Analysis of Enzymatic Systems in the Severe Hepatic Dysfunction

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Cirrhosis of liver, an irreversible and diffuse affection of the liver, is an important cause for morbidity and mortality on a national and international level, with a great impact on patients, on their families, on the sanitary system, on society and economy. The main objective of the study was the assessment of the enzymatic profile and argumentation of the important role of alcoholism has in patients with cirrhosis of the liver. We have performed and observational and prospective study which included 389 patients diagnosed with cirrhosis of the liver, admitted within the Institute of Gastroenterology and Hepatology from Iasi. On the studied group, the average age of the patients was 54.23±5.54 years, with the predominance of the males, representing 65.32% of the total of the patients included in the study. Using the Baveno IV criteria at the registration in the study, from the 389 patients, 152 were in the compensated cirrhosis of the liver stage (39.23%), and 60.77% in the decompensated cirrhosis of the liver stage (237) patients. From an etiologic point of view, 158 patients (40.68%) have presented viral etiology, 59.32% toxic (231 patients) out of which 23.36% viral and toxic etiology. The average CAGE result in patients with ethanol etiology was of 2.177, confirming alcoholism, a systemic affection by the complexity of clinical manifestations with common etiopathogenic basis. Ethanol etiology is predominant, an alarming fact being its presence under 40 years, the implementation of national programs addressed to the young and active population being useful, even necessary. The indirect serological markers may be correlated with fibrosis, but don't typically characterize the fibrogenic biological processes, mostly evaluating the hepatic function and not the metabolism of the extracellular matrix. It presents the advantage of decreased cost and technical facilities. In the patients included in the study, the average ASAT value was of 111.22±7.42 U/L, with limits between 46 and 179 exceeding the values of the healthy individuals ($p < 0,001$) by 3.53 times, and the ALAT value was of 64.65±6.12 U/L (limits between 39 and 198). In a period in which we are witnessing an explosive medical progress, the discovery of serological markers which observe as many criteria of an ideal marker would be of real support in the management of the cirrhosis of the liver. Using these markers could also have an anticipation value through monitoring in dynamics, optimizing the protocols of clinical studies and assessing the efficiency of therapeutic agents.

Keywords: ethanol, enzymatic markers, cirrhosis of the liver, Baveno IV staging

Although medical research from the last decades have evaluated from a macro to a micro degree, being dominated by the valuable discoveries from a genomic and molecular level, pathologies where modern medicine could not develop curative therapies still exist. Cirrhosis of the liver represents the tenth cause of death on a worldwide level according to recent statistics [1]. WHO data show that Romania holds the 2nd place in Europe (after the Republic of Moldavia) for mortality by cirrhosis of the liver (1st place in women). Moreover, it is mentioned that the number of deaths caused by cirrhosis (excluding the hepatocellular carcinoma) is evaluated at approximately 800000/year, more than 70% of the mortality cases by digestive diseases being caused by hepatic chronic affections and cirrhosis of the liver [2].

The main objective of the study was the assessment of the enzymatic profile and argumentation of the important role of alcoholism has in patients with cirrhosis of the liver.

Cirrhosis of the liver, an important public health issue, represents a complex, always current affection, both through the multitude of biochemical and physio-pathological processes, as well as through etiologic variability [3]. The precarious socio-economic status, alcoholism, chronic viral hepatitis, characteristics of contemporary lifestyle have lead to a real *epidemic* of cirrhosis of the liver in the young, active population. No matter the etiology, hepatic insufficiency development and hepatic portal hypertension are the major complications [4-9].

The liver is the center of some complex biochemical processes, the hepatic functional tests showing the particularity of the non-invasive evaluation of patients (in the daily practice there are numerous cases in which the patients refuse invasive explorations, sometimes in situations with vital risk) [7-10].

The Baveno IV consensus proposed a new staging model for cirrhosis of the liver which comprises four different stages depending on the characteristic clinical-imaging traits and the disease's prognostic, appreciating the annual evolution of these patients. Each stage is defined through the presence or absence of three major events which appear in the development of the disease: esophageal varices, ascites and superior digestive hemorrhage (HDS). The I and II stages correspond to the compensated cirrhosis of the liver, and the III and IV stages correspond to the decompensated one. In this vision the element which makes the difference between the compensated and decompensated cirrhosis of the liver is ascites [11].

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The prognosis in patients with cirrhosis of the liver is hard to anticipate but it usually depends on the frequency and significance of the complications [12].

Experimental part

Material and method

The study was performed within the Institute of Gastroenterology and Hepatology from Iasi, being an observational, prospective study. During the selection period, 426 patients with cirrhosis of the liver of different etiologies were evaluated in order to be included in the study. Of the 426 evaluated patients, 389 have fulfilled all the criteria and included in the study and 37 patients (9.52%) have presented criteria of exclusion: age over 75 years (5 patients), hepatic cellular carcinoma (9 patients), acute cardiac insufficiency/chronic cardiac insufficiency class II/IV NYHA (8 patients), other neoplasia (6 patients), obstruction of the superior cava vein (2 patients), mielo/ Lymphoproliferative diseases (3 patients), peritoneal carcinomatosis (4 patients). The patients included in the study were admitted in the hospital within July 01, 2010 - July 01, 2011 and ulterior monitored up until July 01, 2013. The cirrhosis of the liver diagnosis was established based on the clinical exam corroborated with the paraclinical investigations (biochemical and hematologic exam, abdominal ultrasound, superior digestive endoscopy, histological exam ± fibroscan. We did not intervene in the therapeutic conduct applied to the patients.

In order to perform this study we have used as a work instrument the clinical observation chart of the patient, executing special charts with the main parameters monitored in dynamics.

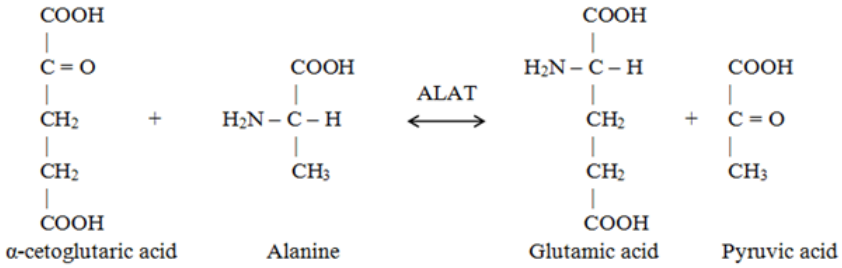
The patients were divided by groups of age, sex, area of origin, causes of admittance, stage and severity of the cirrhosis of the liver.

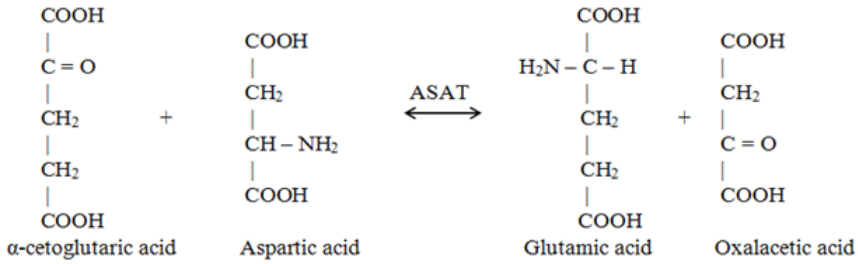
The hepatic enzymatic screening was performed by determining the values of the hepatic transaminases, ALAT (alaninaminotransferasis) and ASAT (asparatamino-transferasis) – enzymes which catalyze the reversible reaction of transfer of the amino group of aminoacids to α- cetoacids, determination of the alkaline phosphatases, lactate dehydrogenase, and dosing the alcohol consume markers (gamma-glutamyl transpeptidase, erythrocyte medium volume and high-density lipoprotein cholesterol – HDLc [13]. Standard testes in compliance with the IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) have been used [14].

Knowing the localization of the enzymes at the level of different cellular organelles is very important, both in understanding the metabolic processes, as well as for interpreting the laboratory results. The enzymatic equipment is different from one organ to another [15].

Alaninaminotransferasis (ALAT), an enzyme with predominantly hepatic distribution, is localized only on cytoplasmic level (unilocular) catalyzing the transamination from alanine to α-cetoglutaric acid, with the generation of the pyruvic acid and the glutamic acid (glutamic - pyruvic transamination) (table 1) [13,15].

Asparataminotransferasis (ASAT), localized on a cytoplasmic and mitochondrial level (bilocular), catalyzes the transamination reaction from the aspartic acid to the α-cetoglutaric acid by forming oxaloacetic acid and glutamic acid [13, 15], as glutamic-oxaloacetic transamination ecuations:



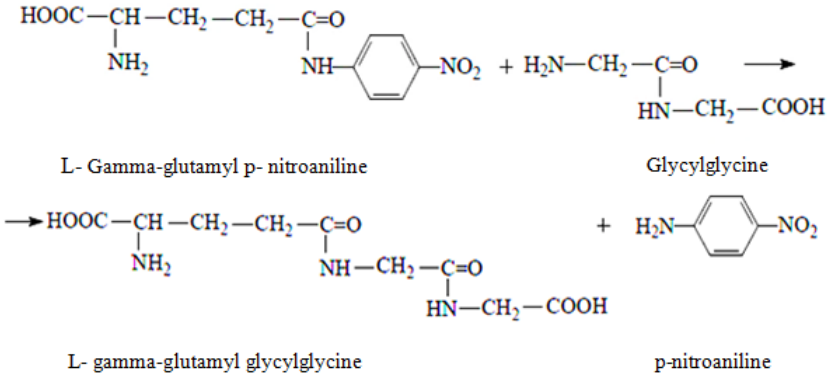


The serum concentration of these lesional enzymes varies depending on several factors: the number of hepatic cells affected the severity of damage on each hepatocyte, damage speed, speed of elimination from the serum (t 1/ 2) of the respective enzymes.

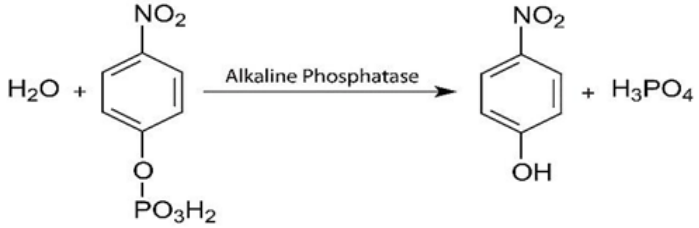
Associated to enzymatic individual growths *variations of the ratio between them interfere*. Thusly, the Ritis rapport (ASAT/ALAT) is modified depending on the etiology of the cirrhosis of the liver.

Gamma-glutamyl transpeptidase (GGT), ubiquitous enzyme, insures the trans-membrane transport of amine acids and peptides. At a cellular level, it is predominantly situated in the membrane, its distribution being specific at the level of the same locus where alkaline phosphatase is also present. The serum activity increase of GGT is parallel to the quantity and duration of the alcohol abuse. This enzymatic behaviour allows the differentiation between heavy drinkers and occasional drinkers. The high serum values of the enzyme plead for the association with chronic hepatic suffering. With a broadly appreciated specificity, 50-100%, and a sensitivity between 25-65%, GGT can be considered as an enzyme which susceptibly and specifically reacts both during the ingestion of small quantities of alcohol and in chronic alcoholism, having a marker role [16].

The determination of GGT activity is performed using L-γ- glutamyl -3-carboxy-4- nitroaniline [15], as underlayer enzymatic mechanism of gamma-glutamyl trans-peptidase:



Alkaline phosphatase, enzyme included in the intermediary metabolism enzyme family, is a esterase which acts at alkaline *p*H (*p*H = 8.6), performing the hydrolysis of phosphate esters [13,15], as enzyme mechanism of alkaline phosphatase:



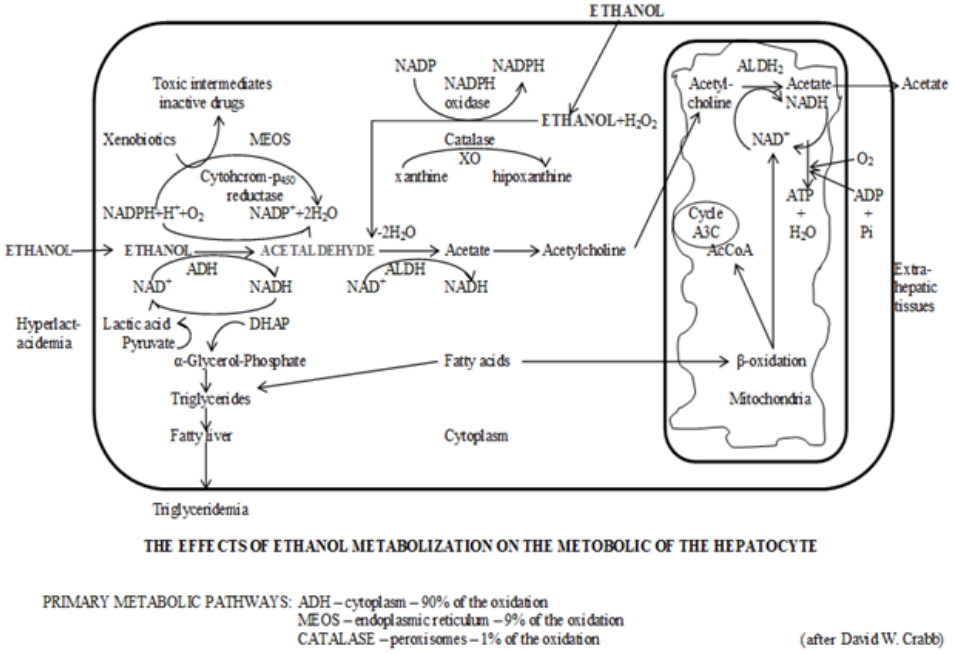
High density lipoprotein cholesterol (HDLc) transports excess cholesterol from different tissues in the liver. Under the influence of even small doses of alcohol, through an enzymatic induction process, this lipoprotein increases in the bloodstream, being generated at a hepatic level. Cirrhosis of the liver leads to a decrease in HDLc levels [17].

The metabolic consequences of alcohol consumption have cascading effects, reflecting the nature of the molecule and its degradation. It is small and easily miscible in lipids and water, thusly being rapidly distributed in the tissues. Its metabolization is compulsory, as another excretion method does not exist (with the exception of small quantities eliminated through respiration and urine). Also, it is not subjected to feedback control. The degradation happens especially at a hepatocyte level. In the same manner as fuel, alcohol supplies 29.5 kJ/6 to 7kcal/g, but the process is dependent on oxygen, resulting in the undesirable production of hydrogen ions. During the metabolic process from alcohol to acetaldehyde and further from acetaldehyde to acetate, the nicotinamide adenine dinucleotide (NAD) acts as a hydrogen acceptor and the resulted $NADH^+$ alters the redox state of the cell, with multiple metabolic effects [18, 19].

Some of the effects of alcohol on the hepatocyte depend on dose and type of ingestion, acute or chronic. Alcohol degradation is mainly catalyzed by alcohol dehydrogenase, a non-microsomal enzyme, and only a small part is controlled by the microsomal enzyme oxidizing system [20].

There are numerous pieces of evidence that the major biochemical lesion produced by alcohol is acetaldehyde accumulation. This molecule produces a vicious circle, disturbing exchanges at a mitochondrial level, essential for the elimination (disposal) of reduction equivalents. The peroxidation of membrane lipids increases and the glutathione decreases, these being decisive factors in the production of cellular deterioration [21].

Three enzymatic systems are involved in alcohol oxidation (fig. 1):



THE EFFECTS OF ETHANOL METABOLIZATION ON THE METOBOLIC OF THE HEPATOCYTE

PRIMARY METABOLIC PATHWAYS: ADH – cytoplasm – 90% of the oxidation
MEOS – endoplasmic reticulum – 9% of the oxidation
CATALASE – peroxisomes – 1% of the oxidation

(after David W. Crabb)

Fig. 1. The effects of ethanol metabolization [21]

- alcohol dehydrogenase (ADH) located in the cytoplasm (the soluble fraction of the cell);
- the microsomal ethanol oxidizing system (MEOS), located in the endoplasmic reticulum;
- catalase, located in peroxiredoxins.

Results and discussions

The average age of the entire patient group was 54.23±5.54 years (ages between 22 and 75 years), comparable to the data from specialty studies (fig. 2), which mention a high incidence of cirrhosis of the liver around the age of 55 years [22]. From a patient distribution by age decade's point of view, within the studied group an increase in incidence is noticed after the age of 40 years, the maximum number of cases being encountered in the age group of 40-59 years (202 patients).

From a sex distribution point of view of the 389 patients with cirrhosis of the liver (fig. 3), 252 were male (65.32%) and 137 were female (34.68%). It was noticed that in the studied group the male sex is more frequently affected, with a male/female ratio of 1.95:1. This case distribution is consistent with the data from specialty literature which shows a higher prevalence of cirrhosis of the liver in males, who represent approximately 2/3 of the cases in different studies compared to females [23].

The relationship with the area of origin of the patients shows that, within the studied group (fig. 4), 234 cases come from rural areas (62.68%) and 146 of the cases from urban areas (37.32%), the lower socio-economic status having a major impact on the prevalence and severity of cirrhosis of the liver [24].

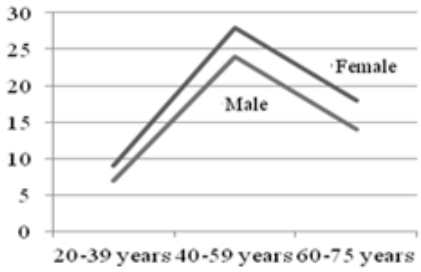


Fig. 2. Structure by age decades

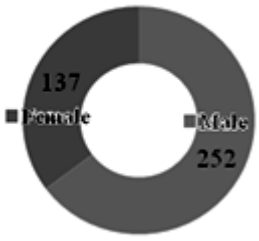


Fig. 3. S distribution

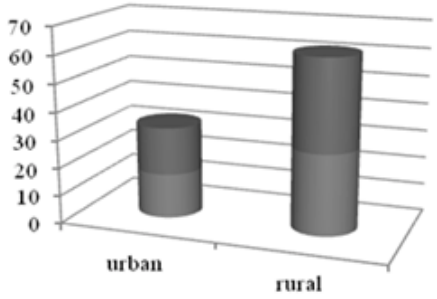


Fig. 4. Distribution by area of origin

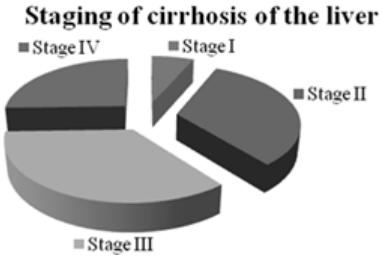


Fig. 5. Patient classification by stages according to the Baveno IV classification

Referring to etiology (figs. 5 and 6), 40.68% (158 patients) from the studied group was exclusively viral, 59.32% toxic (231 patients) out of which 23.36% toxic and viral etiology. Regarding toxic etiology, the ratio was higher in males than females (3.89:1 ratio). Out of the 158 patients (40.68%) with viral etiology, a higher prevalence of viral etiology C is noticed (91 cases, 23.38%) in comparison to viral etiology B (35 patients, 9.12%), coinfection B + D (21 patients, 5.23%) and B+C and B+C+D coinfections being encountered in a low number of cases (11 patients, 2.95%).

In the patients included in the study, the average ASAT value (fig. 7) was of 111.22±7.42 U/L, with limits between 46 and 179 exceeding the values of healthy individuals ($p < 0.001$) by 3.53 times, and ALAT of 64.65±6.12 U/L (limits between 39 and 198) exceeding the normal values by 3.32 times ($p < 0.001$).

GGT determination showed moderately high values (fig. 8) and intensely high values in both sexes (211 males, 83.73% of the total number of analyzed males, respectively 120 females, 87.59% of the total number of the females tested).

A decrease in high density lipoprotein cholesterol (HDLc) in cirrhosis of the liver is noted by many authors. An analysis of HDLc values has shown a predominance of cases with normal and low values of the total cholesterol (table 5).

Macrocytosis, assessed by determining the average erythrocyte volume at the time of study admission of the patients, was detected in approximately ¾ of the patients (table 6).

The average CAGE score in patients with ethanol etiology was of 2.177, confirming alcoholism, systemic disorder through the complexity of clinical manifestations with a common etiopathogenic basis [25, 26].

In the observation chart the presence of comorbidities was noticed (fig. 9), classified by major disorders, apparatuses and systems. A percentage of 50% is allocated

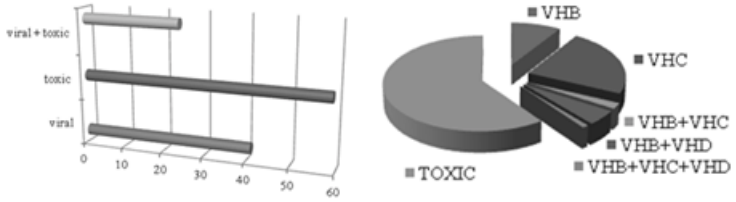


Fig. 6. Group structure by etiology

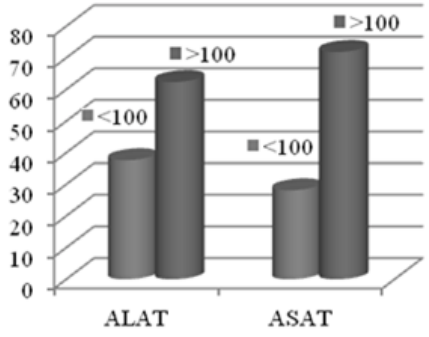


Fig. 7. ALAT and ASAT values in studied patients

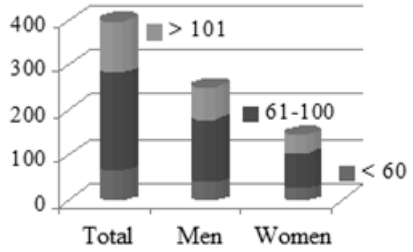


Fig. 8. Group structure by sex for individual GGT values in the studied group

Table 5
HDLc VALUES IN THE STUDIED GROUP

Values (mg/dl)	No of cases	%
Normal (45-75)	129	33.16
Low (< 45)	249	64.02
High (> 75)	11	2.82

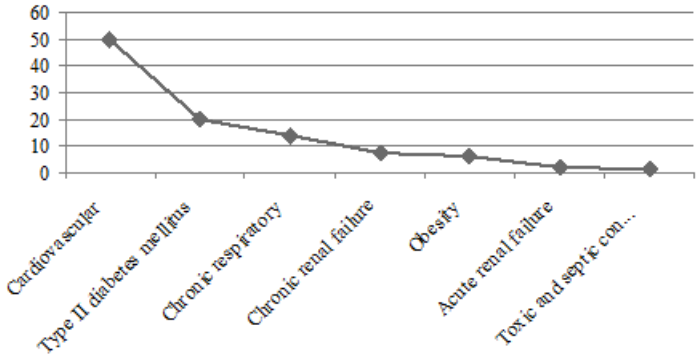


Fig 9. Associated comorbidities

to comorbidities that represent cardiovascular pathology, 20% association to type II diabetes mellitus, 14% representing chronic respiratory pathology, 7% associated to chronic renal failure, 6% different stages of obesity. A low percentage was objectified in the case of sepsis.

Conclusions

Biochemical markers were efficient especially in patient categories considered to have a high risk regarding invasive procedures. Using non-invasive markers is cost-efficient and may optimize the management of patients with cirrhosis of the liver by reducing the risk of decompensation and increasing the survival rate of these patients [27].

Ethanol etiology is predominant, an alarming fact being its presence under 40 years, *the implementation of national programs addressed to the younger population being useful, even necessary* [28, 29]. An interrelationship exists between alcohol consumption (quantity, duration) and the impact on the liver [30, 31].

The results of the study validate the Baveno staging system for cirrhosis of the liver, being proven as an efficient clinical instrument, accessible and reproducible for stratifying the risk of decompensation and death in patients who suffer from cirrhosis of the liver [32].

Cardiovascular affection in patients with cirrhosis of the liver is a frequent complication, which has an important contribution on the increase of morbidity and mortality. Monitoring the cardiac function in patients who suffer from cirrhosis of the liver represents an important component of their management and an important research direction.

Table 6
VEM VALUES IN THE STUDIED GROUP

VEM	No of cases	%
Normal values (80-94 μ^3)	104	26.73
High values (> 95 μ^3)	285	73.26

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