

## Neoplastic Effects of Endogenous Alcohol in Carcinogenesis Process of Hepatocarcinoma

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### Abstract

**Introduce:** Based on the grim statistics, the mortality of alcoholic liver disease was in last years the high in the undeveloped countries but and in developed countries in the statistic compared with last years.

**Aim:** Scope of this work was to discover the patients diagnosed with alcoholic cirrhosis which in time was transformed in Hepatocellular Carcinoma, (HCT) by continue abuse alcohol and which presented, at a previous medical control, with the diagnosis of steatohepatitis after the chronic abuse of alcohol.

**Methodology:** The retrospective study consisted of 180 patients, adult men (mean age 55 years) and females (mean age = 48) hospitalized and investigated for alcoholic liver diseases. From total cases investigated, 54 cases (30% from of cases admitted in hospital, meaning 27 men and 17 women), which were diagnosed with alcoholic liver cirrhosis in last 5-7 years but in next medical controls were discovered liver cancers in the 6.1% percent.

**Results:** The majority of patients with uncompensated liver cirrhosis, (70%), have had elevated AST in average 66 U/L value, (N= 5-38U/L). Also, they have had an AST/ALT ratio 1.36, (N = 1.33) and ratio TGP/TGO = 0.95. In the liver carcinogenesis process the report AST/ALT increased in 2.64 value and ratio TGP/TGO decreased in 0.67 value.

**Conclusions:** The active and rational lifestyle is an essential element in preventing the process of carcinogenesis. Liver disease it's not just how much you drink, but how and when you drink. The treatment of severe alcoholic hepatitis will be necessary in all alcoholic liver diseases centers to prevent hepatic cancer disease.

**Keywords:** Alcoholic Liver Disease, Cytokines, Nutrition, Steroids, Transplantation.

### Abbreviations

ALDH2- acetaldehyde dehydrogenase 2; ALD-alcoholic liver disease; AMPK-AMP-activated protein kinase; AGS-acetaldehyde-generating system; ALC-alcoholic liver cirrhosis; CYP2E1-cytochrome p450; ECM- extracellular matrix; HCC-hepatocellular carcinoma; HBV: hepatitis B virus; HCV: hepatitis C virus;

### Introduce

Alcoholic cirrhosis due to long-term chronic alcohol abuse is on the rise, especially in developed countries. Cirrhosis caused by alcohol requires frequent hospitalizations to prevent the complication of transforming liver cancer and at the same time creates high costs in the public health system [1, 2]. Cirrhosis of

the liver occurs in about 30% of people who consume alcohol for a long time beyond the allowable daily physiological limits. The disease occurs mainly in men who have consumed large amounts of alcohol daily for more than 10 years, [3].

Indirect alcohol biomarkers, which suggest heavy alcohol use by detecting the toxic effects of alcohol, include the following: Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Gamma glutamyl-transferase (GGT), Mean corpuscular volume (MCV). Carbohydrate-deficient transferrin (CDT) which is a biomarker for chronic alcohol consumption of over 60 g ethanol / day. It was considered that the normal limit value is 31.9 U / l for women and 23.6 U / l for men. Elevated CDT levels have been reported in cases of chronic alcohol abuse, with varying degrees of liver damage [4].

A blood alcohol level measured after drinking in the last few hours is not a good indicator of chronic alcohol abuse. [5]. Consumption of alcohol at doses up to 10 g / 1 unit / day after the onset of cirrhosis leads to the development of hepatocellular carcinoma. Also, this amount of alcohol is a risk factor for the development of liver cancer if there are other conditions, such as diabetes or viral hepatitis B or C viruses.

Obesity and diabetes also have a synergistic effect. As in other causes of advanced chronic liver disease, it is recommended that patients with alcoholic cirrhosis should be included in HCC surveillance program so that any tumor can be detected at the earliest possible stage: this strategy has been shown to enable the implementation of curative procedures that can increase survival, [6].

A normal drinking is defined as one 12-oz beer, one 4- to 5-oz glass of wine, or one mixed drink containing 1.5 oz of spirits (80 proof), [1 Oz = 29.57 ml]. The relative risk for the noted maladies with consumption of 4 or more drinks daily is as follows: Cirrhosis - for men, 7.5; for women, 4.8. Drinking outside of meal times increases up to 3 times the risk of alcoholic liver disease. Among older patients with alcoholism, from one third to one half develop alcoholism after age 60 years. Alcohol levels are higher in elderly patients for a given amount of alcohol consumed than in younger patients [7]. Oxidative stress induced by chronic ethanol consumption affects phospholipids and fatty acids in the cell membrane as well as  $\alpha$ -linolenic acid with damage to cell structure [8, 9].

### Aim

By this work of researches, the authors proposed to emphasis, at patients diagnosed with alcoholic cirrhosis, number of cases which

developed Hepatocellular carcinoma, [HCC], by continued of the abuse alcohol and which presented, at a previous medical control, the diagnosis of steatohepatitis after the chronic abuse of alcohol.

### Method

The retrospective study consisted of 180 patients, adult men (mean age 55 years) and females (mean age = 48) hospitalized and investigated for alcoholic liver diseases. From total cases investigated, 54 cases (30% from of cases admitted in hospital, meaning 27 men and 17 women), which were diagnosed with alcoholic liver cirrhosis in last 5-7 years but in next medical controls were discovered liver cancers in the 6.1% percent. In the gastroenterology ward, patients with compensated cirrhosis initially presented with hepatomegaly and / or splenomegaly and at the anatomopathological examination different initial degrees of progressive hepatic fibrosis.

Laboratory analyzes were performed on the Coulter LH 750 hematology analyzer with 26 parameters and on the Vitros, Johnson & Johnson dry biochemistry analyzer.

### Results

The results of liver enzymes in alcoholic hepatocellular carcinoma are shown in Table 1. Most patients with uncompensated liver cirrhosis (70%) had an AST increased by an average of 66 U / L, (N = 5-38U / L). These cases had an AST / ALT ratio of 1.36 (N = 1.33) and the TGP / TGO ratio = 0.95. In the process of liver carcinogenesis, the AST / ALT ratio increased by 2.54 and the TGP / TGO ratio decreased by 0.39. Increase in total bilirubin above 5.6 mg / day L, prolongation of prothrombin time (PT), INR above 4.53 mean value and hypoalbuminemia below 1.2 mg d / L, mean value were markers in the progression of cirrhosis to liver cancer, [Table 1].

**Table 1: Results of liver enzymes in alcoholic Hepatocarcinoma**

Results of cases Mean Value ( $\sum X^-/n$ )	Reference Intervals Adult Females	Reference Intervals Adult males
Platelets, $X^- = 133 \times 10^3 \mu l$ ..... INR = 4.53	140 - 450.0 $\times 10^3 \mu l$ ..... INR = 1.02	140 - 300 $\times 10^3 \mu l$ ..... INR = 1.2
AST = 379.9 U/L ..... ALT = 149.2 U/L	14-36 U/l ..... 9-52 U/l	17-59 U/l ..... 17-59 U/l
LDH = 650 U/L	135-225	135-225
GGT = 123.8 U/L	8-78 U/l	8-78 U/l
Total Bilirubin = 5.6	0,2-1,3 mg/dl	0,2-1,3 mg/dl
ALP = 250 U/L	38-126 U/l	38-126 U/l

The control group of 40 potential health persons, (20 adult men and 20 adult females), presented next follow results in biochemistry field, on Analyzer dry chemistry, Vitos 250, Johnson&Johnson, next results:

Creatinine = 0.7-1.2 mg/dl, [SD=0.15, CV%=29, accuracy [Z] =-1.36]; Iron = 70-100 microgram/dl, [SD=2.88, CV%=1.8, Z=-0.56]; Phosphate = 27-29 mEq/dl, [SD=0.14, CV%=2.2, Z=-0.8]; Urea = 35-40mg/dl, [SD=2.40, CV=2.2, Z=-0.13]; Uric acid = 5-6mg/dl, [SD=0.26; CV=3.2, Z=-0.79].

Patients with cancer liver disease in hematologic field have had leukocytosis cell [(WBC) count =  $13.9 \times 10^3/\mu\text{l}$ , SD = 2.66] and thrombocytopenia (Platelets,  $X^- = 133 \times 10^3/\mu\text{l}$ , SD = 1.91), in 70% from total cases. Leukemoid reactions with counts of  $>100,000$  white blood cells (WBC)/mm<sup>3</sup> in the absence of infection has been seen in patients with hepatocarcinoma was in percent of 10%. The severity of chronic alcoholism is assessed using the Maddrey (MDF) formula, which is calculated by the equation,  $[4.6 \times (\text{PT patient} - \text{PT control}) + \text{total bilirubin (mg / dl)}]$ . If the result of the calculation exceeds the absolute value 32, mortality over time hospitalization of patients can reach over 50%. In the present study, MDF was calculated to have an average value of 20.91,  $[4.6 \times (4.53-1.2) + 5.6]$ .

Mean corpuscular volume (MCV), serum uric acid levels and serum electrolytes are all affected by chronic alcohol consumption. In addition, although symptoms may be nonspecific, increased serum uric acid, hypokalemia, hypomagnesemia and acidosis are indicators that alcohol may play a significant role in liver

disease. Alcohol related bone marrow toxicity and/or splenic sequestration might contribute to macrocytosis (increased MCV) and thrombocytopenia. Leukocytosis is frequent in individuals with alcoholic liver cancer.

## Discussions

Hepatocellular carcinoma is the fifth most common neo-plasm and the third most frequent cause of cancer death. Early stages of hepatocarcinoma development, autophagy acts as a suppressor mechanism, promoting the recycling of defective organelles and unfolded proteins, prevention of oxidative stress, and maintenance of genome stability [10, 11]. However, autophagy can also favor tumor promotion via oncogene-mediated cancer development, and cellular adaptation to different stress, such as hypoxia or starvation [12-15].

Although the crosstalk between autophagy and apoptosis not well defined, a relationship has been established due to the interaction of different autophagy and apoptosis-related proteins [16]. Isolated hyperbilirubinemia as a manifestation of alcoholic liver disease without significant liver abnormalities is rarely observed. Bilirubin may gradually decrease when alcohol consumption is stopped [17]. Acetaldehyde, the result of alcohol metabolism causes liver fibrosis by the deposition of collagen fibers in liver cells. The enzyme alcohol dehydrogenase acts through two molecular mechanisms for ethanol metabolism. The first is the use of a zinc atom used to maintain and position the alcohol group on ethanol and the second is a nicotindiamian cofactor (NAD) that completes the reactions.

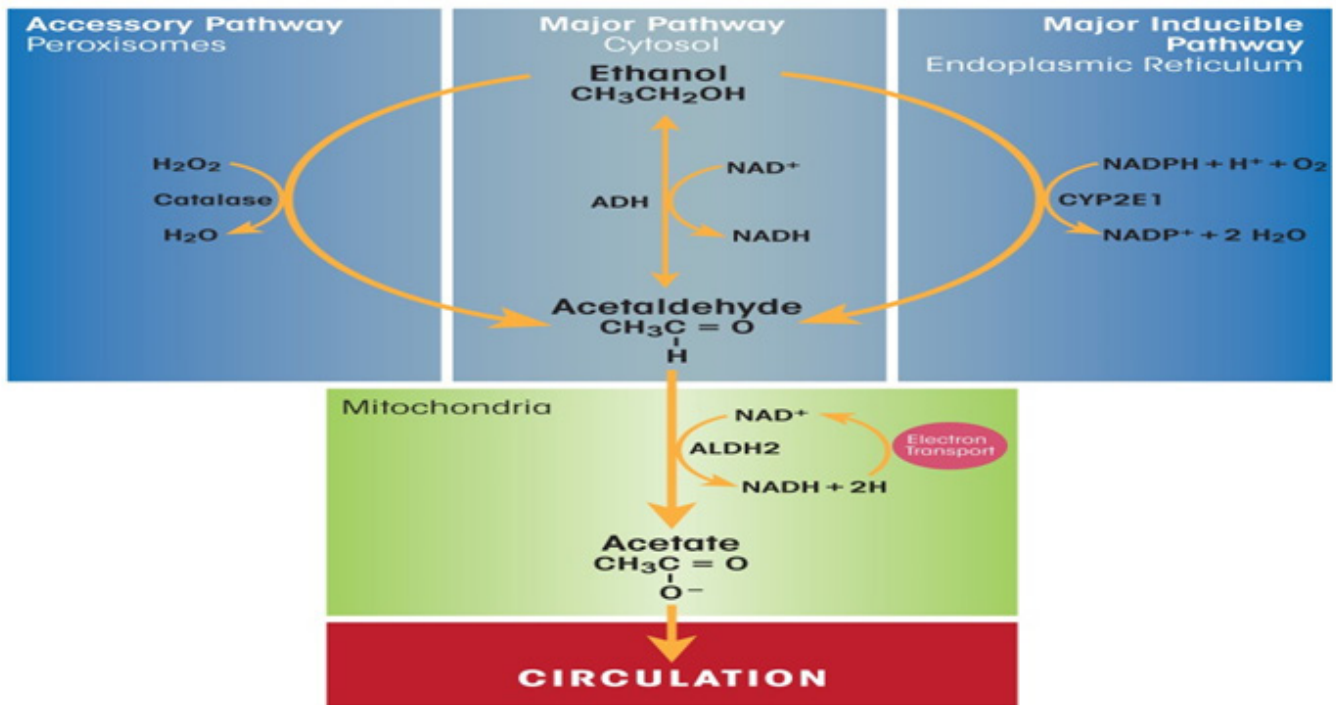


Figure 1: Major pathways of alcohol metabolism in hepatic cells

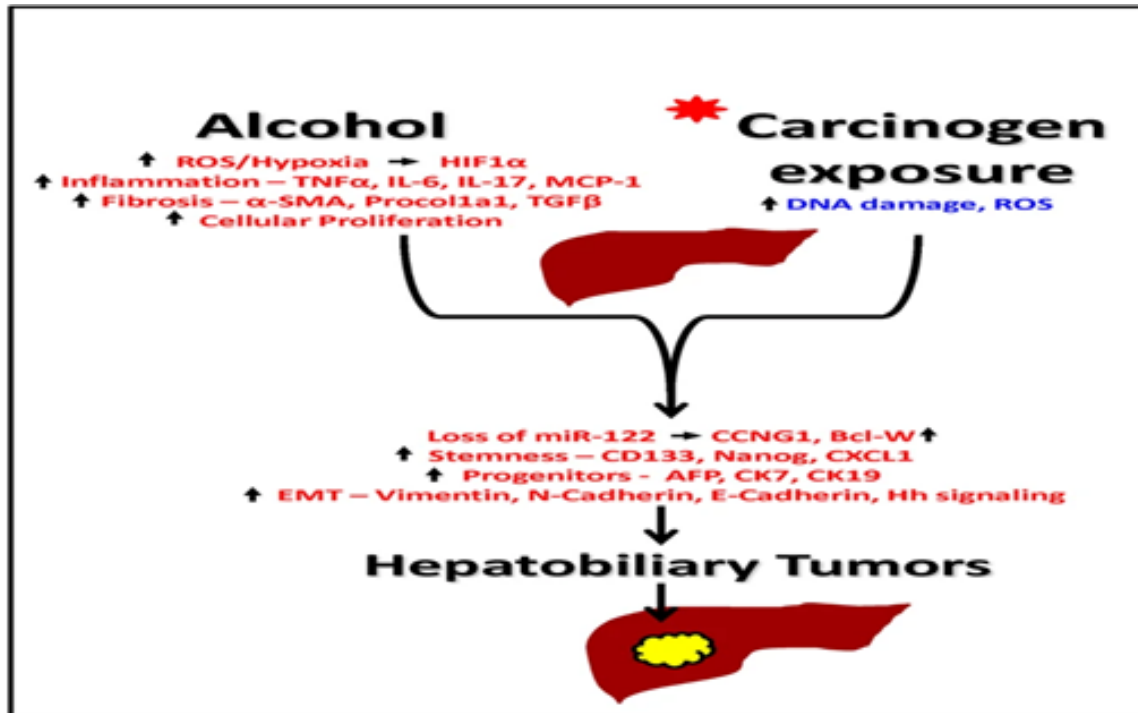
Acetaldehyde is further oxidized to acetic acid and CO<sub>2</sub> and water through the Krebs cycle.

This pyruvic acid, as a result of transamination amino acids, is converted to glucose by gluconeogenesis. People who consume large amounts of alcohol can become overweight through excess NADH which can be used to synthesize glycerol and to synthesize fatty acids with increasing lipid synthesis.

3. NADH in the electron transport chain can also be used for the synthesis of adenosine triphosphate, (ATP), as a source of cellular energy.

Ethanol can inhibit the activity of genes for the synthesis of inducible nitric oxide, in response to the stimulation of bacteria by inhibiting the activity of macrophages, contributing to the contribution to the impairment of antimicrobial defense after alcohol consumption.

The increase in proinflammatory cytokines, IL-6, IL-17, tumoral necrosis factor, (TNF-alpha), plays a central role in the pathophysiology of alcoholism.



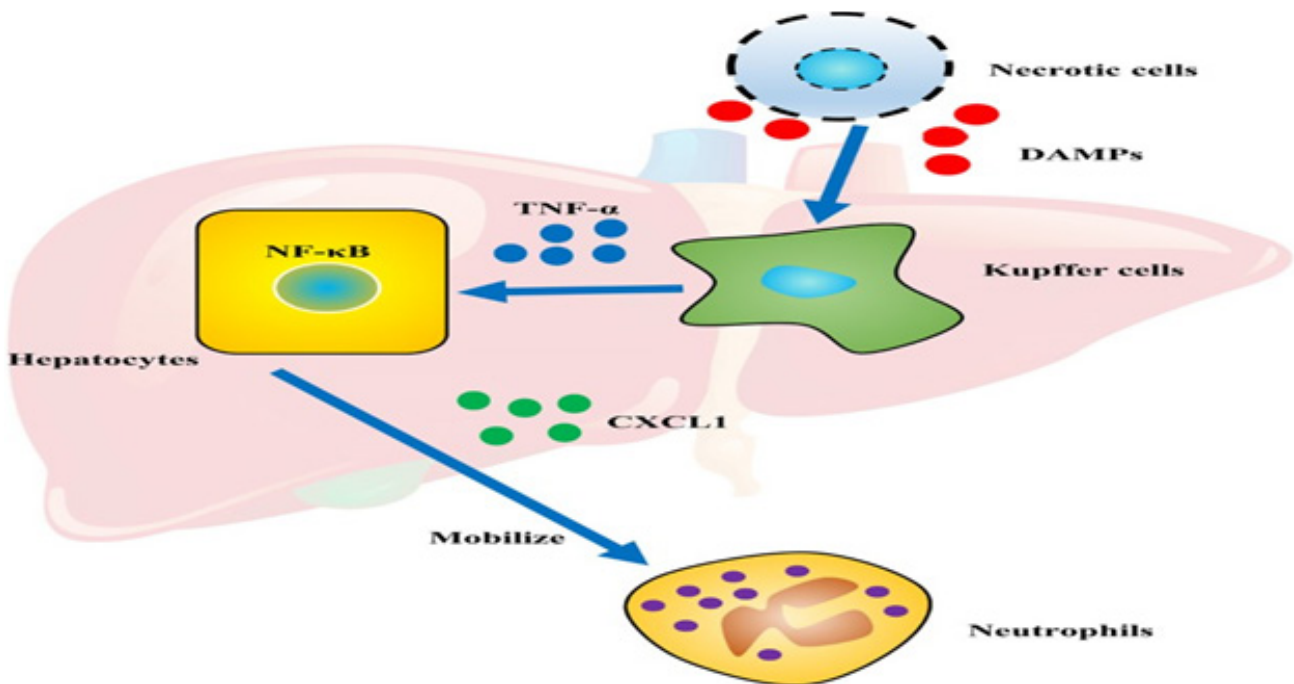
**Figure 2:** Increased proinflammatory cytokines, IL-6, IL-17, particularly TNF alpha, play a central role in the pathogenesis of Alcoholic Liver Disease

In recent years, microRNAs, miR-155, have been found to help increase TNF-alpha production and sensitize liver cells to produce more TNF-alpha in response to liposaccharide activation (LPS) [14]. LPS endotoxin is recognized by the Toll-like receptor complex (TLR) -4 on macrophages or Kupffer cells in the liver and contributes to the production of proinflammatory cytokines leading to liver damage. Precursors to carcinogenesis.

Polymorphisms exist in the enzymes ADH, CYP2E1, and ALDH. Differences in ADH and ALDH certainly contribute to the negative association with ethanol dependence in some Asian populations.

HLA phenotypes, a genetic predisposition toward alcoholism and female gender may also contribute to overall risk.

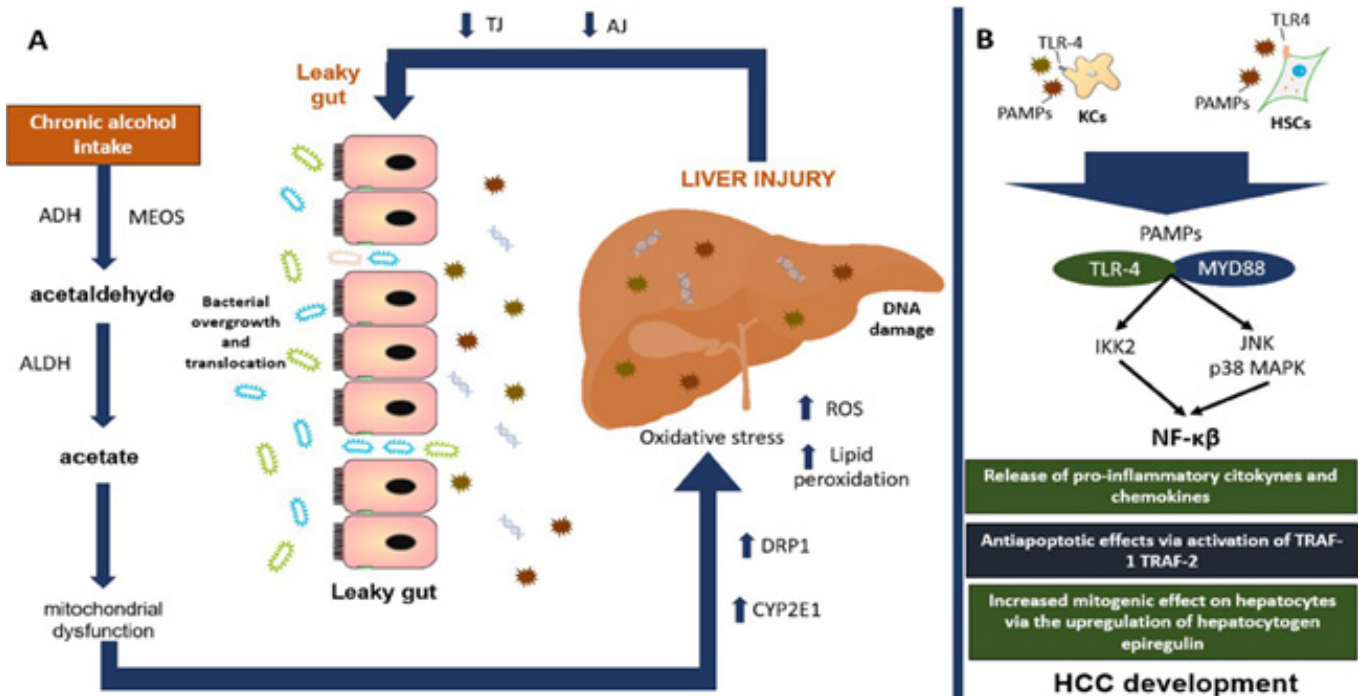
Chronic alcohol abuse increases gut permeability resulting in high circulating endotoxin that reaches the liver via portal circulation. Endotoxin (lipopolysaccharide or LPS) is recognized by the Toll-like receptor (TLR)-4 complex on resident macrophages or Kupffer cells in the liver, leading to production of proinflammatory cytokines, tumor necrosis factor (TNF)-α, and resulting in injury to liver cells (hepatocytes) [15, 16].



**Figure 3:** The typical inflammatory cytokines, such as TNF- $\alpha$ , IL-1, and IL-6, are primarily produced by inflammatory monocytes, macrophages.

Chronic alcoholics have elevated levels of immunoglobulins, especially in IgG and IgA classes with B cell dysfunction. Produce inflammatory cytokines. Recent studies have also shown that

decreased T cell proliferation after chronic alcohol abuse could be caused by impaired monocyte function by producing inflammatory cytokines [18].

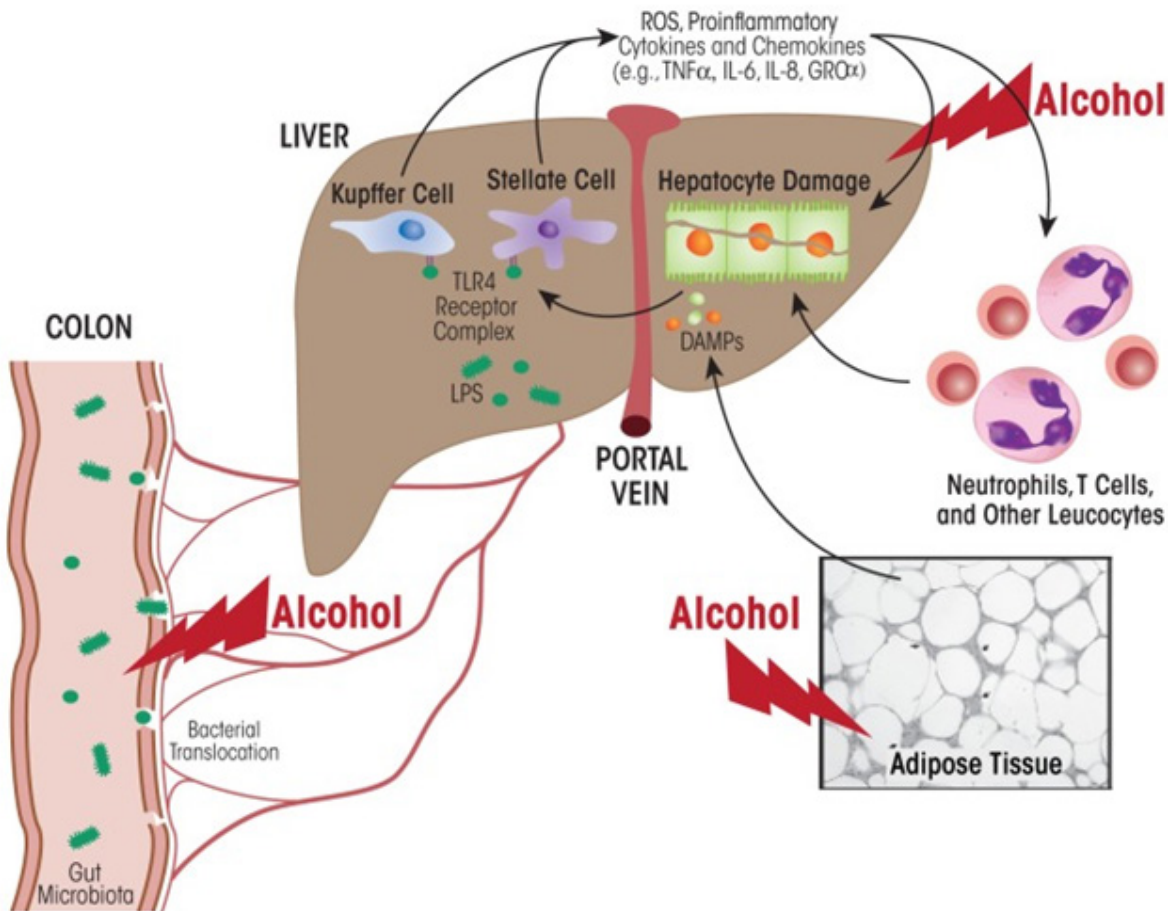


**Figure 4:** Alcohol-induced steatohepatitis is tightly associated with LPS-TLR4 signaling. Among BM-derived cells, Kupffer cells/macrophages highly express TLR4 and produce inflammatory cytokines.

The enzyme ALDH is mainly involved in liver cancer. Overexpression of ALDH enzymes, such as ALDH-1, ALDH-3A1 and ALDH-18A1, gives cancer cells a survival advantage because oxidative stress from high metabolic activity leads to free radical generation (ROS), lipid peroxidation and aldehyde accumulation. Toxic, which can inhibit the proliferation and survival of cancer cells.

As an antioxidant, the enzyme ALDH can decrease apoptosis of immunogenic cells and limit tumor progression by reducing the stress of the endoplasmic reticulum and the production of ROS.

The reduced activity of ALDH-, B1, was found to be protective in patients with hepatocellular carcinoma (HCC), by influencing the oxidation of short-chain aldehydes, including acetaldehyde and propionaldehyde, against hepatocellular proliferation induced liver neoplasm [19].



**Figure 5:** Decreased T cell proliferation after alcohol use might be due to impaired accessory cell/monocyte function

Epigenetic changes in cycle cells include histones by acetylation, phosphorylation, and hypo methylation of DNA. Dysregulation of miRNA biogenesis has been found in non-viral HCC subtypes, and oxidation of ethanol influences the expression of miR-217, miR-155 and miR-212 [20, 21].

In people with alcoholic hepatitis, promoters of liver cancer, the ratio of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) is greater than 2: 1. AST and ALT levels are almost always lower than 500.

The increased AST / ALT ratio is due to pyridoxal deficiency. Phosphate, which is required for the synthesis of the enzyme ALT.

Other laboratory observations include macrocytosis of red blood cells, mean corpuscular volume (VEM), > 100) and increases in serum levels of gamma-glutamyl transferase (GGT), alkaline phosphatase and bilirubin.

Folate levels are low in alcoholic patients due to decreased intestinal absorption, increased need for folate in the bone marrow in the presence of alcohol, and increased urinary loss.

Leukocyte depletion reflects the severity of liver damage. Histological features may include Mallory bodies, giant mitochondria, hepatocyte necrosis, and neutrophil infiltration into the area around the blood vessels [22, 23].



**Figure 6:** Steps which are in progression for liver carcinogenesis

Liver cancer is characterized by an unfavorable prognosis, with a 5-year survival rate of 18%, unless it is discovered at an early stage in the care of treatment includes surgical resection or liver transplantation. For most patients with advanced disease, the drug sorafenib was the only treatment option for systemic therapy for these patients. Currently, the standard of care for patients with advanced HCC involves a combination of immunotherapy between the control point inhibitor, atezolizumab, and the antibody bevacizumab [24].

Embolization can be used to block the flow of blood to a tumor, so that the cancer cells die. Recent studies have confirmed that moderate consumption of red wine is associated with high plasma levels of omega-3 polyunsaturated fatty acids, decreased blood viscosity, increased insulin sensitivity, decreased platelet count and aggregation, and altered plasma coagulation protein levels. HDL-cholesterol, cardio-protector [25].

As for the types of alcoholic beverages, especially wine with a variety of polyphenols, including phenolic acids, tannins, resveratrol, flavonoids, have an anti-carcinogenic antioxidant and anti-inflammatory effects, in contrast to the carcinogenic ethanol [26, 27].

Recent studies have confirmed that moderate wine consumption is associated with high plasma levels of omega-3 polyunsaturated

fatty acids, decreased blood viscosity, increased insulin sensitivity, decreased platelet count, altered plasma coagulation protein levels, and increased HDL-cholesterol. About 50% with the cardio-protective effect and inhibition of the carcinogenic process in 12 types of cancer.

### Conclusions

An active and rational lifestyle is an essential element in preventing the process of carcinogenesis. Liver cancer is not due to the amount of alcohol which a person is consuming, but of the quality alcohol consumed. Treatment of severe alcoholic hepatitis will be required in all alcoholic liver disease centers to prevent liver cancer.

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