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LIVER INJURY IN CHRONIC ALCOHOLISM: CLINICAL, LABORATORIAL AND HISTOLOGICAL CORRELATION

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Liver cirrhosis is responsible for a large number of deaths in our country (Angelo, 1977) as well as in many other parts of the world (Popper, 1975). In Portugal, during the year 1975, cirrhosis was the second most common cause of death among individuals between the ages of 25 and 44 and the third between the ages of 45 and 64 (Angelo, 1977). The mortality rate due to this disease (Fig. 1) is higher in rural areas where the population is predominantly composed of peasants with a low standard of living and where wine production per capita is higher. The districts of Vila Real, Viseu and Guarda are those where the situation is more acute owing to the simultaneous occurrence of both factors (Angelo, 1977).

Most out patients seen in central hospitals, as well as those who are admitted, already display extensive liver damage by the time they first resort to medical advice. Generally the motivation that first leads the patient to present to hospital is due to clinical symptoms derived from the complications of alcohol induced damage to the liver such as ascites, haemorrhage, hepatic encephalopathy and psychiatric disturbances.

Several Portuguese authors studied patients admitted to hospital due to decompensation of cirrhosis (Monteiro 1961; Rosário et al, 1966; Correia et al, 1969), and alcoholic hepatitis (Alves et al, 1974) as well as patients undergoing alcohol withdrawal treatment in psychiatric units (Bordalo et al, 1976).

Following our previous work where we studied patients with latent cirrhosis (Saragoça, 1968; Saragoça et al, 1971), we have now focused our attention upon the clinical, laboratory and histological aspects presented by alcoholic patients selected from an ambulatory and heterogenous population mostly consisting of rural workers attending several consultations in 3 municipalities from the outskirts of Lisbon.

In these municipalities the wine production per capita is high and parallels the mortality rate attributable to cirrhosis, though the values observed in northern discricts are not attained (Angelo, 1977). In our present work, besides the study of the clinical, laboratory and histological alterations displayed by the heavy drinkers, we aimed at the detection of those patients with liver diseases of alcoholic etiology potentialy reversible after withdrawal of the drug.

Although we did not carry on an educational social program, we tried to alert these patients to the serious consequences of drinking, even though the progression of the liver disease towards irreversible damage and cirrhosis cannot always be prevented.

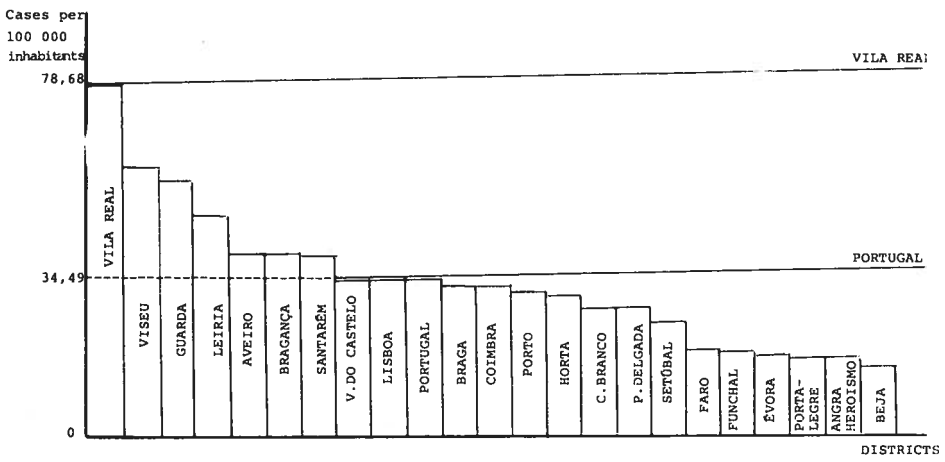

Fig. 1 — Mortality due to cirrhosis (1975, Men and Women)

MATERIAL AND METHODS

We selected for this work outpatients from the social welfare clinics and municipal hospitals from Cartaxo, Azambuja and Rio Maior.

The criterion of selection adopted was based on the daily ingestion of at least 160 g of ethanol for a period of over 10 years and the presence of hepatomegaly defined as the finding of a 2 cm palpable liver below the right costal margin at the mid-clavicular level.

Out of 2149 patients observed we selected 52 male individuals with ages ranging from 37 up to 79 years (Fig. 2).

Twenty eight patients presented a daily alcohol intake between 160 and 320 g while 24 drank over 320 g of ethanol per day. We questioned every patient's feeding habits concerning their daily caloric and alcohol ingestion taking special account of several alcohol-related signs and symptoms of the digestive tract.

The laboratory evaluation of liver function comprised the tests mentioned in Table I-A which were all performed in the same laboratory and by the same technician. In only 37 patients were the tests mentioned in Table I-B also performed since they were not available at the time this work began, which prevented their inclusion in the evaluation of the first 15 patients studied.

All the 52 patients were later notified to present themselves to the local municipal hospital in order to be submitted to another physical examination and to liver biopsy performed with a Menghini needle. Data concerning the alimentary and alcoholic habits were documented by the dietician of the group.

The blood samples taken in a clinic remote from the patient's home were not therefore simultaneous with the physical examination and were much closer in time to the liver biopsy. This fact has obvious implications in the results obtained since many patients after the first consultation undertook a period of alcohol abstinence, which in several cases lasted for three weeks, while others continued to drink.

The liver tissue fragments obtained by biopsy were fixed with formol and sent to the Pathology Institute of the Medicine Faculty of Lisbon. The tissues were then embedded in paraffin, cut in sections of 5 μ of thickness and stained with hematoxylin--eosin, using Perls' technique for iron and the Gordon-Sweat technique for reticulin.

Whenever a *ground-glass* appearance of the hepatocyte cytoplasm was found the tissue was also stained with orcein. The statistical analysis of the data was based on the Student's T Test.

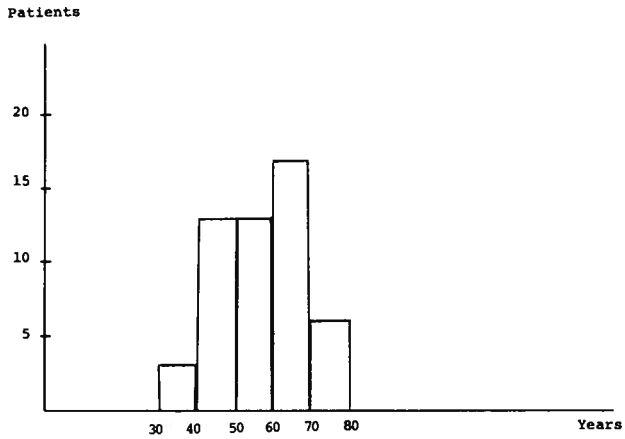

Fig. 2 — AGE (52 Patients)

RESULTS

The nutritional inquiry revealed that the caloric intake, apart those calories furnished by alcoholic drinks, ranged between 1000 and 5000 calories (average 2471,8) with a daily protein consumption between 75 and 165 g (average 89,8 g). The daily average of ethanol intake for the 52 patients was 292,7 g.

From a clinical point of view, we concluded that the majority of the patients presented with spondylitis (26,9%), 5 had arterial hypertension (9,6%), 3 headaches (5,8%), 1 had cardiac failure (1,9%), 1 sinusitis (1,9%), 1 eczema (1,9%), 1 knee trauma (1,9%), 2 respiratory tract infection (3,8%), 4 came to consultation in order to obtain a sanity card (7,7%), 1 was intoxicated with copper sulphate (1,9%) and 18 complained of dyspepsia (34,6%). Five patients had previous episodes of jaundice which was apparent at the time of observation in only two of them. Three patients (5,8%) had intestinal motility alterations in the form of atypical diarrhoea. Low grade ascites was found in two patients (3,8%)and epistaxis in 7 (13,5%), one of whom had had a gastrectomy (Billroth II) 11 years previously after an episode of digestive tract haemorrhage due to peptic ulcer.

The physical examination, besides the presence of hepatomegaly which was constant since it was one of the requisites for the selection of the patients, revealed the

following abnormalities: telangiectasias in 30 patients (57,7%), venous abdominal collateral circulation in 13 (25%), palmar erythema in 18 (75,4%), vascular spiders in 4 (7,7%), splenomegaly in 4 (7,7%) and jaundice in 2 patients (3,8%).

As for the laboratory tests we found out they were alterated in decreasing order of frequency, as follows: glutamic-oxalacetic transaminase (SGOT) (50%), alkaline phosphatase (42,3%), albumin (40,0%), glutamic-pyruvic transaminase (SGPT) (34,6%) and prothrombin time (13,5%). Raised conjugated bilirubin was the least frequent abnormality (5,8%). Table 2 shows the mean values obtained and the extremes of variation.

Table 1a

Liver function tests performed in 52 patients

Analysis	Normal Values
SGOT (Reitman-Frenkel)	up to 19 U/l
SGPT (Reitman-Frenkel)	up to 17 U/l
Bilirubin — 1 m	0,1-0,3 mg/dl
30 m	0,5-1,0 mg/dl
Quick time	80-120%
Alkaline Phosphatase (Bessey-Lowry-Brock)	13-38 U/l
Protein Electrophoresis (Grossman and Hanning)	
Total proteins	6,6-8,7 g/dl
Albumin	3,6-5,4
$\alpha 1$	0,1-0,4
$\alpha 2$	0,5-0,9
β	0,5-1,1
γ	0,9-1,7

Table 1b

Liver function tests performed only in 37 patients

Analysis	Normal Values
GGTP (Sasz) (U. V. 25.º)	6- 28 U/l
LAP	8- 22 U/l
CHE	3000-9300 U/l
BSP (Rosenthal-White)	0- 5% retention 45 min.

Table 2

Average values of the analysis. The extremes of variation and the percentage of abnormal values found in the patients

Analysis	Average values	Extremes of variation	Abnormal values	
			N.º Patients	%
SGOT	30,7 U	2-120	26	50
SGPT	21,6 U	5-89	18	34,6
Bilirubin 1'	0,2 mg	0,01-1,6	3	5,8
30'	0,6 mg	0,2-3,2	5	9,8
Alkaline Phosphatase	40,3 U	16-113	22	42,3
Quick time	95,8	44,2-113%	7	13,5
Albumin	3,64 g	2,83-4,67	21	40,4
*GGTP	117,5 U	5-593	22	59,5
*LAP	19,3 U	8-44	8	21,6
*CHE	5025 U	2383-7569	1	2,7
*BSP	8,2	0-20%	15	40,4

* Only 37 cases

In those 37 patients subjected to bromsulphalein (BSP) test and determination of serum levels of gammaglutamyl transpeptidase (GGTP), leucineaminopeptidase (LAP)and cholinesterase (CHE), we inferred that the GGTP was the most frequently alterated biochemical test (59,5%). In this group, SGOT presented abnormal values in 15 cases (40,5%) (average 19,6), alkaline phosphatase in 15 (40,5%) (average 37,4), glutamic-pyruvic transaminase (SGPT) in 9 (24,3%) (average 13,9), serum albumin in 9 (24,3%), conjugated bilirubin in 3 (8,1%)and prothrombin time in 6 (16,2%).

Concerning the interpretation of histological data, we aimed at the individual evaluation of each biopsy as a whole and its subsequent classification according to the chief histological diagnosis. (Table 3). Liver cirrhosis was found in 8 cases and steatosis in 18 biopses, 9 of which were classified as moderate (i. e. vacuoles were present in over 1/3 of the liver cells). Two biopsies displayed an histological pattern ascribed to alcoholic hepatitis without cirrhosis, i. e. degenerative hepatocyte alterations including Mallory hyalin bodies and polymorphonuclear infiltration.

The alterations were not specific in 14 patients and consisted of areas of necrosis in isolated hepatocytes, inflammatory infiltration of mononucleated cells and Kupffer cell proliferation. Parenchymal siderosis constituted the main finding in two cases while in another eight cases no alteration was to be seen or merely a large quantity of lipofuscin or a *ground-glass* appearance of the cytoplasm was reported. The parenchymal changes are presented in Table 4 and the portal ones in Table 5. The most meaningful alterations found were steatosis, fibrosis, inflammation, focal necrosis, centro-lobular collapse and *ground-glass* appearance.

Table 3

Chief histological diagnosis

Diagnosis	N.° Patients	%
Cirrhosis	8	15,4
Fatty Liver	18	34,6
Alcoholic Hepatitis	2	3,8
Non Specific	14	26,9
Haemosiderosis	2	3,8
Without Alterations	8	15,4

Table 4

Parenchymal abnormalities found in the 52 biopsies

Parenchymal Changes	N.° Biopsies	%
Steatosis	41	78,8
mild	17	
moderate	12	
severe	12	
Centrolobular Collapse	15	28,8
Mallory Bodies	6	11,5
Focal Necrosis	20	38
Vesicle Like Nuclei	11	21,5
Ground-Glass	13	25
Parenchymal Siderosis	12	23,1
Lipofuscin Increase	5	9,6
Focal Inflammation	29	55,7

Table 5

Portal changes in 52 biopsies

Portal Changes	N.º Biopsies	%
Fibrosis	37	71,1
mild	22	
moderate	12	
severe	3	
Inflammation	29	55,7
Duct Proliferation	3	5,7

Comparing the history and the physical examination with the histological data, we found out that only one patient with cirrhosis sought medical advice because of dyspepsia and this patient presented moderate ascites and jaundice. The other patients sought medical advice owing to copper sulphate intoxication (1 case), routine examination in order to obtain a sanity card (2 cases), knee trauma (1 case), spondylitis (2 cases) and lung disease (1 case).

The other patient with mild ascites and jaundice had lesions of alcohol induced hepatitis. As for the three patients with a past history of jaundice, the cause seemed to be acute viral hepatitis in one of them while the etiology could not be discovered in the other two cases.

Comparing alcohol intake and nutritional data with the histological patterns observed, in patients with or without cirrhosis, no statistically significant difference was detected between the average values of the calories, proteins and alcohol ingested per day in these two groups of patients (Table 6).

Table 6

Alcohol intake and nutritional data in patients with and without cirrhosis

	Cirrhosis	Without Cirrhosis
Alcohol P(t=1,7669, n=50)=0,0833>0,05	387,8 g	321,3 g
Proteins P(t=0,7609, n=50)=0,4503>0,05	83,9 g	91,1 g
Calories P(t=0,7151, n=50)=0,4779>0,05	2859,8 g	2640,0 g

According to laboratory data, those 19 patients with daily alcohol intake between 160 and 320 g presented a mean GGTP (gamma-glutamyl-transpeptidase) value of 94 U/l, while the enzyme concentration reached 142,03 U/l in those 18 patients whose daily ethanol intake exceeded 320 g. However, the disparity between the mean values of GGTP concentration in both groups was not statistically significant P (t=0,9575 n=35)= =0,3449)>0,05.

Taking the whole group of 52 patients and comparing the biochemical features with the histological presence or absence of cirrhosis, it was evident that no statistically significant difference could be found except for the mean concentration of conjugated bilirubin among patients with (8) or without (44) cirrhosis ($P<0,005$) (Table 7).

Table 7

Biochemical features in patients with and whithout cirrhosis

Biochemistry	Cirrhosis	Without Cirrhosis
SGOT		
P(t=1,0332, n=50)=0,3065>0,05	37,5	27,0
SGPT		
P(t=0,3656, n=50)=0,7162>0,05	22,25	19,6
AP		
P(t=1,6209, n=50)=0,1113>0,05	50,6	38,8
BSP		
P(t=1,9853, n=35)=0,0550>0,05	10,2	5,7
LAP		
P(t=2,7857, n=35)=0,0086<0,05	27,3	17,3
BIL 30'		
P(t=3,5499, n=50)=0,0028<0,05	1,001	0,502
GGTP		
P(t=2,8568, n=35)=0,0072<0,05	259,4	82,1

Statistical analysis (T test of student)

In those 37 patients submitted to BSP test and whose serum levels of (GGTP), (LAP) and (CHE) were evaluated, the only statistically important difference concerned the mean values of GGTP ($P<0,005$) and LAP ($P<0,005$) among the six cirrhotic and the thirty one non-cirrhotic patients.

DISCUSSION

The population we studied was made up of rural and factory workers and business men, of a similar socio-economic distribution to the population studied by Bordalo (Bordalo et al 1976).

Owing to the time limitation imposed upon one of us who was working in the province and to the lack of personnel and technical resources, the number of patients observed had to be restricted. We chose, therefore, for this study only patients with a daily ethanol intake superior to 160 g and with hepatomegaly though the latter does not necessarily indicate the presence of histological abnormalities. We did not pay attention to patients with a daily alcohol intake between 80 and 160 g although several authors consider it potentially dangerous (Pequignot 1961).

No female patient took part in our study because their alcohol intake was denied by the patients themselves and by their families.

As for the male individuals the situation was quite different considering that all patients admitted, in both interviews performed, heavy daily alcohol intake and their families confirmed this habit.

We suppose that the refusal of the women to admit alcohol abuse can be ascribed to social dogmas which condemn the females who drink.

As for the nutritional inquiry, we found that all but five patients had a daily caloric intake above 2000 calories not taking into account those furnished by the alcohol. In most patients the quantity of proteins, fats and carbohydrates was within the limits considered by the World Health Organization as reasonable.

On the other hand, no statistically significant discrepancy was detected between the feeding habits of the patients with and without cirrhosis.

Neither the clinical history nor the physical examination showed any correlation with the morphological features observed and this fact can be ascribed to the particular

characteristics of the population studied, mostly consisting of patients with few symptoms and signs of chronic liver disease.

From the biochemical point of view, besides the routine liver function tests, we also performed other enzyme estimations not usual in our current practice such as the gammaglutamyltranspeptidase (GGTP). This enzyme catalyses the transfer of glutamyl radicals from the peptides where they originally exist to other peptides and to L--aminoacids (the glutathione acts as substrate) and has been the subject of several reports which proved its production in various liver diseases.

This is the most frequently disturbed enzyme in toxic liver diseases and in drug addicts (Pateland 1975). The determination of serum levels of GGTP has been the subject of increased attention in alcoholic individuals since the concentration of this enzyme is raised in 75% of heavy drinkers. This test has also been used as a screening test for the evaluation of such patients.

The activity of GGTP is raised as long as the patient continues to drink while abstinence is followed by a decrease in activity according to an exponential law with a half life of 5 to 17 days. The enzyme activity measurements have been, therefore, successfully employed for the control of patients attending withdrawl programmes in specialized recuperation centers (Lamy et al 1975).

In our series the gammaglutamyl transpeptidase (GGTP) was raised in 22 out of 37 patients (59,5%). This percentage is a bit lower than that reported by other authors but this can be attributed to the fact that some patients stopped drinking just after the first consultation. The lapse of time between this first consultation and the taking of the blood samples was responsible for the return of the enzyme serum activity to normal.

No statistically significant difference between the average values of GGTP in both groups of individuals with diverse alcohol intake was recorded in our patients. A statistically significant difference between GGTP average values in patients with and without cirrhosis has been found.

For leucinoaminopeptidase (LAP) values a statistically significant difference between cirrhotic and non-cirrhotic patients was observed though the abnormal values of LAP were less frequently detected than the GGTP ones, which is in accordance with data published by other authors (Levinson et al 1974).

Cholinesterase is a liver-synthesized enzyme and a fall in its concentration implies extensive damage of the liver since the functional reserve of this organ is quite large. In serious forms of hepatitis the enzyme activity is, therefore, very low which connotes a bad prognosis. In spite of this we decided to include it in our study since it is inhibited by a variety of insecticides, carbamates and organophosphates (Smith 1974) to which our patients were often exposed. In only one patient, who also had contact with insecticides, the serum level of the enzyme was found to be low.

Comparing our histological results with those from other groups of chronic alcoholics, we observed that the incidence of cirrhosis in our patients (15,4%) is similar to that reported by Lelbach in 1976 and by Christofferson and Nielsen in 1972 from their study on 330 chronic heavy drinkers (16%). The incidence of steatosis (78%) was a bit lower than that reported by Christofersen but similar to the incidence found by Bordalo (76,6%).

The low number of cases of alcoholic hepatitis in our serie (3,7%) can be explained by the fact that the patients were ambulatory and sought medical advise for reasons other than their alcohol ingestion. The time which elapsed between the initial observation and the biopsy could have also contributed to the regression of this type of histological pattern.

It was not possible to investigate immunologically the ground-glass features of the hepatocyte cytoplasm, which were detected in 13 out of 52 biopsies (25%), from the point of view of the hepatitis surface antigen; however, the orcein stain was negative.

As reported in the literature, these alteration can be ascribed to contact with several drugs and toxins and are due to reticuloendothelial system hypertrophy (Wincker et al 1976; Thomsen et al 1976).

Owing to this fact we undertook a systematic inquiry in order to investigate the sort of toxins to which the patients were exposed and which could be responsible for the above mentioned histological features. The results of this work will be published subsequently.

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