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THE RELATIONSHIP BETWEEN ALCOHOLIC LIVER DISEASE AND NON-ALCOHOLIC LIVER DISEASE WITH OBESITY, DIABETES AND PROTEIN MALNUTRITION

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THE RELATIONSHIP BETWEEN ALCOHOLIC LIVER DISEASE AND NON-ALCOHOLIC LIVER DISEASE WITH OBESITY, DIABETES AND PROTEIN MALNUTRITION

By Maytham Jalil Fadheel FADHEEL

June 2022

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ABSTRACT

THE RELATIONSHIP BETWEEN ALCOHOLIC LIVER DISEASE AND NON-ALCOHOLIC LIVER DISEASE WITH OBESITY, DIABETES AND PROTEIN MALNUTRITION

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In patients undergoing bariatric surgery, the prevalence of NAFLD ranged from 37 to 91%, with the increase in this prevalence being linearly proportional to the increase in BMI, with the presence of central obesity. Alcoholic hepatitis is due to major liver damage with inflammation and destruction of liver cells; appears more abruptly and can be very serious. Patients typically present with yellowing (jaundice), malaise, nausea, vomiting, abdominal pain, and fever. It is important to highlight those numerous studies have been carried out with a proteomic approach in tissue samples from animal models of obesity, which have provided relevant information to clarify the molecular mechanisms involved in this disease; however, it is necessary to consider the limitations of animal models in the interpretation of the studies and their extrapolation to obesity in humans. Finally, cirrhosis associates a significant destruction of liver cells with a marked scarring process (fibrosis) that significantly alters the architecture of the liver, compromising its functions. Initially, patients may have almost no symptoms, but if their evolution continues, they may present accumulation of fluids in the abdomen (ascites), digestive bleeding, behavioural disorders (encephalopathy) and even a liver tumor.

2022, 68 pages

Keywords: NAFLD, BMI, Lipid profile, ALT, GGT, Liver enzymes

ÖZET

ALKOLLÜ KARACİĞER HASTALIĞI İLE ALKOLSİZ KARACİĞER HASTALIĞININ OBEZİTE, DİYABET VE PROTEİN YETERSİZ BESLENME İLE İLİŞKİSİ

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Bariatrik cerrahi uygulanan hastalarda NAYKH prevalansı %37 ile %91 arasında değişmekte olup, bu prevalanstaki artış, santral obezitenin varlığı ile VKİ'deki artışla lineer orantılıdır. Alkolik hepatit, karaciğer hücrelerinin iltihaplanması ve yok edilmesiyle oluşan büyük karaciğer hasarından kaynaklanır; daha ani görünür ve çok ciddi olabilir. Hastalar tipik olarak sararma (sarılık), halsizlik, bulantı, kusma, karın ağrısı ve ateş ile başvururlar. Obezitenin hayvan modellerinden alınan doku örneklerinde proteomik bir yaklaşımla yürütülen ve bu hastalıkta yer alan moleküler mekanizmaları açıklığa kavuşturmak için ilgili bilgileri sağlayan çok sayıda çalışmanın altını çizmek önemlidir; bununla birlikte, çalışmaların yorumlanmasında hayvan modellerinin sınırlamalarını ve bunların insanlarda obeziteye yönelik çıkarımlarını göz önünde bulundurmak gerekir. Son olarak, siroz, karaciğer hücrelerinin önemli bir tahribatını, karaciğerin yapısını önemli ölçüde değiştiren ve işlevlerini tehlikeye atan belirgin bir yara izi süreci (fibrozis) ile ilişkilendirir. Başlangıçta hastalar neredeyse hiç semptom göstermeyebilir, ancak evrimi devam ederse karında sıvı birikimi (assit), sindirim kanaması, davranış bozuklukları (ensefalopati) ve hatta karaciğer tümörü gösterebilirler.

2022, 68 sayfa

Anahtar Kelimeler: NAFLD, BMI, Lipid profili, ALT, GGT, Karaciğer enzimleri

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Maytham Jalil Fadheel FADHEEL
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CONTENTS

LIST OF SYMBOLS

%	Percent
±	Plus-minus
°C	Degrees Celsius
dL	Deciliter
g	Gram
IU	International unit
kg	Kilogram
L	Liter
m^2	Square-meters
mg	Milligram
mL	Milliliter
mmol	Millimole
ng	Nanogram
U	Unit

LIST OF ABBREVIATIONS

ALD	Alcohol-induced liver disease
BMI	Body mass index
CLD	Chronic liver disease
FAS	Fatty acid synthetase
HbA1	Hemoglobin a1c
HCV	Hepatitis c virus
HDAC4	Histone deacetylase 4
HPR	Haptoglobin-related protein
MHO	Metabolically healthy obese
NAFLD	Non-alcoholic fatty liver disease
NAFSH	Non-alcoholic fatty steatohepatitis
NASH	Non-alcoholic steatohepatitis
PBMC	Peripheral blood mononuclear cells
TSP1	Thrombospondin 1
VLDL	Very low-density lipoprotein

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

Several studies from the twentieth century established a well-documented link between steatosis, cirrhosis, obesity, diabetes, and alcohol; however, Ludwig *et al.* published a Mayo Clinic study in 1980 that established the non-alcoholic nature of hepatic changes in steatosis and steatohepatitis, coining the term NASH ("Non-alcoholic steatohepatitis"). The condition known as chronic liver disease (CLD) is a leading cause of death and disability in the world. It causes liver failure, portal hypertension, encephalopathy, ascites, hepatorenal syndrome, and gastrointestinal bleeding owing to oesophageal varices, among other consequences (1). Osteoporosis and bone fractures are also at increased risk due to CLD. "Hepatic osteodystrophy" (López-Larramona *et al.* 2011) has been used as a broad term to describe the many bone changes that occur in chronic liver illness. Osteopenia, osteoporosis, and osteomalacia are all included in this category. Mineral density decreases, and bone microarchitecture weakens, which increases the risk of fragility fractures in patients with the first two of these conditions. Only a very small percentage of patients with CLD are diagnosed with osteomalacia defined by low bone mineralization. Predominantly in chronic cholestatic illnesses (PBC and PSC), BMD loss has been linked to an increased risk of fracture, which has been documented in both the early and late stages of cirrhosis. Many studies have shown that BMD abnormalities in liver illnesses of diverse origins and stages, including viral hepatitis, nonalcoholic fatty steatohepatitis (NAFSH), alcohol-induced liver disease (ALD), and hemochromocytosis, have been published in recent years (Hashimoto *et al.* 2019).

1.1 Objectives of the Study

- To assess the prevalence of Alcoholic liver disease and non-Alcoholic liver disease and associated risk factors in individuals undergoing medical follow-up in primary health care centres and Baghdad Teaching Hospital, Baghdad, Iraq during the period of March 2021 to February 2022.

- To Estimate the number of patients who are known to have liver disease and who were referred for specialized consultation.
- To assess the association between hepatic steatosis and meat consumption.
- To recognize the importance of ALD, its high prevalence, incidence and mortality.
- To identify the main risk factors and their contribution.
- To identify the main symptoms and signs of ALD.
- To recognize the main diagnostic methods of ALD, their characteristics, indications, advantages and disadvantages.
- Establish the best therapeutic regimen according to the patient's clinical condition.
- To identify the main future perspectives regarding the management of patients with documented or suspected ALD and NAFLD patients.
- To determine ALT Enzyme in ALD and NAFLD patients
- To determine total protein in ALD and NAFLD patients.
- To determine serum Albumin in ALD and NAFLD patients.
- To determine serum glucose in ALD and NAFLD patients.
- To investigate the relationship between serum total protein, Albumin, in patients and the control group.
- Assess and classify nutritional status through the assessment of body mass index and waist circumference.

2. LITERATURE REVIEW

2.1 Obesity

WHO defines obesity as a disease in which excess body fat can reach levels capable of affecting health? Excess fat results from successive positive energy balances, in which the amount of energy ingested is greater than the amount of energy expended. The factors that determine this imbalance are complex and include genetic, metabolic, environmental and behavioral factors. When this imbalance is perpetuated, obesity becomes a chronic disease (Albuquerque *et al.* 2017). To classify obesity, the WHO adopted a criterion based on BMI, which is defined by calculating body weight, in kilograms, divided by the square of height, in square meters (BMI = Kg/m^2), and also by the risk of associated mortality. For better classification, the WHO divided obesity into grade I when the BMI is between 30 and 34.9 kg/m^2, grade II when the BMI is between 35 and 39.9 kg/m^2 and grade III when the BMI is higher. than 40 kg/m^2 (Klatsky *et al.* 2017). The individual is considered morbidly obese when their BMI is greater than or equal to 40 kg/m^2 or BMI is greater than or equal to 35 kg/m^2 with the presence of comorbidities such as diabetes, cardiovascular diseases, arthritis, shortness of breath. Gallbladder disease, fatigue or disability (Kitahara *et al.* 2014). The diagnostic criteria for childhood and adolescent obesity are controversial, but in 2009 the General Coordination of Food and Nutrition Policy of the Ministry of Health of Iraq adopted the curves developed, which include curves of BMI from infancy to 19 years of age and the cutoff points for overweight and obesity are considered to be the 85th and 97th percentiles, respectively. Such curves are fundamental both for the diagnosis and for the evaluation of the patient's evolution during the treatment. It is only by viewing the child's graph that we can verify how significant small variations in weight and, consequently, in BMI can be. (Rolland-Cachera and Péneau 2011)

2.2 Pathological Anatomy and Pathogenesis

To a large extent, the pathogenesis of AH is oriented towards explaining the location and nature of the histological alterations. It is therefore logical to treat these two aspects of the disease together.

The centrilobular zone, or perivenular area, is where AH lesions originate in both humans and experimental animals (zone 3 of the acini). The usual reduction in oxygen tension from zone 1 to zone 3 may be exacerbated as a result of alcohol metabolism, according to one theory. Hepatocytes may be more vulnerable to hypoxia as a result of this, or the intensity may be high enough to cause hypoxic injury (Huang and Chen 2010). Zone 3 hypoxia may accentuate the effects of alcohol metabolism, whereas the low oxygen tension in the perivenular zones may be the real cause of the alcohol-induced redox shift. As the damage to the liver worsens, microcirculatory changes occur, reducing the amount of oxygen available. On the other hand, chronic administration of alcohol increases the permeability of cell membranes in the centrilobular zone. This was observed experimentally and in patients with alcoholic fatty liver, which could explain the increase in glutamic dehydrogenase in about half of alcoholics. Indeed, this enzyme is preferentially located in the centrilobular area.

Lately, attempts have been made to clarify this phenomenon by means of ultrastructural morphometry. Hepatocyte volume was shown to be significantly larger in the centrilobular area than in the periportal area in patients with and without alcoholic disease. However, the magnitude of hepatocyte enlargement in the centrilobular area was more prominent in alcoholic liver disease than in non-alcoholic liver disease. The average microtubule volumes, expressed for each hepatocyte, in both areas of the cases with alcoholic liver disease were significantly lower than those corresponding to the areas of non-alcoholic liver disease. These results indicate that a decrease in microtubules in the centrilobular area could play an important role in the genesis of hepatocyte alterations and explain the preferential centrilobular location in AH (Kourkoumpetis and Sood 2019).

Interpretation of liver biopsy samples: in EHA there are three objectives in the interpretation of liver biopsy samples:

Differentiate between alcoholic and non-alcoholic liver disease.

Determine the stage of ALD (Fatty liver, AH, cirrhosis).

Evaluate the possible evolution of liver injury by means of serial biopsies.

One of the reasons for this review has been that there is no universally accepted criterion for the histological diagnosis of ALD. Although none of the lesions considered to be characteristic of AH is pathognomonic of its etiology, their presence is very useful in the interpretation of a liver biopsy. Its absence does not exclude the alcoholic origin of liver disease, but caution is required before considering this etiology. The evidence of replication of the B virus in alcoholic patients with cirrhosis and eventually with AH, suggests that in a certain proportion of these patients the lesion is of viral aetiology (Rehm *et al.* 2021).

Treatment: Despite recent advances in understanding the pathogenic mechanisms of the disease, the mainstays of treatment remain alcohol abstinence and nutritional support. The tested drugs are still in the experimental stage.

Abstinence: Abstinence appears to have a beneficial effect on survival if done early in the disease. There is a point where the disease becomes irreversible; This is when survival is determined by major complications, such as portal hypertension with bleeding esophageal varices and ascites, rather than by hepatocellular injury. In these patients, abstinence represents very little because it is too late.

During the detoxification period, sedatives or tranquilizers are commonly used. A few years ago, diazepam or chlordiasepoxide were recommended, being preferable to use the oral or intravenous route (in acute cases) since the intramuscular route can generate poor absorption. However, it is important to keep in mind that the dose of any sedative should be reduced to between 25% and 50% of the usual dose, and they should not be administered to patients with encephalopathy. Once the patient has recovered from the acute illness, abstinence from alcohol is the most important factor determining survival time.

Nutrition: clinical and experimental research works on the use of an adequate caloric regimen in the treatment of AH, gave controversial results. Lately this has been an area of considerable activity. The metabolic effects of an enteral feeding formula have been studied in patients with moderate and severe AH, reporting its benefits, highlighting that enteric infusion does not have a negative effect on fluid balance or degree of encephalopathy. To determine if there is an improvement in survival and liver function, a controlled trial of parenteral nutrition has been performed in moderate and severe AH. The conclusions were: parenteral nutrition does not provide benefits in moderate AH; rapidly improves morbidity (liver tests) and probably liver function in severe AH; it does not improve early mortality and has no deleterious effect on encephalopathy or ascites. A multicenter prospective study has tested the infusion of insulin and glucagon in patients with acute AH, with promising results. Although these therapeutic modalities are not within our reach, we must remember that alcoholics develop deficiency pictures of various vitamins, especially thiamin and folic acid. Therefore, it seems prudent to administer to these patients a balanced, hypercaloric diet, rich in vitamins, mainly thiamin, B6 and folic acid, although we know that diet by itself does not prevent the progression of the disease.

Drugs: the pharmacological treatment of AH is mostly symptomatic and, in any case, empirical. Several initial tests suggested that corticosteroids could be useful due to their known effect in lowering serum bilirubin and increasing albumin. In addition, the pathogenic evidence that an abnormal immune response was processed to generate and maintain the disease, gave grounds for its use. However, the benefit was only for a small group of patients who had a severe form of AH (Das and Vasudevan 2005). This result was assumed to be due to a significant improvement in caloric intake, an indirect effect of prednisone on its ability to improve appetite and sense of well-being. Recently, methylprednisolone therapy has been tested in a group of patients with similar clinical characteristics, i.e., severe AH, with spontaneous hepatic encephalopathy. The results can be described as good, because there was a marked decrease in immediate mortality. As can be noted, steroid therapy would be indicated only in severe forms of the disease.

The hypermetabolic state that characterizes AH has given rise to the therapeutic use of an antithyroid drug. propylthiouracil. Its beneficial effects have been revealed in a decrease in mortality and recovery of liver function (Das *et al.* 2019). Some facts suggest that this drug would not act by decreasing the hypermetabolic state, but by decreasing the hepatic response to adrenergic agonists. Indeed, rats previously treated with propylthiouracil, or reserpine are less susceptible to hepatic necrosis caused by tetrachloride. The protective effect of both drugs could depend on the decrease in adrenergic activity. Reserpine could produce a decrease in catecholamines, while less adrenergic sensitivity could be expected with propylthiouracil, since adrenergic receptor sensitivity is increased in hyperthyroidism and decreased in hypothyroidism.

2.3 Obesity and Liver Disease

Obesity is a chronic disease that usually begins in childhood or adolescence and occurs as a result of an imbalance between energy intake and expenditure. Genetic and environmental factors are involved in its origin, which determine a systemic metabolic disorder that leads to an excessive accumulation of body fat. In current clinical practice, the most widely used criteria for the diagnosis of overweight and obesity, whether in children or adults, are based on the body mass index (BMI).

Epidemiological studies carried out in different countries show that 5-10% of school-age children are obese, in the adolescent population the proportion increases to 10-20% and in adults the obesity prevalence figures range between 7, 7% in Switzerland and 32.2% in the United States 1,2. Even more eloquent are the figures provided by the World Health Organization, which estimates the number of obese adults in 2005 at 400 million, while forecasting that in 2015 there will be around 700 million obese adults in the world 3, which indicates that obesity currently represents a major global health problem (De and Duseja 2021).

In recent years, clinical and epidemiological evidence has shown that obesity, in addition to being a common risk factor for various diseases, such as diabetes, cardiovascular disease and certain types of cancer, is associated with an increased risk of present liver disease due to fatty deposits not related to alcohol abuse (NAFLD) and contributes to the progression of liver diseases of different aetiologies, such as chronic hepatitis due to the hepatitis C virus (HCV). In this review, special emphasis will be placed on the existing clinical and epidemiological evidence linking obesity to liver disorders and on the pathogenic mechanisms of obesity-associated liver disease.

2.4 Obesity is Associated with Liver Disorders

In the classic study (Dhanda and Collins 2015), which was carried out in a population in northern Italy, found that 76% of non-alcoholic obese people and around 15% of non-obese people had ultrasound evidence of fatty liver. In subsequent studies, however, it was found that the prevalence of fatty liver varies considerably depending on the diagnostic method used, race and sex. Thus, in a study carried out on 2,287 inhabitants of different American cities, in whom the hepatic content of triglycerides was determined by means of a sophisticated method of proton magnetic resonance spectroscopy, it was observed that approximately one third of the population had hepatic steatosis and that this was more frequent in people of Latin American origin (45%) than in those of white (33%) or black (24%) race, as well as in men (42%) compared to women (24%). An interesting finding of this study was that the higher frequency of steatosis in the Hispanic population was due to a higher prevalence of obesity. A similar trend in relation to the higher prevalence of steatosis in men than in women has been observed in populations of Asian origin as well as small variations in body weight (between 1.3–2.5kg on average) are associated with significant variations in the ultrasound pattern of hepatic steatosis, 7 indicating that the degree of obesity influences the accumulation of fat in the liver.

Some epidemiological studies have indicated that increased serum concentrations of liver enzymes are a sensitive indicator of hepatic steatosis, and for this reason they have been used in population studies as a marker of liver damage. With this strategy, different population studies carried out in different geographical areas have observed that the higher the BMI, the higher the prevalence of elevated liver enzymes and that the presence of visceral obesity, measured by the ratio between the waist circumference and the hip, is a determining factor of the significant association between obesity and increased serum concentrations of aminotransferases (Polyzos *et al.* 2019).

Obesity has not only been related to the initial phases of NAFLD, but also to the risk of progressing to steatohepatitis and also to cirrhosis and hepatocellular carcinoma (HCC). In this sense, a study carried out in the United States and based on the first national health and nutrition examination survey found that hospitalizations or deaths related to liver cirrhosis were more frequent in obese people (0.81/1000 people per year) and in those who were overweight (0.71/1000 person-years) than in those with normal weight (0.45/1000 person-years), regardless of alcohol intake 11. On the other hand, a prospective population study that evaluated 900,000 American adults who were cancer-free at the start of the study in 1982 and who were followed for an average of 16 years showed, in both men and women, a correlation relationship between BMI and death from different types of cancer. Specifically, a BMI greater than 35kg/m2 was associated with a relative risk of death from HCC of 4.52 compared to people with a normal BMI (Hruby and Hu 2015). Along the same lines, a study conducted in patients transplanted for HCC also found that obesity was an independent predictor of HCC in patients with alcoholic cirrhosis and in patients with cryptogenic cirrhosis. The pathogenic association between obesity and HCC is unclear, but experimental evidence from animal models of obesity indicates that obesity-related metabolic disturbances, rather than cirrhosis per se, may be involved in the induction of hepatocarcinogenesis during obesity.

2.5 Obesity is an Etiological Factor of Non-Alcoholic Fatty Liver

A detailed review of the large population-based epidemiological studies indicates that obesity increases the risk of having elevated serum concentrations of liver enzymes by 2 or 3 times, while the risk of sonographic steatosis increases 3 times in overweight people and up to 15 times in the presence of obesity. All these data have been confirmed in different studies carried out in cohorts of patients with morbid obesity 15–18, in which it has been observed that the majority of these patients have hepatic steatosis (91%, range: 85–98%), about a third have histological signs of steatohepatitis (37%, range: 24–98%), of which 20-40% present an advanced stage of fibrosis and even cirrhosis in around 2%. The concept that obesity is an aetiological factor of NAFLD has recently been reinforced in view of the results obtained in longitudinal studies of cohorts of patients with morbid obesity, who have been studied metabolically and histologically after surgery. bariatric Weight loss in these patients correlated with improvement in metabolic disturbances, such as insulin resistance (IR) and serum concentrations of adipokines (Li et al 2016).

Although NAFLD is considered to be a slowly progressive liver disease compared to other chronic liver diseases, the probability of progressing to cirrhosis is increasingly being recognized. In this sense, different studies have evaluated the presence of metabolic risk factors in cohorts of cirrhotic patients and have found that the prevalence of obesity or diabetes, or both, in patients with cryptogenic cirrhosis was similar to that of patients with non-alcoholic steatohepatitis. (NASH), but significantly higher than in patients with cirrhosis of viral or alcoholic aetiology, indicating that cryptogenic cirrhosis may represent the final stage of NASH. The fact that patients with cryptogenic cirrhosis frequently develop obesity and post-transplant NAFLD supports the concept that obesity is an aetiological factor of NAFLD and that NAFLD can potentially evolve into advanced forms of liver disease such as cirrhosis. There is also clinical evidence indicating that the risk of HCC in patients with cirrhosis associated with NAFLD is similar to that of patients with alcoholic cirrhosis or chronic HCV infection. More specifically, another retrospective study found that 27% of obese patients with cryptogenic cirrhosis had HCC compared to 21% of those with HCV cirrhosis, indicating that the carcinogenic potential of obesity is similar to that of HCV in presence of cirrhosis.

In the last decade, numerous longitudinal studies have been carried out, with serial liver biopsies, whose main objective has been to evaluate the progression of fibrosis and the associated risk factors in cohorts of patients with NAFLD. Thus, (Rafiq *et al.* 2008) observed, after a mean follow-up of 11 years, that fibrosis progression was much more frequent in patients with steatohepatitis (8%) than in those with simple steatosis (0%). One of the factors that seems to be involved in the progression of fibrosis is obesity, since in another more recent longitudinal study it was shown that obesity was significantly more prevalent in patients with progressive fibrosis (86%) than in those in which fibrosis remained stable (27%) 30. In addition to obesity, the presence of diabetes has been related to an increased risk of fibrosis progression in patients with NAFLD. Finally, there are few data on the long-term prognosis of NAFLD, but in a study of 129 patients, with a mean follow-up of 13.7 years, it was found that patients with steatohepatitis had a significantly higher mortality rate than those with steatohepatitis. patients with simple steatosis, and that the most frequent causes of death are cardiovascular disease and advanced liver disease.

2.6 Epidemiology and Risk Factors

NAFLD cases have increased significantly, reaching a worldwide prevalence of 25%, ranging from 13% in Africa, 24% in North America, 30% in South America and almost 32% in the Middle East (Glassner *et al.* 2017). However, the different histological parameters used for the diagnosis of NAFLD as well as the different populations studied make it difficult to accurately assess the prevalence. Depending on the population and methods used, studies have shown results ranging from 10 to 35%, and in potential liver donors in the USA 20% were diagnosed with NAFLD, as well as in Korea, where 51% of donors had hepatic steatosis.

Less invasive studies using abdominal ultrasound such as the one carried out in Iraq showed a prevalence of NAFLD in 33% of men and 20% of women (Abdulghani and

AL-Abachii 2021). In India, a study (De and Duseja 2021) carried out with middle-aged and elderly individuals found a high frequency, representing 35.2% of the sample. In a meta-analysis involving 35,599 patients, the prevalence of NAFLD was verified in 59.67% of patients with type 2 diabetes mellitus (DM2), with results ranging from 29.6 to 87.1%. Recently, a meta-analysis, which analyzed data from more than 24 million individuals, found a higher risk of severe liver disease in individuals with T2DM.

Patients with NAFLD have a high prevalence of MetS, similarly, patients with MetS have a high prevalence of NAFLD, which is considered a hepatic manifestation of MetS. A cohort that analysed 3613 individuals with NAFLD found higher mortality in those with MS and the increase in the number of MS components was associated with lower survival (Niriella *et al.* 2021). The prevalence of MetS in individuals with NAFLD found in Asia, Europe, the Middle East, North America and South America was 34%, 62%, 31%, 33% and 37%, respectively, as reported in a recent systematic review. Age, as already demonstrated in other studies, has also been a predisposing factor for NAFLD, as age increases the risk of developing NAFLD also increases, whereas in relation to sex, the data are controversial.

Studies have evaluated NAFLD in individuals with a BMI <25 kg/m², although it is prevalent in overweight and/or obese individuals, individuals considered thin have also presented the disease, but in the absence of traditional risk factors, it remains poorly recognized. It should be noted that NAFLD "in thin" encompasses a different pathophysiology. With genetic predisposition, diet rich in fructose and cholesterol, visceral adiposity, endocrine disorders such as polycystic ovary syndrome, hypothyroidism and growth hormone deficiency and dyslipidemias could play a central role in development. This population tends to develop a less severe form of the disease, but may pose a risk of metabolic disorders, cardiovascular morbidity or general mortality (Kumar and Mohan 2017). A meta-analysis with more than 300,000 individuals found, in the general population, 11.2% of NAFLD in individuals with a BMI <25 kg/m² and 25.3% of those with NAFLD were considered thin (Young *et al.* 2020).

Excessive alcohol consumption also leads to this condition, with the threshold for reliable alcohol consumption that could distinguish NAFLD from alcoholic liver disease still controversial, but most studies use the cutoff point of ≥30 g/day in men or ≥ 20 g/day in women (Chalasani *et al.* 2018).

2.7 Literature Review

In order for clinical proteomic studies to be feasible and extensive, it is preferable to use fluids such as blood, since they are biological samples that are routinely obtained and do not involve invasive or costly procedures. However, the main disadvantage of using a blood sample to separate plasma or serum is that these fluids contain a large amount of albumin and immunoglobulins. These proteins correspond to 95% of the total sample, while the remaining 5% belongs to a wide variety of proteins such as cytokines, hormones, enzymes, and cytoplasmic and nuclear proteins (Ahmed *et al.* 2003).

The abundance of albumin and immunoglobulins can background any proteomic analysis and mask significant changes in the proteins of interest. These proteins can be removed from serum using various methodologies, such as column chromatography, to help identify protein biomarkers that have lower levels of expression. However, since albumin carries various proteins, it is possible that, by removing it, proteins of interest related to obesity are also unintentionally removed. On the other hand, serum lipids are frequently increased in obese people and may mask proteins that may be potential biomarkers. In some cases, it is also necessary to remove lipids from serum samples.

Published a study that compared the serum proteome of obese women with metabolic alterations and metabolically healthy obese (MHO). Their findings showed 20 proteins differentially expressed in women with metabolic disorders; overexpression of various hemoglobin subunits (HbA1, P5), haptoglobin-related protein (HPR), APOB100 and APOA4 apolipoproteins, RBP4 protein, and CRP protein were found, while the MHO phenotype was associated with low levels of molecules proinflammatory and higher levels of anti-inflammatory biomarkers. These findings suggest that an anti-inflammatory state and protection from lipid metabolism dysregulation were the main molecular features of the MHO phenotype (Doumatey *et al.* 2016).

It has been reported that an intense physical exercise regimen lasting only 2 weeks can induce changes in the proteomic profile in obese and overweight individuals, although it is important to evaluate the permanence time of these changes. In this study, it was determined that after 6 or 7 sessions of intense exercise, proteins related to chronic inflammation such as IL-6, MCP-1, TNF-α, ICAM-1 and IL-10, have a notable decrease, as well as than fatty acid synthetase (FAS). On the other hand, annexin 2 protein in adipose tissue also presented a different expression pattern before and after physical activation. Despite these changes, no changes were detected in the BMI of the individuals analyzed or in insulin sensitivity (Tatsumi *et al.* 2015).

Published a study in which they performed proteomic analysis of peripheral blood mononuclear cells (PBMC). PBMC samples were obtained from normal weight and obese individuals, in order to identify and quantify the differentially expressed proteins between both groups. In this study, the mechanisms underlying the progression of obesity and the possibility of reducing it with physical activity were analyzed. Forty-seven differentially expressed proteins were identified, of which thrombospondin 1 (TSP1) increased its expression, while histone deacetylase 4 (HDAC4) showed a reduction. The patients were evaluated again after 3 months of supervised physical activity and found that the expression levels of TSP1 and HDAC4 were reversed, observing expression levels similar to those present in subjects with normal weight. Derived from this finding, the possibility arises of using drugs that modulate the activity of HDAC4 to combat obesity (Abu-Farha *et al.* 2013).

The pathogenesis of IR is complex, involving genetic polymorphism, which influences the degree of insulin secretion and the action of environmental factors such as a sedentary lifestyle and dietary habits that promote obesity and the onset of MS (Marchesini *et al.* 2003).

As a result of insulin resistance, there is increased synthesis and retention of triglycerides within the hepatocyte, leading to macrovesicular steatosis. It is likely that lower fatty acid oxidation by mitochondrial dysfunction may contribute to this change. On the other hand, IR-associated hyperinsulinemia decreases the synthesis of apolipoprotein B-100, an essential component of very low-density lipoprotein cholesterol (VLDL-chol), thus reducing the transport of triglycerides out of the hepatocyte. Chronic hyperinsulinemia promotes de novo lipogenesis through the regulation of lipogenic transcription factors and can activate fibrotic cytokines such as connective tissue growth factors promoting inflammation and hepatic fibrosis (Tamura and Shimomura 2005).

The correlation between obesity and more advanced liver disease, evidenced by the presence of fibrosis, has also been the subject of more accurate investigation. Some studies have detected a positive association. In other studies, in which the research was carried out in the morbidly obese population, BMI was not among the independent predictive factors for histologically more severe disease. In their study, using the NHANES III database, (Ruhl and Everhart 2003) concluded that in the multifactorial logistic regression analysis, BMI was not independently related to the increase in ALT in this population studied.

Controversies have always occurred and continue to occur about the contribution of obesity to liver pathology. An important question is whether liver damage would occur due to obesity itself or to factors associated with it, such as, for example, insulin resistance. Type 2 diabetes: The association of type 2 diabetes and NAFLD dates back to the recognition of this syndrome and has been repeatedly confirmed over the years, being considered, after obesity, the most strongly associated risk factor. Type 2 diabetes is reported by 10% to 55% of patients with the syndrome. Prevalence studies of NAFLD in random diabetic patients revealed that about 25% of them had sonographic alterations compatible with hepatic steatosis.

Abnormalities in liver serological tests, such as aminotransferases, are common findings in patients with type 2 diabetes, with reports close to 40% of these alterations, attributed to the presence of hepatic steatosis, already in old publications. More recently, in a population study, using data from NHANES III, hyperinsulinemia was reported as a strong determinant of ALT.

In another study by (Idalsoaga *et al.* 2020) it was observed that the elevation of aminotransferases associated with alcohol intake was more evident in patients with overweight or obesity. in a logistic regression analysis of 1604 alcoholics, identified a body mass index greater than 25 kg/m^2 for at least 10 years as an independent risk factor for steatosis, alcoholic hepatitis and cirrhosis. This conclusion is particularly intriguing, as an experimental study by Yang et al demonstrated that genetically obese rats are at increased risk of endotoxic liver injury. Since it has been suggested that endotoxins derived from the gut flora may contribute to increased inflammation in alcoholic liver disease, this latter information may provide a potential mechanism for the risk associated with obesity (Protopapas *et al.* 2021).

3. MATERIALS AND METHODS

3.1 Type of Study

A Cross sectional, observational case series study with a comparison group of clinical cases of Alcoholic Liver Disease (ALD) and Non-Alcoholic Fatty Liver disease (NAFLD) patients

3.2 Location of the Study

Government Primary Healthcare centre, and Baghdad Teaching Hospital (BTH), Baghdad, Iraq. Period of the Study: March 2021 to February 2022

3.3 Sample (Patients) Selection

Patients scheduled to undergo reduction gastroplasty, after consultation with a surgeon, anaesthesiologist and nutritionist, were interviewed by the clinician, and those who met the criteria were invited to participate in the study. Patients were duly informed about the need for intraoperative liver biopsy, as well as the additional risks inherent to the procedure. All those who accepted the invitation signed the informed consent form. Those who entered the study underwent a careful clinical evaluation, the clinical data were recorded in a standardized form, attached. At the time of the initial evaluation of the patient, he already had the biochemical tests requested. The other complementary laboratory tests, necessary to rule out the presence of other hepatic pathologies, were requested as required by the study protocol. All results were recorded on a standardized form.

3.3.1 Selection of NAFLD patients

Patients with an ultrasound diagnosis of NAFLD aged between 20 and 59 years of both sexes and patients with a negative ultrasound diagnosis of NAFLD participated in this study. All subjects were evaluated by a single sonographer. Participants who had one of the following findings were excluded: alcohol consumption (>20g/day for women and >30g/day for men), drug addiction, schistosomiasis, hepatitis B or C or other chronic liver diseases, age ≥60 years, severe weight loss, BMI ≤18.5Kg/m^2, use of drugs known to cause steatohepatitis (Gunn 2021).

3.3.2 Selection of ALD patients

The approach to alcohol ingestion was carefully performed by a hepatologist, explaining to the patient the importance of data accuracy for the correct diagnosis of the pathology. Whenever accompanied, family members were asked to share information inherent to the drinking habit. Data referring to alcohol consumption were checked with those recorded in the anaesthesiologist and nutritionist records.

Patients were considered diabetic only when they already had a previous diagnosis and were controlled with diet and/or oral hypoglycaemic agents and/or insulin therapy. Those who had fasting glycaemia in the range between 110 mg and 125mg/dL were classified as having altered fasting glycaemia; those who had blood glucose above 125mg/dL and were not among those with a confirmed diagnosis were classified as probable diabetics and, for statistical purposes, were associated with patients with altered fasting blood glucose. This description takes into account that the diagnosis of diabetes requires confirmation of fasting glucose above 126mg/dL on two different days.

As hypertensive patients, patients who already had a previous diagnosis, those with documented use of antihypertensive therapy and those with resting systemic blood pressure greater than or equal to 140/90 mmHg, in at least two assessments (cardiologist and anaesthesiologist) were included.

All patients who did not fit the desirable profile were considered as having an alteration in the lipid profile, as follows: TC < 200mg/dL, HDL-C>40, LDL-C<100 and

TG<150mg/dL, according to the laboratory classification of the WHO Guidelines on dyslipidaemias.

The criteria used for metabolic syndrome were those of the “National Institute of Health”: fasting glucose greater than or equal to 110mg/dL; central obesity (waist circumference greater than 102 cm in men and greater than 88 cm in women); blood pressure greater than or equal to 130/85mmHg or treated pharmacologically; triglyceride levels higher than 150mg/dL or when using fibrates, and HDL-cholesterol lower than 40mg/dL (man) and lower than 50mg/dL (woman). Alcohol consumption was assessed using the following formula, obtained from data published by the National Institute on Alcohol Abuse and Alcoholism. The description of the formula is.

$$\text{Ethanol} = \frac{(\text{days})(\text{quantity})(\text{size})(\text{factor})}{365} \tag{3.1}$$

According to alcohol intake, individuals were classified into four groups: (0) no or sporadic consumption; (1) up to 10g/day; (2) between 10-20g/day; (3) between 20-30g/day. Obesity time was divided into three categories: (1) between 5-10 years; (2) between 10-15 years; (3) over 15 years old. Liver biopsy was performed by the surgeon in all individuals at the beginning of the surgical procedure, in the left lobe of the liver, using a 16 gauge-22mm biopsy needle (Bard – Max Core). In a second stage, without the presence of the patients, the results of the requested complementary laboratory tests and the histological data were transferred to standardized exam sheets.

3.3.3 Quantification of alcohol intake in groups ALD, NAFLD and control.

For the investigation of the amount of ethanol consumed in grams, the amount invested in millilitres and the type of beverage were evaluated, considering the percentage of ethanol, with volume correction, multiplying the estimated value by 0.8 for the correction of density alcohol (mL x %ethanol x 0.8); in addition to the amount of ethanol consumed, the time of consumption of the same was recorded.

3.3.4 Dietary survey

24-hour and seven-day Recall Test: The 24-hour recalls and semiquantitative frequency of the food consumed were usually applied at two different times by a single nutritionist.

The amounts of macro and micronutrients were estimated from the Software Avanutri Online®.

3.3.5 Body composition

Body composition was evaluated by electrical bioimpedance (Biodynamics®310®), and lean mass, resistance, reactance and biologically active mass of cell membranes were recorded. This examination was performed at the first consultation of the patient, and provided that there was no contraindication for it.

3.3.6 Data collection

Data were collected through the application of a structured questionnaire, which addressed socio-demographic, clinical, biochemical, anthropometric and food consumption information. Patients referred from the BTH outpatient clinic took part in this research, as well as voluntary participants, who were assigned to the case or comparison group, according to the NAFLD/ALD diagnosis.

Anthropometric assessment: The diagnosis of nutritional status was performed by the Body Mass Index (BMI), which is the ratio between weight (in kilograms) and height (in meters) squared. The result found was compared with the reference values established by the World Health Organization. Abdominal obesity was found in patients who presented the value of waist circumference in line. According to BMI, obese people are categorized into three classes: (1) class I- BMI between 30.0-34.9 kg/m^2; (2) class II- BMI between 35.0-39.9 kg/m^2; (3) class III- BMI greater than or equal to 40.0

kg/m^2. All obese patients included in the study belonged to classes II and III. Waist circumference was considered at risk when greater than 102 cm in men and greater than 88 cm in women. The waist-hip ratio (WHR) was evaluated by measuring the waist circumference divided by the hip circumference and was used as an indicator of visceral adiposity. WHR was defined as risk when greater than 0.95 cm for men and greater than 0.80 cm for women.

Finally based on the above screening practices we have selected 100 patents and 20 control subjects (Detailed cloudification given in the coming sections) and classified as three groups as follows:

1. Group- A: 50 Men.
2. Group- B: 50 Women.
3. Group- C: 20 healthy subjects without liver disease.

3.4 Exclusion criteria

- Alcohol intake > 20g/day or 140g/week for men and > 10g/day or 70g/week for women
- Patients with known to have liver diseases: alcoholic liver disease; viral hepatitis B and C; drug-induced liver disease; autoimmune hepatitis; autoimmune cholangiopathy; primary biliary cirrhosis; obstructive bile duct disease; Wilson's disease; primary hemochromatosis; α-1-antitrypsin deficiency.
- Use of tamoxifen, amiodarone, corticosteroids, high doses of oestrogen. methotrexate for a prolonged period of time or within the last six months.
- Use of other potentially hepatotoxic drugs in the last six months.
- Large small bowel resection surgery or jejunoileal bypass.
- Total parenteral nutrition at the time of the biopsy.
- Malignant diseases.

3.5 Biochemical Evaluation

The analysis of each sample was done in triplicate, reporting its average value. For each patient, a file was prepared in which their personal data and the results obtained were recorded. The enzymatic activities of the enzymes studied are expressed in International Units per Litre (IU/L or U/L), which is equivalent to the micromoles of product formed (or substrate transformed) by the enzyme in one minute per litter of solution. (µmol/min). The presence of hypercholesterolemia (≥200mg/dL) and elevated LDL-c (≥160mg/dL) was verified according to the criteria of the IV Guideline of the American Society of Cardiology (Arnett *et al.* 2019). For HDL-c, triglycerides and fasting blood glucose were considered the values recommended by WHO. Liver function was assessed by comparing serum ALT, AST, GGT and AF; Serum Total protein, Serum Albumin and Serum glucose, for testing the all-parameters Beckman Coulter ODR3021 analyser was used to determine each variable in our laboratory.

Blood collection and conservation: For the collection, conservation and processing of blood samples, the Manual of Laboratory Procedures in Basic Haematology Techniques was taken into consideration. The samples were collected in the early hours of the morning with the patient fasting and in a sitting position. Blood was obtained from the visible antecubital veins, for which a tourniquet was applied above the elbow flexion. The area was cleaned with 70% alcohol and blood was drawn with needles and vacutainer tubes without anticoagulant in an approximate volume of 5 mL. The samples, duly labelled, were placed in the thermo-refrigerated rack containing ice and thus were transported for processing.

Obtaining the blood serum: After approximately 2 h of collection, the tubes containing the blood were centrifuged at 3000 rpm for 15 min. The obtained supernatant (serum) was decanted into plastic vials, which were kept frozen (-20 ^{o}C) until analysis. Haemolyzed samples were discarded.

Diagnosing the Metabolic Syndrome: The presence of comorbidities related to the Metabolic Syndrome such as: hypertriglyceridemia, glucose intolerance, diabetes mellitus, arterial hypertension, abdominal adiposity and low HDL-c was evaluated by the team responsible for the research. According to the criteria of the I Guidelines for Diagnosis and Treatment of Metabolic Syndrome, a patient with MS was diagnosed with a combination of at least three components of those reported above.

Dietary Assessment: Food intake was assessed using the Semi-Quantitative Food Frequency Questionnaire (FFQ) previously validated by (Bredin *et al.* 2018), which contained foods commonly used in the region. Dietary consumption was analysed using the DietSys software version 4.01 (National Cancer Institute, Bethesda, MD, USA) using the US Department of Agriculture Food Chemical Composition Table as a database. Some foods not included in the program were inserted from product label data.

3.6 Statistical Analysis

Excel 2013 software and SPSS version 13.0 were used for statistical analysis. The results are presented in the form of a table and/or graphs with their respective absolute and relative frequencies. To verify the existence of an association between the categorical variables, Pearson's chi-square test and Fisher's exact test were used. Continuous variables with normal distribution had their means compared using the “t” student test and the Mann-Whitney test was used when the criteria for normality and/or homoscedasticity were not met. Correlation tests were used to verify the occurrence of correlation between anthropometric variables (BMI and AC) and biochemical and food consumption data. For the variables with normal distribution, the Pearson's Correlation was used and the ones with non-normal distribution, the Spearman's Correlation. A significance level of 5% ($p<0.05$) was used to reject the null hypothesis.

4. RESULTS AND DISCUSSION

4.1 Study on NAFLD with Obesity, Diabetes Patients

4.1.1 Prevalence of NAFLD

Of the 55 (Men=10; Female 45) patients recruited for the study, nine were excluded due to inconsistency in the data, failure to complete the forms, and failure to return to the outpatient clinic with the biochemical results. The final sample consisted of 55 patients, from the case group (with NAFLD) and 20 from the comparison group, with a negative diagnosis of NAFLD.

In the case group, nine patients (40.90%) were male and 11 (59.20%) were female. In the comparison group, 10 (18.18%) and 45 (81.81%) were male and female, respectively. The groups were matched by gender (Chi-square, p = 0.469) and age (t-Student, p = 0.909).

By the criteria of (Brunt *et al.* 1999), samples with NAFLD were classified in grade and stage, with mild in 80%, moderate in 15% and severe in %. Portal-associated fibrosis was present 7.5% of the population, 5.0% in stage and 2.5% in stage 3.

Regarding education, the comparison group had a better level of education when compared to the case group (p=0.009), since one patient in the comparison group (2.3%) was illiterate, 14.0% knew how to read and write, 7.0% had the Completed Elementary School, 48.8% Completed High School, 27.9% Higher Education. In the case group, 2.4% patient was illiterate, 43.9% knew how to read and write 14.6% had Completed Elementary School 29.3% Completed High School and 9.8% Higher Education.

The case group had a characteristic BMI of obesity, while the comparison group had overweight (32.00 ± 4.498 x 26.53 ± 2.926 kg/m^2, p<0.001). As for the AC variable, all patients in the case group had a very high risk of developing cardiovascular disease (102.21 ± 10.180 cm). In both groups, the presence of hypercholesterolemia was observed (206.33 ± 45.392mg/dL in the case group and 210.68 ± 34.096mg/dL in the comparison group). Hypertriglyceridemia was found in the case group [170.0mg/dL (Q1 = 104.4; Q3 = 223.2)]. In the comparison between the groups, there was a statistical difference in all the anthropometric and biochemical variables analysed, except for the values of total cholesterol and LDL-c Table 4.1.

The clinical profile of patients with NAFLD points to a higher prevalence of diabetes mellitus (17.1%, p=0.0259), abdominal obesity (75.7%, p<001) and metabolic syndrome (56.1%, p<0.001) compared to the comparison group Table 4.2.

Food consumption of patients with NAFLD was statistically higher in all variables studied when compared to the group without NAFLD, except for lipid intake, which was similar in both groups Table 4.3.

In the correlation between the anthropometric variables and the biochemical profile of the patients, a positive correlation was verified with a statistical difference in most of the analysed parameters. As expected, we found a negative correlation between anthropometric variables and HDL-c. As for TC, there was a negative correlation with BMI and AC, however without statistical difference, with LDL-c we showed a negative correlation with BMI and AC, but with statistical significance only with the latter. Regarding lipid intake, there was a positive correlation between BMI and AC, but this did not show a statistical difference Table 4.4.

4.1.2 Factors associated with NAFLD

We studied the correlation of demographic, anthropometric and laboratory variables with the degree of hepatic steatosis in two groups, the first comprising grade I steatosis and the second associating grade II and III steatosis. The association of moderate and severe degrees (II+III) was due to the fact that only six patients were in the last category, and both were considered higher steatosis scores, when classifications ranging from zero to four stages were used (Lee 1989).

In the univariate analysis of association between the demographic variables with the two groups of steatoses mentioned, statistical significance was found for the highest mean age in the second group (p=0.036), but not for age over 49 years (p=0.415). Although there was a trend towards the predominance of females, there was no statistical significance (p=0.074). The presence of metabolic syndrome was associated with group two (p=0.000). In the correlation analysis between the anthropometric variables and the degree of steatosis, the only variable that showed a statistically significant association with the highest degrees of steatosis was the mean waist circumference (p=0.0308). Among the laboratory variables, five showed statistically significant associations: HDL-C (inversely) (p=0.0290); triglycerides (p=0.0046); ALT (p=0.000) and γGT (p=0.006). Using logistic regression analysis, GGT was the only parameter with a significant independent association for the degree of steatosis, OR 0.04, 95% CI 0.003 - 0.63, P=0.022. The variable ALT approached significance with OR 0.15, 95% CI 0.02 – 1.07, P=0.059.

Among the quantitative parameters analysed, statistical significance for association with NAFLD was found for triglyceride levels (p=0.029); although a trend towards higher levels of glycosylated Hb was detected among patients with NAFLD, the difference was not statistically significant (p=0.064). Among the categorical variables, the following parameters were associated with NAFLD: the group formed by type 2 diabetes, plus those with altered fasting glucose (p=0.044); fasting glucose greater than 110mg/dL (p=0.034); the type 2 diabetes group shows a trend without being statistically significant (p=0.067); altered ALT (p=0.044); there is also a trend towards a higher percentage of women in the steatohepatitis group, but there was no statistical significance (p=0.062).

No statistical significance was found between the following parameters and the presence of NAFLD: age; obesity time; alcohol consumption; use of zero to seven medications; BMI; WHR; waist circumference; number of pathologies; arterial hypertension, metabolic syndrome and other laboratory parameters other than ALT.

Logistic regression analysis did not identify any factors independently associated with NAFLD. The univariate analysis for the association of clinical, anthropometric and laboratory factors with perisinusoidal fibrosis was compromised as it consisted of a sample of only three cases. Taking into account that, in morbid obesity, isolated portal fibrosis has been highlighted (Abrams *et al.* 2004), for the purpose of statistical analysis, the two groups were associated, and isolated portal fibrosis was also analysed separately. In the univariate analysis of the association between the independent variables: demographic, anthropometric and laboratory with all cases of fibrosis (perisinusoidal, isolated portal and perisinusoidal associated with portal) and with isolated portal fibrosis, only the increase in GGT showed a statistically significant association with the group composed of all cases of fibrosis (p=0.035). When submitted to logistic regression analysis, γGT did not remain as an independently associated factor, however, it showed values close to statistical significance, OR 5.69, 95% CI 0.99 – 32.73, P=0.051.

4.1.3 Factors associated with histological aspects present in NAFLD

The analysis of associations between clinical-demographic independent variables and histological findings of inflammation and ballooning did not show statistical significance. There was an association between type 2 diabetes and dyslipidaemia with the presence of Mallory bodies (p=0.036 and p=0.017 respectively). The association analysis between the anthropometric independent variables and the histological findings of inflammation, ballooning and Mallory bodies did not find associations with statistical significance. The analysis of association between independent laboratory variables and histological findings of inflammation, ballooning and presence of Mallory bodies showed a statistically significant association between the continuous variable HDL-C (inversely) and ballooning (p=0.0236). The presence of Mallory bodies showed statistically significant associations with the continuous variables, blood glucose and HbA1C (p=0.0080 and p=0.0273 respectively) and with the categorical variables ALT and ferritin (p=0.004 and p=0.040 respectively) (Figure 4.1).

Age was found to be an independent factor in the occurrence of ballooning, OR 0.89, 95% CI 0.79 – 0.91, P=0.029, when these values were entered into a logistic regression model. In the association with ballooning, the type 2 diabetes variable did not approach statistical significance, but the values are close to significance, OR 21.06, 95 percent CI 0.89-498, P=0.059. A significant correlation between dyslipidaemia and the presence of Mallory bodies was found, with ORs of 0.05, 95 percent confidence intervals of 0.002-0.75, and P=0.031, respectively.

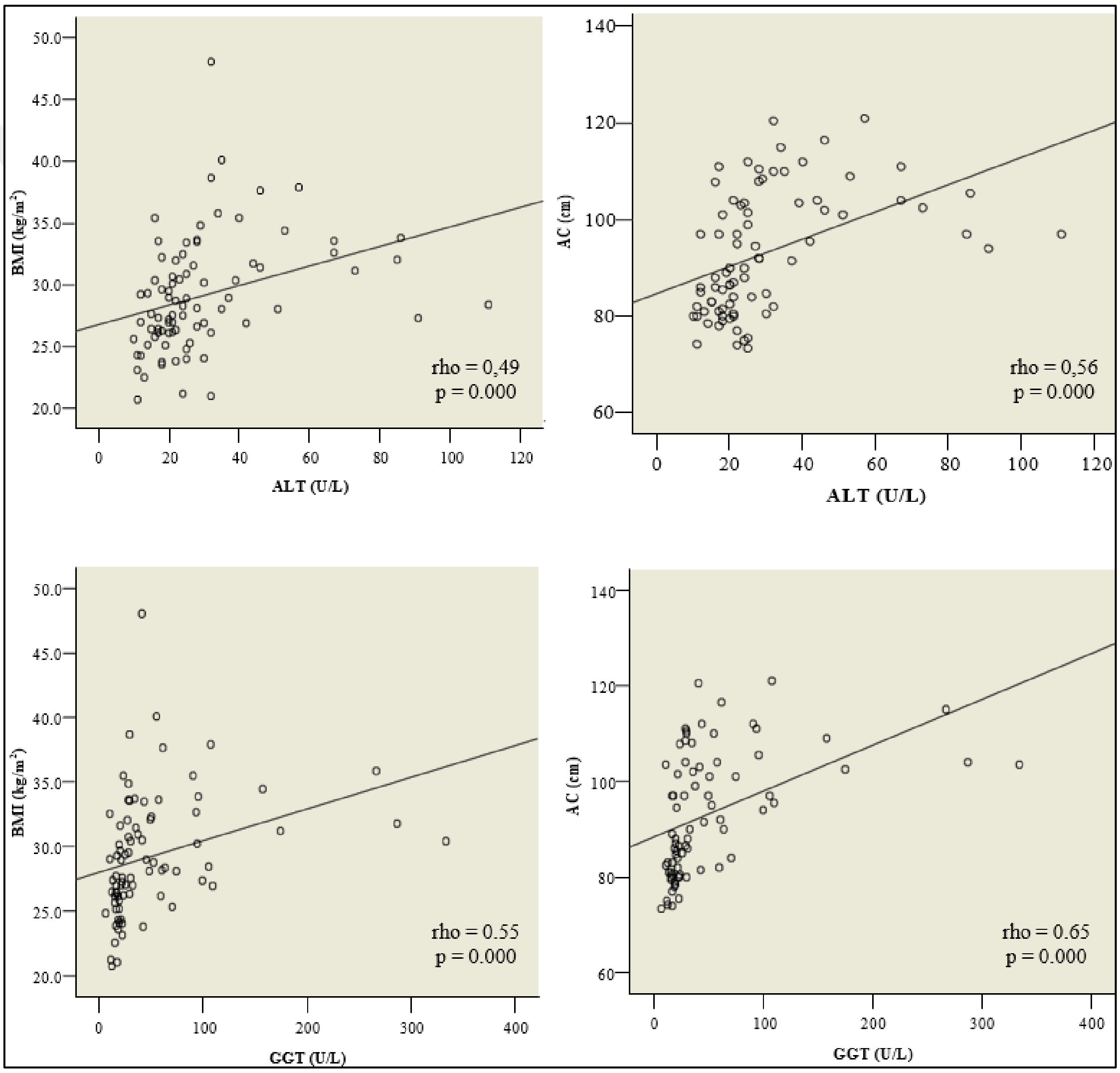

Figure 4.1 Correlation between anthropometric variables and ALT Vs BMI and AC; GGT vs BMI

Table 4.1 Anthropometric and biochemical characteristics of the case and comparison groups

Variables	**NAFLD group**	**Control group**
	Mean ± SD	Mean ± SD
BMI (body mass index) (kg/m^2)	31.98 ± 4.39	27.63 ± 2.821
Abdominal circumference (AC) (cm)	103.37 ± 11.191	86.40 ± 9.406
TC (total cholesterol) (mg/dL)	210.62± 44.434	209.98 ± 36.196
LDL (low density lipoprotein) (mg/dL)	115.91 ± 30.614	125.10 ± 27.514
HDL (high density lipoprotein) (mg/dL)	50.61 ± 14.205	64.82 ± 17.31
AP (alkaline phosphatase) (U/L)	94.36 ± 30.621	79.36 ± 36.36
	Median (Q1; Q3)	Median (Q1; Q3)
FBG (fasting blood glucose) (mg/dL)	103.0 (99.3; 113.1)	93.6 (88.9; 99.9)
TG (triglycerides) (mg/dL)	169.0 (103.6; 222.3)	99.1 (71.6; 145.8)
ALT (alanine aminotransferase) (U/L)	33.0 (23; 49.3)	21.2 (16.9; 26.0)
(AST) aspartate aminotransferase) (U/L)	26.31 (18.96; 37.0)	19.26 (15.6; 21.7)
GGT (gamma glutamyl transferase) (U/L)	51.0 (30.0; 63.4)	23.2 (17.9; 28.0)

Table 4.2 Clinical profile of the case and comparison groups

Variables		**NAFLD Group**	**Control**	**p-value**
Diabetes mellitus	Yes	17.10%	2.30%	0.22*
	No	82.90%	97.70%	
Systemic arterial hypertension	Yes	41.50%	27.30%	0.249**
	No	58.50%	72.70%	
Abdominal obesity	Yes	75.70%	18.60%	<0**
	No	24.30%	81.40%	
Abdominal obesity	Yes	56.10%	11.40%	<0.001**
	No	43.90%	88.60%	

(*) Fisher's exact, (**) Chi-Square

Table 4.3 Usual daily intake of the main dietary components of the case and comparison groups

Variables	**NAFLD Group**	**Control**	**p-value**
	Mean ± SD	Mean ± SD	
Calories (cal/day)	3482.8 ± 1414.750	2747.6 ± 1044.371	0.008*
Carbohydrates (g/de)	533.0 ± 210.515	407.2 ± 152.741	0.002*
Proteins (g/day)	115.3 ± 47.267	96.9 ± 33.633	0.040*
	Median (Q1: Q3)	Median (Q1:Q3)	
Lipids (g/day)	84.02 (61.3; 136.61)	82.0 (51.2; 97.61)	0.218**

(*) t Student, (**) Mann-Whitney

Table 4.4 Correlation between anthropometric, biochemical and food consumption variables of both groups

Variables	**BMI (kg/m^2)**		**AC (cm)**	
	Coefficient	p-Value	Coefficient	p-Value
BMI (kg/m^2)*	1.01	-	0.8888	0
AC (cm)*	0.8888	0	1.01	-
TC (mg/dL) *	-0.1111	0.3131	-0.1616	0.1515
LDL (mg/dL) *	-0.1818	0.1111	-0.2525	0.0303
HDL (mg/dL)*	-0.2727	0.0101	-0.3434	0
AP(U/L)*	0.1717	0.1212	0.2323	0.0404
calories *	0.3232	0	0.3737	0
proteins *	0.3636	0	0.4343	0
FBG (mg/dL)**	0.2626	0.0202	0.3232	0
TG (mg/dL)**	0.3737	0	0.3535	0
AST (U/L)**	0.3737	0	0.404	0
Lipids **	0.1414	0.2222	0.1717	0.1313

(*) Pearson's Correlation, (**) Spearman's Correlation

4.2 Clinical and Demographic Characteristics of Alcoholic liver disease Patients

The demographic, clinical and laboratory aspects of the 45/100 patients and 20 control subjects who remained and completed the study are shown in Table 4.5.

Table 4.5 Main characteristics of ALD patients (N=100) and control group (n=20)

Variable	**ALD***			
	Male	Female	Control (n=20)	
Sample Number	40	5	20	Median
Age (years)	18-59	19-61	18-60	
Obesity time (years)(%): 0 to 10	20	3		21.5
10 to 15	12	1		12.5
>15	8	1		8.5
BMI (Kg/m^2)	61	63	36	92.5
WHR	59	62	1.22	90
Waist circumference (cm)	65	68		99

*ALD: Alcoholic liver disease/ BMI, body mass index; WHR, waist-hip ratio;

The mean age ±S.D. of all patients was 38.4 years ± 1.67 years (range, 19 –61 years). The vast majority of patients had been obese for more than 0 to10 years (62.2%).

4.2.1 Alcoholic habit details of the selected population

The observation in Table 4.6 shows the studied characteristics of alcoholic patients with pancreatitis and individuals in the control group.

Table 4.6 Alcohol consumption data

	ALD group	Control Group	P-Value
Most consumed acholic drink	sugarcane brandy 50%	nil	
	beer 40%	nil	
Time	21.95 ± 9.14	nil	0.486
Gram's ethanol/day (Retrospective information.)	252.52±229.12	nil	0.0263
Diabetes	27 (26 Male/1 Female)	nil	

The time of alcohol consumption is not significant and the number of grams of ethanol ingested per day is approximately double in relation to the no alcoholic, which was statistically significant (p=0.026), as shown in Table 4.7.

Table 4.7 Enzymatic activity in ALD patients

Variables	Average Values	Minimum	Maximum	Standard Deviation (SD)
ALT	10.97	7.8	13.45	1.8
AST	14.04	10.3	17.12	2.32
GGT	15.92	12.26	19.85	2.47
ALP	122.05	101	141.4	11.92
AST/ALT	1.28	1.32	1.27	1.29

4.2.2 Enzymatic activity

The results found, with respect to the measurement of the enzymatic activity of the enzyme analysed, are found in Table 4.7 and whose respective statistical parameters are presented in Table 4.7. The analysis of variance (ANVA) of the activity of each enzyme (Table 4.8) shows that, in terms of the clinical condition of the patient (alcoholic and non-alcoholic), there is a highly significant difference ($P \leq 0.01$) in the five enzymes studied, the activity of ALT, AST, GGT and ALP being greater in alcoholics than in healthy people. Regarding the enzymes ALT, AST, ALP do not show significant difference (P>0.05); and for GGT significant difference (P≤0.05). In the ANVAs of the studied enzymes, no interaction effect was found for patient level (P>0.05). This means that both factors studied are independent.

Table 4.8 Statistics of serum alanine aminotransferase (ALT or GPT) enzymatic activity according to the patient status (U/L)

Aminotransferase (ALT or GPT) enzymatic activity according to patient status (U/L).			
Patient's status	Average ± SD	Covariance (%)	minimum-maximum
Control	10.97 ± 0.41b	16.41	7.8 - 13.45
ALD	67.67±1.42a	18.49	50.61 - 85.62
Serum aspartate aminotransferase (AST according to patient status (U/L).			
Control	13.90 ± 0.46 b	16.54	10.20 – 16.95
ALD	144.78 ± 3.21 a	11.08	112.08 – 169.58
γ-glutamyl transpeptidase (γ-GT or GGT) in blood serum according to patient status and (U/L).			
Control	15.76 ± 0.49 b	15.53	12.14 – 19.65
ALD	117.02 ± 2.96 a	12.65	96.44 – 142.88
Alkaline phosphatase (ALP) in blood serum according to the patient's condition (U/L).			
Control	120.84 ± 2.36 [b]	9.76	100 – 140
ALD	357.32 ± 3.56 [a]	4.99	315 – 390
AST/ALT ratio			
Control	1.30 ± 0.06 [b]	21.86	0.78 – 2.18
ALD	2.22 ± 0.07 [a]	15.5	1.79 – 2.97

4.2.3 AST/ALT ratio

The results of the AST/ALT ratio of the study groups and their statistical parameters are shown in Table 4.8. The analysis of variance (ANVA) indicates that there is no effect (P>0.05) but a highly significant difference between alcoholics and control (P≤0.01), being higher in alcoholics with a ratio of 2.18 and lower in control 1.39.

4.2.4 Enzymatic correlation

Using Pearson's correlation coefficient, the correlation between the activities of the five enzymes analysed has been determined. The results are presented in Table 4.9.

Table 4.9 Pearson's correlation coefficient for the different enzymatic activities

Enzymes	ALT	AST	GGT	ALP
ALT	1	0.9864#	0.9878#	0.9659#
AST	-	1	0.9789#	0.9789#
GGT	-	-	1	0.9698#
ALP	-	-	-	1

#The correlation is significant at the 0.01 level (2-tailed).

It is observed that for all the enzymatic cases studied, there is a high and positive correlation between the enzymes studied, except for cholinesterase with the other enzymes where there is a high negative correlation (P≤0.01).

Table 4.10 shows the mean values for the inflammatory markers in the three groups studied, and the analysis of CRP of amyloid protein and leptin showed no differences when comparing the three groups.

Table 4.10 Inflammatory markers in patients in ALD, and NAFLD and in individuals in control group

	ALD	**NAFLD**	**Control**	**P-Value**
CRP (mg/L)	4.76 ±7.19	3.76±4.44	2.74±1.85	0. 9514
Serum amyloid A protein (mg/L)NV**up to 6.4mg/L	5.52 ±5.92	5.10 ± 4.26	4.38 ± 3.05	0. 9478
Leptin (ng/mL) NV- For BMI between 18 and 25kg/m²: Women: 3.7 to 11.1 ng/mL Men: 2.0 to 5.6 ng/mL	4.82±5.67	7.62 ± 13.18	6.03 ±3.79	0. 2669

*NV - normal value

4.2.5 Serum indicators of nutrition and metabolism

Table 4.11 shows the mean values of the nutritional markers researched in the three groups of patients studied and their observation shows that, as a rule, the variables were within the normal range. As an exception, the cholesterol of group A (ALD) was significantly lower than that of group B (NAFLD) (p=0.0198); whereas that of group C (Control) was lower than that of B, but not significantly. Blood glucose was high in Group A, as expected, due to the presence of 60% of diabetics in this population.

Table 4.11 Nutritional markers in patients in groups A and B and in individuals in group C

	ALD group	NAFLD Group	Control Group	p-Value
Albumin (g/dL) (NA-3.4 to 4.8g/dL)	3.95 ±0.47	4.17 ± 0.44	4.26 ± 0.26	0.0882
Haemoglobin (g/dL) (NV-13 to 18g/dL)	14.53 ±1.20	14.94 ± 0.65	14.94 ± 1.07	0.4124
Blood glucose (mg/dL) (NV- 70 to 100mg/dL)	137.50 ± 63.57	93.58 ±14.05	94.31 ±12.51	0.0202

4.2.6 Nutritional and body composition findings

Table 4.12 shows the energy and macronutrient values obtained in the seven-day dietary survey of patients in groups A and B and of individuals in group C.

Table 4.12 Energy and macronutrient values obtained in the seven-day dietary survey of patients in groups A and B and in individuals in group C

	ALD group	**NAFLD Group**	**Control Group**	**p-Value**
Kilocalories (Kcal)	1637.17± 546.35	1644.82± 309.67	1584.46± 502.71	0. 8969
carbohydrates)	224.21± 86.82	242.77± 49.54	222.03±77.19	0. 7594
Proteins (and)	70.74± 24.01	61.93±13.61	67.87±18.02	0. 5154
Lipids (g)	50.58±17.11	48.28 ±13.96	47.20± 20.61	0. 8558

In the three groups, the average of calories and lipids ingested was similar. Groups A and C also showed similarity in relation to carbohydrate and protein intake. Group B had the lowest consumption of these nutrients, but not statistically significant.

5. DISCUSSION

The three groups can be considered homogeneous, with all individuals being male and with similar average age. In the present investigation, sugarcane spirit was the beverage most consumed by group A and the second most consumed by group B. As this beverage has a high ethanol content (50% ethanol in 1000 mL), it becomes clear that these patients received a high calorie content but did not do so through food intake. Alcohol consumption tends to reduce food intake, especially the components of healthy eating. The amount of ethanol used by patients in groups A and B was able to double the caloric gain in the former and almost triple it in the latter. This fact could induce chronic disorders in eating habits, in the biochemical profile and in the body composition of the population. However, the seven-day recall did not show differences, thus demonstrating that if there were distortions in food intake, they were regularized with the interruption of alcohol consumption. The 24-hour recall was not presented because, as it was performed on Mondays, it led patients to refer to what they had ingested the day before, a Sunday, an atypical day on a diet.

The patients in group A presented clinical manifestations such as diabetes, and it can be assumed that their appearance contributed to the dietary distortions already mentioned. Such clinical manifestations possibly also promoted a reduction in alcohol consumption, which would not have occurred with group B. The reasons to suspect that some residual alcohol consumption persisted in Group B were the increase in total cholesterol and in that linked to low-density lipoproteins (LDL), in the presence of the same dietary regimen, which raises the hypothesis of non-alcoholic ingestion. revealed from ethanol. In Group B, GGT, a parameter commonly used to assess sustained alcohol intake, was elevated, but not significantly. Obesity is a worse prognostic factor in chronic pancreatitis and not just in severe acute pancreatitis. As recorded, truly obese patients were not included in this series, with only occasional examples of overweight being perceived. In the correlations performed, a significant result was observed when fat mass was compared with leptin in groups A and B, suggesting that these patients are more inflamed ($p=<0.0001$).

In the investigation presented here, the leptin concentration did not increase appreciably when compared to the control group (group C), a finding similar to that of two studies by (Adrych *et al.* 2008), in which the authors suggest that their results are independent of the etiology of chronic pancreatitis.

Conventional inflammatory marker CRP tended to increase not only in group A, but also in group B, without statistical significance when compared with group C. (Pelli *et al.* 2008) used CRP as a diagnostic criterion for patients with alcoholic CP. In severe acute pancreatitis, CRP is naturally an established indicator, showing significant elevations, but it is usually not used to assess CP. CRP indicates the existence of inflammation and also indicates response to treatment, showing three hypothetical situations: infection, acute or chronic inflammation and metabolic risk. With regard to C-reactive protein, both common sense and Pearson's test indicated that the longer the time of alcohol consumption, the greater the elevation of such protein, and it can be assumed that with the amyloid serum, which usually accompanies CRP, the same happens (Dayer *et al.* 2007).

5.1 Analysis of NAFLD Patients Category

In the present study, patients with NAFLD had a BMI $\geq$ 30.0 Kg/m^2, which is characteristic of obesity, confirming the relationship between such disorders, and corroborating (Rocha *et al.* 2009) who, in a series of cases with 81 patients with a clinical and/or histological diagnosis of NAFLD, found obesity to be the most frequent risk factor for NAFLD, affecting 40% of patients.

Patients with NAFLD showed a significant increase in BMI and AC in relation to patients without NAFLD, similar results were found by (Soler *et al.* 2008), in a cross-sectional study with 22 patients with NAFLD and 38 patients without NAFLD, where they showed a significant increase in BMI and AC in patients with NAFLD.

There was an association between NAFLD and diabetes mellitus, abdominal obesity and metabolic syndrome, but not with arterial hypertension, this can be explained by the lack of measurement of blood pressure during the research, since, in a similar study, except for the measurement of blood pressure during data collection, (Soler *et al.* 2008) showed an association between NAFLD and arterial hypertension, diabetes mellitus, metabolic syndrome, and among other cardiovascular risk variables. The measurement of waist circumference is an anthropometric parameter capable of identifying the presence of central obesity. This, in turn, is related to visceral fat, insulin resistance, increased free fatty acids and metabolic syndrome. (Márquez *et al.* 2008) found that NAFLD was independently associated with abdominal obesity, insulin resistance, and hypertriglyceridemia, and the association remained strong after controlling for age, gender, and other potential risk factors. In the same study, the authors observed an association between BMI and NAFLD in the univariate analysis, which did not occur in the multivariate analysis, demonstrating that NAFLD is more associated with central obesity than with general obesity.

Regarding gender, there was a higher prevalence of women in both groups, which may have occurred due to the outpatient and voluntary nature of the research, since women have a greater demand for health care. Females seem to be more consumers of health care than males throughout life and the curves as a function of age indicate that this difference is more important at times when gynecological-obstetric problems are more frequent.

Food choice was associated with the individuals' level of education, given the high occurrence of individuals with low education in the case group. It is known that the low level of education is associated with the significant consumption of foods considered at risk for the development of cardiovascular diseases. In this way, the low level of education presented and the asymptomatic characteristic of NAFLD becomes even more aggravating, since the low level of education contributes both to the determination of inadequate eating habits, as well as to the lower perception of the unsatisfactory state of health. As for the biochemical findings, we found significantly increased values of fasting glucose, triglycerides, ALT, AST, GGT, FA and decreased HDL-c in the

NAFLD group, whereas total cholesterol and LDL-c values did not differ between groups. Other studies found similar results comparing the variables of the lipid profile and fasting glucose in individuals with and without NAFLD. Regarding liver markers, our results corroborate the findings of (Adrych *et al.* 2008), except for alkaline phosphatase, since these authors did not perform the analysis of that enzyme, so they observed statistically higher values of serum ALT, AST and GGT in patients with NAFLD. Evaluating 408 patients (210 with and 198 without NAFLD), found higher values of BMI, WC, ALT, AST, CT, TG and lower HDL-c values in the NAFLD group. According to (Bajaj *et al.* 2009) in a case-control study, significant increases are observed in the values of BMI, WC, FG, CT, TG in patients with NAFLD. These authors also showed higher prevalence of obesity, glucose intolerance and metabolic syndrome in patients with NAFLD, while blood pressure, measured during the research, did not differ between the groups (Jun *et al.* 2008).

Regarding the correlation analysis between the anthropometric variables (BMI and WC) and the biochemical findings, our results demonstrate a greater exposure of NAFLD patients to cardiometabolic events, as well as a lower interference of excess weight and abdominal fat in the elevation of blood levels of TC and LDL-c, similar to the results found by (Rezende *et al.* 2006), in a cross-sectional study with 231 individuals, evaluating the association between BMI and WC with cardiovascular risk factors, where they found a positive correlation with a statistical difference between the aforementioned anthropometric variables and FG and TG, HDL-c correlated There was a negative correlation with BMI and WC, but this was significant only with WC, while TC showed a positive correlation with BMI and WC without statistical difference and with LDL-c there was a positive correlation with both anthropometric indices, however with statistical difference only with CC.

As for the correlation between BMI and AC and liver markers, we found positivity with a statistical difference in all analyzed variables, except for AF, which did not show statistical significance, these findings should be used as an incentive to promote abdominal fat loss, since (Samadi *et al.* 2007) also found a positive correlation between AC and liver enzymes ALT ($p=0.01$), AST ($p=0.02$), FA ($p=0.01$) and GGT ($p=0.05$) in adults and suggested that elevated liver enzymes may indicate potentially reversible liver inflammation.Evaluating the association between BMI and liver enzymes (ALT, AST and GGT) in 3167 individuals, 2373 men and 794 women, respectively, observed that the serum values of these markers increased in obese individuals when compared with eutrophic individuals in both sexes, showing the relationship between BMI and serum activity of liver enzymes (Salvaggio *et al.* 1991).

In the dietary analysis, we can infer that patient with NAFLD have high caloric, protein and glucose consumption in relation to individuals without NAFLD, while in lipid intake there was no difference between the groups with and without NAFLD. In view of this, therapeutic measures based on the modification of dietary habits should aim not only at the quantitative consumption of food, but above all at the nutritional quality of the components in the ingested meals. (Cortez-Pinto *et al.* 2006), evaluating, among other variables, the food consumption of 45 patients with NAFLD, using a semi-quantitative food frequency questionnaire and, comparing with the data of 856 individuals, they found lower consumption of carbohydrates and higher consumption of lipids in patients with NAFLD, indicating that high lipid consumption seems to be associated with the most advanced form of the disease. When comparing the consumption of macronutrients between patients with hepatic steatosis and NAFLD, observed a higher lipid intake, and a trend towards a higher total consumption of carbohydrates in patients with NAFLD than in patients with simple steatosis, and caloric and protein intakes showed no difference. statistics between individuals with steatosis and NAFLD, suggesting, again, the relationship between NAFLD and high lipid consumption (Vilar 2006).

The participation of lipids in the severity of the disease is still a controversial topic in the literature, since (Toshimitsu *et al.* 2007) when evaluating food consumption by 3 consecutive days recall in patients with NAFLD and simple steatosis and comparing the results with a group of randomized Japanese, found no difference between groups in total caloric, carbohydrate, lipid and protein intake in the correlation between the anthropometric variables (BMI and AC) and food consumption, we found a positive correlation with a statistical difference in caloric, protein and carbohydrate intake, and lipid consumption was also positively correlated, but there was no statistical difference, suggesting that the high consumption caloric intake is related to the accumulation of abdominal fat. Similar results regarding the correlation between AC and caloric consumption, but not with that of lipids, were found by (Vilar 2006), since evaluating patients with simple steatosis and NAFLD and anthropometric, clinical and dietary variables found a positive correlation between WC and caloric and lipid intake in patients with NAFLD. A limitation of the present study is due to the diagnosis of NAFLD performed only by ultrasound, since the use of liver biopsy, considered the gold standard, would be relevant to relate the dietary characteristics of NAFLD patients with the degree of disease progression. In conclusion, the results suggest that patients with NAFLD are at increased risk for the development of cardiometabolic diseases and high dietary intake of carbohydrates and lipids, requiring intervention aimed at losing weight and modifying the diet of these patients.

In the present study, the clinical variables that were shown to be associated by univariate analysis with the highest steatosis scores were: older mean age (over 40 years); highest average waist circumference; presence of metabolic syndrome; HDL-C (inversely); triglycerides, ALT, γGT and ferritin. In the univariate analysis, there was also a tendency to accentuate hepatic steatosis in females, but there was no statistical significance ($p=0.074$). After logistic regression analysis, only γGT showed an independent association with the degree of hepatic steatosis.

Although the literature recognizes that fatty changes can affect people of any age, prevalence studies in the general population, based on ultrasound and accidental death series, found a higher prevalence of fatty liver over fifty years. In our study, older age did not show a significant correlation with more pronounced degrees of steatosis (II and III) in the adjusted analysis, which is compatible with the results of other studies, also in selected groups of morbidly obese. After adjusted analysis, the sex factor persisted with no independent association with the degree of steatosis.

In a study with a sample from morbidly obese individuals, WHR was correlated with the presence of NAFLD, regardless of total adiposity. It was also verified that, in individuals with normal weight, those with higher WHR had a higher prevalence of hepatic steatosis. The data together support the idea that central adiposity is a more relevant factor for NAFLD than total adiposity.

Another study, in patients from bariatric surgery, showed, in a binary logistic regression analysis, diabetes and WHR as clinical predictive factors and insulin resistance and ALT as laboratory predictors, associated with higher degrees of steatosis (scores 3 and 4). However, when analyzed together, only the laboratory factors remained significant. In the present study, no independent association was found between anthropometric variables and the degree of steatosis, after adjusted analysis. This fact could probably be explained by the fact that it is a homogeneous sample, in relation to these aspects, which should obtain more relevance in more heterogeneous groups regarding the determinants of these variables. This last assertion is reinforced by findings in a study of a non-selective sample for obese individuals, in which BMI was an independent predictive factor for the presence of steatosis.

Dyslipidemia, particularly hypertriglyceridemia, has been shown to be associated with the degree of steatosis in some studies. We found, in the univariate analysis, that triglycerides and HDL-C (inversely) showed an association with the degree of hepatic steatosis, with the correlation disappearing in the adjusted analysis. These results are in agreement with those of (Dixon *et al.* 2001), in whose study the insulin resistance index and ALT were the only laboratory variables identified as independent predictors of higher degrees of hepatic steatosis.

ALT remains the test most uniformly reported to be associated with NAFLD and has even been used in population-based studies to predict the disease. Literature data place the elevation, usually moderate, in ALT as the laboratory finding most correlated with NAFLD, in normal weight, overweight and obese patients.

In our results, ALT was significantly associated with the degree of steatosis in the univariate analysis, and in the adjusted analysis, despite the values deviating from statistical significance, they were positioned close to it. The association was not demonstrated in a study that also investigated this correlation (Angulo 2007). It is also known that normal aminotransferase levels do not exclude the disease, and normal levels have already been demonstrated, even in advanced cases of fibrosis and cirrhosis. It is important to report that, in our study, aminotransferases were analyzed as categorical variables, since we worked with different normality values for these enzymes, which may have masked a greater relevance of this variable.

An interesting result of our study is the fact that the increased expression of γGT was the only laboratory parameter that showed an independent association with the degree of steatosis, in contrast to data from other studies in morbidly obese patients (Dixon *et al.* 2001).

Although it is impossible to say that there was no omission of data regarding the most abusive consumption of alcohol, AST did not show an association with the degree of steatosis, even in the univariate analysis; MCV was normal in all patients, and the AST/ALT ratio was less than one in 90% of cases. These aspects do not corroborate the association of alcohol consumption and the significant expression of γGT. The use of medication is another relevant factor in the analysis of γGT elevation. Despite having been excluded from the study, all patients, currently using or in the last six months of drugs known to be hepatotoxic, we have to take into account that these are patients with associated comorbidities, resulting in concomitant use of drugs. However, the analysis of the association between the use of medication and the presence of hepatic steatosis in this study did not show statistical significance.

It is recognized that γGT levels may be elevated in NAFLD, but data are still scarce in the literature regarding the frequency and levels of elevation (Cusi *et al.* 2017), which does not allow us to make comparisons.

γGT is expressed on the outer surface of many cells, being responsible for mediating the entry of glutathione. Knowing the relevant action of glutathione as an antioxidant at the intracellular level, a link is established between oxidative stress and increase in γGT levels. In their study, demonstrated, in a follow-up of 4,088 healthy individuals for a period of four years, that the increase in γGT concentrations, within the normal range, proved to be a sensitive and early marker for the onset of diabetes in the period studied (Lee *et al.* 2007).

The connection between increased expression of γGT and the insulin resistance syndrome has been demonstrated by other authors. A study carried out with a sample of 11,884 male Japanese, divided into three groups by age group, analyzed the correlation of age, alcohol consumption and various aspects that make up the metabolic syndrome, with the increase in γGT. After submitting the results to logistic regression analysis, correcting the alcohol factor for each group, the authors found that alcohol consumption > 40g/day, BMI >25.9 kg/m² and triglyceride levels >150mg/dL were important associated factors. an increase in γGT activity, regardless of age. In the group between 40-59 years of age, the factors arterial hypertension, hypercholesterolemia, hyperuricemia and increase in A1C glycosylated hemoglobin levels were also correlated with an increase in γGT activity. These results led the authors to conclude that there is an association of increased γGT expression with the state of insulin resistance (Yokoyama *et al.* 2003).

Finally, considering that insulin resistance has established itself as the basis of the metabolic alterations associated with NAFLD, the finding of the independent association of γGT with higher degrees of hepatic steatosis, in the present study, reinforces the associations already made between insulin resistance and increased expression of γGT and alerts for a greater relevance of this enzyme in the follow-up of patients with NAFLD.

The actual prevalence of NAFLD in the general population is even more elusive than that of NAFLD. Difficulties increase as enzymatic alteration criteria and imaging methods for presuming NAFLD are even more limited and inadequate than for presuming NAFLD. The lack of a reliable marker for the diagnosis of this pathology maintains the biopsy procedure as the gold standard for this purpose. Another major difficulty is the lack of consensus on the definition of NAFLD, creating an obstacle to the correct comparison between different studies. Using the degree and stage criteria by

Brunt *et al.* we found a prevalence of NAFLD of 66.7% in the sample studied, being mild in thirty-two patients (80%) of the sample with NAFLD. Zone 3 perisinusoidal fibrosis was detected in only three of these cases (7.5%). NAFLD prevalence data, published in the literature, come from hospital autopsy studies, or from patients selected by various criteria. A study of 351 hospital autopsies in non-alcoholics found NAFLD in 2.7% of the lean and 18.5% of the obese. In studies of population subgroups, such as those of morbidly obese patients undergoing bariatric surgery, literature data point to NAFLD prevalences ranging from 2.7% to 69.5%. It is observed, however, that the criteria used to define NAFLD are not homogeneous, which makes comparisons very difficult. The criteria employed by (Nasrallah *et al.* 1981) required the presence of steatosis associated with inflammatory exudate and moderate to severe focal or confluent necrosis, thus finding a percentage of 9%. These are stricter criteria than those used by (Garcia-Monzón *et al.* 2000). The latter used the (Brunt et al 1999) histological lesion scoring system. However, as a minimum criterion, for mild NAFLD, the sum of steatosis and inflammation scores ≤4 points was applied; and, for moderate, above 4 points, not requiring the presence of ballooning. (Marceau *et al.* 1999) do not define criteria for NAFLD. They refer to the percentage of lymphocytic infiltrate of the portal tracts and did not exclude viral hepatitis from the sample, through serological tests. (Dixon *et al.* 2001) used, as a definition of NAFLD, the presence of steatosis associated with two of the following three criteria: (1) intralobular necro-inflammatory infiltrate; (2) ballooning with or without Mallory bodies; (3) perisinusoidal fibrosis in zone 3, based on (Lee 1989).

The prevalence of NAFLD of 66.7%, found in our study, is higher than the average seen in the literature in relation to the morbidly obese group. It equates to the results of (Garcia-Monzón *et al.* 2000), however the criteria used by this author are less restrictive than those used in this study, which applied the degree and stage criteria by (Brunt *et al.* 1999). The series by (Dixon *et al.* 2001, Beymer *et al.* 2003), who used, as in the present study, more restricted criteria for the diagnosis of NAFLD, presented lower prevalence (25% and 33%, respectively), when compared to our result (66.7%). We analyzed the most relevant demographic and laboratory characteristics in the samples from these three studies: age, sex, BMI, diabetes, hypertriglyceridemia, arterial hypertension, elevation of AST and ALT. The age and sex distribution variables are shown to be approximate. The BMI averages of the two studies in comparison were even higher when compared to ours. We could observe, however, that the percentages of patients with type 2 diabetes associated with changes in fasting glycemia and hypertriglyceridemia were higher in our sample than in the aforementioned studies. Arterial hypertension, which was present in 38% of the sample by (Dixon *et al.* 2001) and was an independent predictive factor for NAFLD in this study, it was present in 56.7% of our cases, but did not show statistical significance regarding the association with NAFLD. The percentages of elevation of aminotransferases were approximate in this study and in that of (Dixon *et al.* 2001) and was not reported in (Beymer *et al.* 2003).

The higher prevalence of NAFLD in our study may be due to characteristics inherent to our sample, such as a greater number of individuals with diabetes, arterial hypertension and hypertriglyceridemia. Although an independent association of these factors with NAFLD was not demonstrated in our study, they have already been cited as associated in other study reports in the literature. We must consider the possibility of regional characteristics of the sample, requiring comparative studies at this level to assess this aspect. A study carried out in Rio Grande do Sul (Moretto *et al.* 2003) analyzing seventy-seven patients undergoing bariatric surgery, showed only 2.7% of steatohepatitis, but did not refer to the criterion used for the diagnosis, making the tests unfeasible. comparisons in this regard. It is recognized that NAFLD can evolve differently with lesser and greater severity. Hepatic steatosis results from high levels of free fatty acids in the liver, however it is generally accepted that a second insult or trigger is necessary for the evolution of the simple presence of fat in the liver to the state of necro-inflammation, called as EHNA.

Many factors have been reported to be associated with a worse outcome within the pathology spectrum. Obesity, considered from the beginning; dyslipidemia (mainly hypertriglyceridemia and low HDL-C levels); type 2 diabetes; insulin resistance; arterial hypertension and visceral obesity, all components of the metabolic syndrome, are the most commonly reported factors in the literature as associated with the evolution to NASH, having all these factors, as a basis, insulin resistance.

The associated factors alternate in the different studies, often depending on particularities in the selection of samples, the design of these studies and, in other publications, no reason is apparent for the divergence of results. Hence the need to increase the volume of assessments on the influence of these factors, until the trends begin to be, definitively, sedimented.

In our study, in the univariate analysis, the clinical variable, composed of the group formed by diabetics and those with changes in fasting glucose, was significantly associated with NASH, as were the laboratory variables ALT, triglycerides and blood glucose >110mg/dL. In the adjusted analysis, however, none of these variables remained as independently associated factors.

Regarding gender, fourteen of sixteen men (87.5%) and twenty-six of forty-four women (59.09%) in the sample had NASH. There was a predominance of NASH in men in the sample compared to women. The results, however, did not show statistical significance in the univariate analysis or after logistic regression analysis. For the most part, studies pointed to a predominance of NASH in women. This data, however, has been questioned, and other researchers have reported approximately equal and even higher prevalence in males. It is true that studies have emerged in the literature that support the female sex as a predictive aspect of progression to more severe disease.

The assessment of alcohol consumption in these and other studies has obvious limitations. The lack of a safe marker to rule out its use makes it impossible to prove non-alcoholic status. Based on all available information, patients were selected within the limits set out in the study methodology. There was no association between alcohol and NASH in this study. The result is in agreement with literature data, which evaluated the association of alcohol consumption, within the limits accepted as not sufficient to cause liver damage, with NASH and/or disease-related fibrosis.

In the study, in a sample of morbidly obese, by (Dixon *et al.* 2001) the moderate use of alcohol seems to have even reduced the risk for the appearance of steatosis, NASH and Mallory bodies. This finding is supported by the fact that moderate alcohol consumption promotes a decrease in insulin resistance and may, therefore, reduce the risk for the development of type 2 diabetes.

In the present study, BMI did not prove to be a factor independently associated with the development of NASH, a fact that was also verified in other studies on samples of morbidly obese individuals. Differently, other studies have demonstrated associations of this factor with the pathology, including more serious outcomes such as liver fibrosis and cirrhosis. A fundamental difference between these studies that found an association with BMI is the fact that they were carried out in samples including a BMI between normal and high. On the other hand, the three studies mentioned in the first, which did not find the referred correlation, whose results we equated, were carried out in samples of morbidly obese patients. In the present study, WHR and waist circumference also did not show significance as factors associated with greater severity in the NAFLD spectrum of the morbidly obese. We can infer from these results that, as the sample is more homogeneous in relation to these factors (all are morbidly obese), anthropometric aspects may lose relevance in this group of patients.

We found that dyslipidemia, as a whole, was not shown to be a factor independently associated with NASH in the morbidly obese. Although, in the univariate analysis, high levels of triglycerides showed a statistically significant correlation with this variable, in the adjusted analysis, hypertriglyceridemia did not remain as a factor independently associated with NASH, in contrast to what has been demonstrated in other studies.

Hypertriglyceridemia was identified as an independent factor associated with NASH and was included in a clinical-biological score composed of four independent variables: BMI, age, ALT and triglycerides (BMI, age, ALT, triglycerides - BAAT), proposed by (Ratziu *et al.* 2000).

(Dixon *et al.* 2001) proposed another score, based on their results, including arterial hypertension, elevated ALT and insulin resistance (hypertension, ALT, insulin resistance - HAIR), the three independent predictors, found by the authors, for the development of more severe forms of EHNA. These authors, in their considerations, comparing their findings with those of raised the possibility that hypertriglyceridemia could be a more relevant factor in the category of patients with a lower BMI. In relation to the findings by (Ratziu *et al.* 2000), the authors considered the possibility of a selection bias for this factor, as the patients in this study, in a significant proportion, were selected through the detection of elevated aminotransferases, when submitted to investigation due to dyslipidaemia.

In the present study, the dyslipidaemia and BMI variables were introduced according to the same criteria of other authors, morbidly obese patients undergoing bariatric surgery, and it is confirmed the no independent association of triglycerides with NASH in the morbidly obese population.

Retrospective study by (Angulo *et al.* 2007), who investigated factors associated with fibrosis, in patients with a histological diagnosis of NASH, evaluated hyperlipidemia with which they did not find a statistically significant association, but triglycerides showed an association with the degree of hepatic fatty infiltration.

Although in some studies older age has been identified as a risk factor for more severe pathology, which could be justified by a longer period of illness, this aspect was not relevant in our sample. We observed that the three cited studies found a correlation between age and the appearance of fibrosis and the mean age of the three studies was: 50.5; 48.9 and 41.1, respectively. In their work (Angulo *et al.* 2007), found based on the ROC curve, significance of the age aspect from forty-five years old. The logistic regression analysis of the data by (Ratziu *et al.* 2002) pointed to age $\geq$50 years as one of the independent predictive factors for septal fibrosis.

In our study, in relation to the age factor, we observed that the mean age of our sample (38.4) was lower than that of the studies mentioned above, and the prevalence of perisinusoidal fibrosis was very low. As these are cross-sectional studies, causal relationships cannot be established. Taking into account, however, the associations of fibrosis and older age in these studies, the low mean age in our sample may be a determining factor for the low percentage of fibrosis. Among the histological alterations found in NAFLD, Mallory's bodies, detected in nine of the sixty cases (15%), were the most associated with the variables studied. The presence of this histological finding in NAFLD has been reported with wide variation, as low as 9.5% and as high as 90%, in adult series. In the present study, the presence of Mallory bodies was shown to be independently associated with dyslipidaemia and ALT. It has been discussed the hypothesis that free fatty acids would act at the intracellular level as a detergent, causing the rupture of cytoskeletal membranes with underlying formation of Mallory bodies. The presence of Mallory bodies in NAFLD seems, therefore, to be a consequence of alterations at the metabolic level. Hepatocytic ballooning, histological aspect corresponding to lytic degeneration of hepatocytes, showed an independent association with age, and values close to statistical significance were found for the independent association of ballooning with type 2 diabetes.

5.2 ALD Category Studies

5.2.1 Alanine aminotransferase

The results indicate that ALT activity does not differ between individuals from 38.76 U/L to 41.58 U/L) ($P>0.05$); but there is a highly significant difference between healthy (10.75 U/L) and alcoholics (69.59 U/L) ($P\leq0.01$). According to different authors, normal ALT levels vary from laboratory to laboratory, but they range between 0 and 40 U/L, a range in which the ALT activity of healthy individuals of the present is found. study (10.75 U/L), but the value of the alcoholic group (69.59 U/L) is above the upper reference limit. The high ALT activity in people with alcohol abuse and dependence in this study would be directly attributed to the liver damage suffered by alcoholics. As is known, ALT is a cytoplasmic enzyme, highly concentrated in the liver, which is why it is more specific for liver injury than AST, and when there is injury to these organs, ALT is released into the blood and appears elevated in tests, as stated by (Burti *et al.* 2021).

Also state that the level of ALT in the blood increases when liver cells are being damaged or destroyed at a faster rate than normal by alcohol, drugs and other substances; Likewise, the death of liver cells also increases the level of ALT, therefore, ALT activity in serum can be correlated with the degree of cell death or inflammation of the liver, which can be used to assess the extent of liver damage. In this regard, (Kuntz and Kuntz 2006) point out that ALT elevation is the most sensitive indicator of hepatocellular damage.

(Satapathy *et al.* 2004), when studying ALT levels in alcoholic cirrhosis, found values of 85 IU/L, a value that is slightly higher than that found in the present study. Herrera et al (1999) when studying the effect of chronic alcoholism on ALT activity in rats, found that "alcoholic" rats presented statistically higher values (53.96 IU/L) than controls (28.62 IU/L), a result similar to that of humans, the object of this study.

5.2.2 Aspartate aminotransferase

AST activity, like ALT, was not different between individuals 79.34 U/L to 84.71 U/L) ($P>0.05$), but there was a statistical difference between healthy people (14.59 U/L) and alcoholic people (149.46 U/L) ($P\leq0.01$). The normal range of AST in serum, according to (Detmer *et al.* 1997, Kuntz and Kuntz 2006) varies between 0 and 35 IU/L, a range in which the one found in the present study is found for the case of non-alcoholic people (14.59 U/L). Similarly, (Romero *et al.* 2006) reports levels of 120 to 500 U/L for patients with alcoholic hepatitis, a range in which the AST activity of the alcoholic patients in this study is found (149.46 U/L). On the other hand, (Satapathy *et al.* 2004) found values of 160 U/L in alcoholic cirrhosis, a value slightly higher than that found in the present study for the case of alcoholics.

As in the case of ALT, the high activity in alcoholics is attributed not only to liver damage, but also to the fact that several organs and tissues are being damaged by the direct effect or by the metabolism of alcohol, considering that AST is present in the heart, liver, skeletal muscle and kidneys, as pointed out by (Braunwald *et al.* 2001). Other authors such as (Lara-Carrillo 2012) state that when the cells containing AST are injured or the permeability of the cell membrane is altered, it is released into the plasma along with other enzymes, increasing its serum concentration, so elevated AST levels will also be found in cardiomyopathies, myopathies, kidney disease, and other diseases.

5.2.3 γ-glutamyl transpeptidase

Unlike transaminases, the levels of GGT found in the present study are statistically higher in the inhabitants from 70.58 U/L to 66.39 U/L) (P ≤0.05). Likewise, GGT activity is significantly higher in alcoholics (121.01 U/L) than in non-alcoholics (15.95 U/L) (P≤0.01).

The normal range of GGT is between 12 and 55 IU/L and according to (Kuntz and Kuntz 2006) for men it is <60 IU/L. The GGT activity for healthy individuals was 15.95 U/L, a value that is within the range established by the two indicated authors. On the other hand, (Satapathy *et al.* 2004) reports a value of 240 IU/L of GGT in alcoholic cirrhosis, a value higher than that found in the alcoholic group (121.01 U/L) of the present study.

Since GGT is the most widely used biochemical marker in the diagnosis of chronic alcoholism, the high GGT activity in alcoholics found in this study confirms liver damage due to the effect of alcohol and its toxic metabolites and is also an indicator that these per- Some would be suffering from hepatobiliary diseases. This assumption finds support in the versions of (Kuntz and Kuntz 2006) who indicate that alcohol induces liver damage which raises serum GGT after regular and prolonged alcohol consumption and, therefore, GGT has become the most widely used biochemical marker for alcohol, although it is not specific, since between 20-30% of alcoholics have normal GGT values. (Takahashi *et al.* 1993) in his experiments support the results found, since the author found that the levels of GGT in rat plasma increased after chronic alcohol consumption, in the same way, volunteers who ingested ethanol increased their GGT in 6 to 7 times, concluding that increases in GGT in liver and plasma are induced by ethanol. (Kumar and Clark 2005) Also confirm the assumption by indicating that in the case of liver disorders, GGT is an indicator of toxic aggression, however, given its non-specificity, the determination of GGT only has clinical value when its values are compared with those of other enzymes with higher specificity. The latter is ratified by (Romero *et al.* 2006) who indicate that as a marker of alcoholic liver disease, GGT is deficient when used alone, being safer when combined with transaminases. In our case, the three transferases were elevated, confirming the alteration of the liver due to the effect of alcohol.

However, it must be considered that the elevation of GGT is also elevated in other alterations. (Kuntz and Kuntz 2006) point out that GGT is elevated in cholestasis, porphyria, pancreatitis, pancreatic cancer, myocardial infarction, nephrotic syndrome, diabetes mellitus, nicotine abuse, among others. In such a way that the alcoholics studied could concomitantly be suffering from any of these diseases as a direct or indirect consequence of the alcoholism they suffer from.

5.2.4 Alkaline phosphatase

In this regard, the results indicate that the serum levels of ALP between individuals from 238.98 U/L to 239.08 U/L do not differ statistically ($P>0.05$). But there is a difference between groups of patients with different clinical conditions, being higher in alcoholics (357.46 U/L) than in non-alcoholics (122.50 U/L) ($P\leq0.01$).

The normal range for serum ALP activity is between 40 and 130 IU/L (Kuntz and Kuntz 2006). The serum activity found in healthy people (121.50 U/L) in this study is within the indicated range.

The superiority of ALP activity in alcoholics is also attributed to liver dysfunction suffered by alcoholics, since, as pointed out by (Caballería 2000) in alcoholic liver disease it is increased, being a sign of obstruction. Although ALP is distributed in many tissues and organs, the liver is the main source of ALP present in serum, which is why it is a biochemical marker of liver disease. Mention that under pathological conditions, ALP is a sensitive marker for the diagnosis of hepatobiliary diseases and cholestasis, diseases that, as stated above, would also be present in the alcoholics studied (Dawson *et al.* 2002).

5.2.5 AST/ALT ratio

The ANVA shows that there is no statistical difference in the AST/ALT ratio between 1.76 to 1.80 ($P>0.05$). But the clinical condition of the patient has a significant effect on this ratio ($P\leq 0.01$), being higher in alcoholics (2.18) than in healthy people (1.39). This means that in alcoholics there is a greater increase in AST than ALT, (Wilson 2007) confirms this statement by pointing out that when the AST/ALT ratio rises, the necrotic involvement is deeper and more severe. (Kumar and Clark 2005) Explain that this is due to the location of the enzymes, while ALT is located exclusively in the cytoplasm, AST is more mitochondrial (80%) than cytoplasmic (20%), so that when there is serious involvement tissue, as in necrosis, there will be release of mitochondrial enzymes into plasma.

Corroborate the fact that the elevated enzymatic activity found in alcoholics is a sign of great liver damage, since when there is damage to the liver cell or some interference in the bile flow, the aminotransferases and ALP are elevated, as found in the study (Braunwald *et al.* 2001).

Another reason why the quotient is greater than 2 in alcoholics is attributed to nutritional deficiencies or malnutrition suffered by these people. As point out, an elevation of the ratio to >2 is suggestive of alcoholic liver disease; a low serum ALT level is due to alcohol-induced pyridoxal phosphate deficiency, which raises the AST/ALT ratio (Braunwald *et al.* 2001).

It is likely that many alcoholic patients who have been included in the study have a cirrhotic phase. In this regard, (Cotran *et al.* 1999) indicate that in this phase serum transaminases are elevated, there is hyperbilirubinemia and an increase in ALP, hypoproteinemia and anemia. Also, (Kasper *et al.* 2015) indicate that in cirrhosis the AST/ALT ratio is > 2; (Pushilal and 2009) state that the increased activities of transaminases in cirrhosis vary with the degree of the cirrhotic process and rise between 4 and 5 times higher than the upper limit, with an AST/ALT ratio greater than 1, such like the one found in the present study.

5.3 Enzymatic Correlation

The high and positive correlation existing between the five enzymes of the present study ($P \leq 0.01$) would be suggesting that the liver, as well as other organs and systems, in healthy people function adequately while in alcoholic subjects the Liver injury is taking place because its function is being impaired by the damage it has been suffering.

In this regard (Coila Añasco 2010). and Seilnacht K. indicate that in alcohol-induced hepatitis the enzymes GOT and GPT are increased, GGT very intensely increased, and cholinesterase decreased, results that agree with those found in the present study.

mentions that the liver, as a fundamental organ in metabolism and detoxification, is subject to the direct toxicity of ethanol and its degradation products; In addition, autoimmune mechanisms can contribute to its damage. This leads to liver failure which, although it may be due to sudden and massive destruction of liver tissue, is usually the final stage of progressive organ damage (Cotran *et al.* 1999).

Point out that chronic alcohol consumption produces multiple biochemical changes in liver metabolism, which can vary in intensity in individuals. In addition to changes in hepatic metabolism, morphological changes are observed at the subcellular and cellular levels, depending on the amount and duration of alcohol consumption. This leads to an aggravation of liver damage and ultimately to extensive transformation processes (Kuntz and Kuntz 2006).

(Beers and Berkow 2000) mention that alcohol is a hepatotoxin whose metabolism causes profound liver cell alterations. Concentrations above 25 mmol/L of ethanol in portal blood produce focal hepatocellular necrosis, in part due to vasoconstriction. hepatic. Therefore, this ethanol-induced microcirculatory disturbance may be involved in the pathogenesis of alcoholic liver disease.

In conclusion, the results found in this study allow us to deduce that there is no effect of environmental altitude on liver damage and function, both in healthy individuals and in individuals suffering from alcoholism. This means that liver damage and impaired liver function caused by alcohol.

However, it is necessary to take into account what was indicated by Braunwald *et al.* that no test allows to correctly evaluate the total functional capacity of the liver since this liver carries out thousands of biochemical functions, most of which they cannot be easily measured by blood tests, and laboratory tests measure only a limited number of these functions (Braunwald *et al.* 2001).

6 CONCLUSIONS AND RECOMMENDATION

6.2 Conclusion

The comparison of the nutritional and dietary assessment and the inflammatory profile of chronic alcoholic patients with pancreatic injury (ALD Group -A) and without pancreatic injury (NAFLD Group -B), with non-alcoholic individuals (Group-C) showed:

1. Chronic alcoholic pancreatitis (Group A) was not associated with a drop in dietary intake, however, even so, there was a reduction in lean mass, evidencing protein malnutrition.
2. Non alcoholics with pancreatitis (Group B) had lower lean mass compared to patients in Group C
3. There was success in demonstrating that body composition and inflammatory profile are distinct and relevant, not only in alcoholic chronic pancreatitis (Group A), but also in alcoholics without pancreatic injury (Group B), which should also be better studied and monitored. throughout its clinical course.

Based on the above results and discussion following conclusion were made in the current study:

- Patients with NAFLD had a BMI characteristic of obesity, while patients without NAFLD were overweight, confirming that obesity is the common clinical finding on physical examination.
- The measurement of abdominal circumference reveals the presence of a very high cardiometabolic risk in individuals with NAFLD. The occurrence of hypercholesterolemia, hypertriglyceridemia and low HDL-c further contributes to the risk of developing cardiometabolic events.

- The serum glucose levels of the NAFLD group were higher than the comparison group, suggesting that there are alterations in the glucose metabolism of patients with this pathology.
- Blood concentrations of ALT, AST, GGT and FA were higher in NAFLD patients, supporting the need to verify these data in the biochemical investigation of these patients.
- A positive association was found between NAFLD and the presence of comorbidities such as: diabetes mellitus, abdominal obesity and metabolic syndrome. However, our findings showed no association with arterial hypertension. This reflects an increased risk of morbidity and mortality for these patients if these changes are not treated.
- Patients with NAFLD had higher caloric, carbohydrate and protein intakes than the group without NAFLD. However, there was no difference in lipid consumption. These data suggest abnormalities in the food consumption of these individuals, facilitating positive energy balance and the accumulation of total body fat.
- Correlation analyzes confirm the relationship between excess weight and the risk of developing cardiometabolic events. As for liver enzymes, the results suggest a relationship between NAFLD and excess weight. The increased food intake of calories, carbohydrates and proteins confirms the relevance of excessive food consumption in weight gain.
- The correlations between AC and lipid and glucose profile suggest that this anthropometric marker of abdominal obesity is quite useful in determining the risk of developing comorbidities for these patients. And the correlations between CA and the analyzed liver enzymes make evident the involvement of abdominal obesity as a risk factor for NAFLD. As for the correlation between AC and food consumption, it is suggested that excessive nutrient intake predisposes to the accumulation of body fat, especially in the abdominal region.
- In view of the above, complementary and specific studies are needed on the relationship between NAFLD and food consumption variables, using their own sample and methodological designs, in order to clarify with greater certainty, the discordant results of the literature.

- We suggest the development of research aiming to deepen the knowledge of etiological factors related to NAFLD, since epidemiological studies of the association type only assume direct and indirect risk factors for the occurrence without explaining its causal mechanism, as well as complementary studies aimed at deepening the points still open about the relationship of NAFLD, as well as additional research with the aim of establishing the resulting medium and long-term effects for patients with different stages of the disease.
- In the area of service provision, it is important to consider prevention, diagnosis and treatment as a matter of routine care, ensuring material resources and training of personnel so that these objectives are put into effect. And that, contrary to what the norms of most health services establish, the nutritional assessment (anthropometric and food consumption) of patients with steatosis should be considered in its own way as a unique and systematic component of cardiometabolic risk assessment and that the NAFLD is included in the discussion group for structuring the specific guideline for monitoring services.
- Effect of alcoholism on enzymatic activity: Chronic alcoholism greatly increases the activities of: ALT, AST, GGT and ALP in blood serum, their activities being higher in relation to the enzymatic activity of non-alcoholic people ($P \leq 0.01$). In contrast, CHE activity is lower in alcoholics than in healthy people ($P \leq 0.01$).
- The AST/ALT ratio is significantly higher in alcoholics (2.18) than in non-alcoholics (1.39) ($P \leq 0.01$).
- There is a high and positive correlation between the five enzymes considered in the study ($P \leq 0.01$), except for cholinesterase, which is high and negative with the other enzymes.

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