromes are more clearly expressed (TAS in serum registers the lowest values for the patients which also have a better expressed parenchymal decompensation).

References

Başa M., Roşoiu N., Dumitru G., Rus A.S., Roşoiu R. D., (2010), - *Comparative biochemical serum-ascitic fluid study of hepatic ascitic cirrhosis*, Archives of the Balkan Medical Union, 45(2), 105-110.

Buligescu şi col., (1999) - *Tratat de HepatoGastroEnterologie*,vol.II, Ed.Medicală Amaltea, Bucureşti.

Ciurea T., Săftoiu A., (2004) – *Sindromul ascitic* in *Tratat de Hepatologie,* de Grigorescu M. Editura Medicală Nationalã, Bucureşti, 265-282.

Frances Fischbach, (2004), - *Chemistry Studies, A Manual of Laboratory and Diagnostic Tests*, Lippincott Williams & Wilkins, USA, 7, 316 – 455.

Friedman L.S., Martin P., Munoz S.J., (2003) – *Laboratory evaluation of the patient with liver disease*. In Zakim D., Boyer T., (eds), Hepatology Textbook of liver disease, 4th Edition, Elsevier Science (USA), 661-708.

Grigorescu şi col.,(2004) - *Tratat de Hepatologie*, Ed. Medicală Naţională, Bucureşti.

Henriksen J. H., (1995) – *Cirrhosis: Ascites and hepatorenal sindrom. Recent advances in pathogenesis*, J.Hepatol., (Suppl 1), 25-30.

Mody şi col., (2000), - *Biochimie Clinică*, Ed. All Educational,Bucureşti.

Rosoiu N., (2002), - *Biochimie Clinică – Capitole speciale*, Editura Muntenia & Leda, Constanţa, Romania.

Roşoiu N., Verman G. I., (2008), - *Biochimie Clinică*, Ed.Muntenia, Constanţa.

Zălaru M., Blaj Ş., Nedelcu D., (2001) *Ascitele,* Ed.Universitară "Carol Davila", Bucureşti.