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Introduction and Objectives: Metabolic reprogramming is a hallmark of cancer cells. Fructose metabolism is decreased in liver cancer cells to counteract the oxidative environment induced by fructose. Ketohexokinase (KHK) A is overexpressed, and its switch confers advantages to cancer cells. The **objective** was to investigate the effect of fructose metabolism on the aggressiveness of liver cancer cells.

Materials and Patients: KHK isoform expression was measured by qRT-PCR in Huh-7 and HepG2 cells. Metabolic characteristics of liver cancer cells (Huh-7 and HepG2) treated with a fructose (1mM) for 48h in a high glucose DMEM media (11mM) was developed using Mito Fuel Flex assay and Glycolysis Rate Assay using SeaHorse technology. To prove the hypothesis that fructose metabolism enhances aggressiveness, we performed proliferation and enzymatic assays. Chemoresistance assays (*in vitro* and *in vivo*) was developed using Huh-7 cells previously treated with Fructose (1mM) for 72h. Then, we applied Fructose (1mM), Cisplatin (CDDP, 22,11μM for in vitro assays or 100μM for in vivo assays) or Fructose (1mM) + CDDP (22,11μM for in vitro or 100μM for in vivo) for 48h.

Results: ***Huh-7 cells expressed higher levels of khk-a compared to HepG2 cells. The isoform switch was*** associated with improved fructose uptake and higher proliferation in Huh-7 cells. We did not detect differences in mitochondrial glucose or fatty acid oxidation capacity, but glutamine oxidation capacity was lower in Huh-7, indicating the overall dependence of this cell line on the glutamine pathway. However, we only detected differences with fructose-treated (Fru-treated) cells with less dependence on fatty acid oxidation in hepatoma cells, suggesting that fructose metabolism has a different effect with respect to the differentiation level of the cells. Next, we evaluated the glycolytic pathway in the aggressive cell line (Huh-7), and the analysis showed that Fru-treated cells contributed less to media acidification, suggesting the activation of alternative pathways by fructose. The pentose phosphate pathway was affected by fructose and inhibition of glutathione reductase abolished the benefits gained. We then assessed survival to CDDP treatment, and found that both, in vitro and in vivo, fructose treatment improved survival and resistance to CDDP therapy.

Conclusions: Fructose promotes a metabolic remodeling leading to the sustained proliferation of liver cancer cells. Specifically, fructose metabolism promotes alternative metabolic pathways that contribute to the aggressiveness of HCC cells. In addition, fructose may increase cancer cell survival and the treatment failure.

Ethics statement: All the cell lines were obtained from the American Type Culture Collection (ATCC). The material was obtained legally, ethically, and with due consideration for the well-being, privacy, and dignity of the donor.

Declaration of interests: None.

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Neutrophil-lymphocyte ratio as a prognostic factor in patients with alcoholic hepatitis.

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Introduction and Objectives: The neutrophil-lymphocyte ratio (NLR) has been used as a predictor of survival in critically ill patients. However, there are scarce studies that evaluate the relationship between NLR and alcoholic hepatitis. Thus, we aimed to determine the association between NLR with mortality and the degree of acute-on-chronic liver failure (ACLF).

Materials and Patients: Longitudinal, retrospective, observational and descriptive cohort study of a hospital center. Patients who attended from March 1, 2022, to April 30, 2024, to Gastroenterology service were included. The subjects met criteria for alcohol hepatitis established by the National Institute on Alcohol Abuse and Alcoholism: alcohol consumption >40 g/day (women) or >60 g/day (men) for six or more months, jaundice during the previous eight weeks, AST > 50 IU/L, AST/ALT ratio > 1.5, and both values < 400 IU/L, BT > 3.0 mg/dL. Patients with concomitant infections or conditions that could alter the NLR (steroid use, pancreatitis, hemorrhage, neoplasms) were excluded. Statistical analysis was performed with the SPSS version 26 program. To compare clinical values, Student's T-test or Mann Whitney U test were performed according to the distribution of the data. The association analysis between NLR and 30-day mortality, as well as the association between NLR and ACLF degrees, were carried out using a point-biserial correlation. Cohen's d test was performed to determine the effect size.

Results: This study included 58 patients with alcoholic hepatis (98% men). The mean of the INL was 24.3. There was significant difference between patients who died within 28 days compared with those who survived (Table 1). The main differences were observed in the following data: leukocytes ($p < 0.001$), creatinine levels ($p = 0.007$), BT ($p < 0.001$); as well as, in the indexes: INL ($p < 0.001$), CLIF SCORE ($p < 0.001$), MELD ($p = 0.02$) and MELD Na ($p = 0.01$). (Table 1). The mean NLR value in patients who survived was approximately three times the value presented in patients who died within 28 days [23.0 (19.1, 28.6) vs. 8.0 (5.0, 11.0); ($p < 0.001$)]. A gradual increase in severity-dependent NLR was identified based on the CLIF SCORE scale (significant difference among the three groups considering CLIF SCORE 0). In addition, significant associations between NLR and 28-day mortality ($p < 0.001$), and between NLR and the degree of ACLF ($p < 0.001$) were found. According to Cohen's test, the effect size of the NLR was moderate (0.678).

Conclusions: The association between high NLR levels and mortality within 28 days is confirmed. Furthermore, there is an association between NLR and the severity of ACLF. Therefore, the NLR could be a useful prognostic factor in the clinical practice for alcoholic hepatitis. However, more studies with larger sample sizes are required.

Ethical statement: The protocol was registered and approved by the Ethics Committee. Patient confidentiality was maintained, and informed consent was obtained.

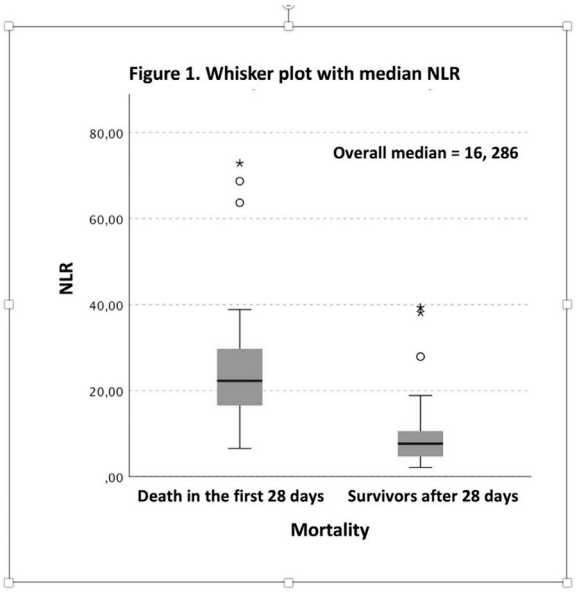
Declaration of interests: None.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Table 1
Comparison of clinical data and severity scales between surviving and non-surviving subjects at 28 days.

Variable	Death in the first 28 days (n=33)	Survivors after 28 days (n=25)	*p*
Age	46.0 ± 8.7	41.8 ± 10.1	0.094
Leukocytes	21.6 (15.0, 29.6)	9.6 (7.7, 13.2)	< **0.001**
Platelets	168.9 ± 97.1	138.8 ± 91.9	0.234
PT	23.0 (19.1, 28.6)	22.2 (17.9, 25.7)	0.236
BT	24.4 ± 9.3	15.0 ± 10.1	< **0.001**
INR	2.00 (1.79, 2.70)	1.94 (1.50, 2.27)	0.118
Cr	2.16 (1.30, 3.19)	1.30 (0.82, 2.09)	**0.007**
NLR	23.0 (18.0, 34.0)	8.0 (5.0, 11.0)	< **0.001**
CLIF SCORE	56.18 ± 6.28	46.88 ± 6.35	< **0.001**
MADDREY	71.3 (55.3, 99.1)	65.6 (33.4, 74.5)	0.059
MELD	35.7 ± 12.5	25.0 ± 8.6	< **0.001**
MELD NA	39.2 ± 15.8	29.7 ± 13.8	**0.021**

BT, Total bilirubin; CLIF SCORE, Chronic Liver Failure score; Cr, Creatinine; INR, International Normalized Ratio; MADDREY, Maddrey's discriminant function; MELD, Model for End-Stage Liver Disease; MELD NA, MELD sodium; NLR, Neutrophil-lymphocyte ratio; PT, Prothrombin Time.



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Importance of the profile of inflammatory and anti-inflammatory cytokines in patients with Hepatitis C according to degrees of fibrosis.

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Introduction and Objectives: The cure for chronic Hepatitis C (HCV) is a milestone for humanity, however, patients could still progress toward hepatocellular carcinoma even after receiving direct-acting antiviral treatment [1]. Therefore, determining the inflammatory (IFN-γ, TNF-α), and anti-inflammatory (IL-10- IL-1RA) profiles in patients with different degree of liver fibrosis are the important aim in researching of new biomarkers.

Materials and Methods: Prospective, cross-sectional and multicenter study; approved through the ethics committee of the UNAM, and the Hospital General de Mexico, patients with chronic Hepatitis C (CHC), and healthy subjects (control group) were included. A personalized survey of chronic and recent pathologies, as well as alcohol consumption, was performed in all the subjects. Biochemical and hematological laboratory test were performed, including Fibroscan and/or Fibrotest to determinate the degree of fibrosis. In addition, the cytokine profile (IFN-γ, TNF-α, IL-10 and IL-1RA) was quantified in the serum by multiple suspension array. The data was analyzed by Kruskal-Wallis, Mann-Whitney U and ANOVA statistical tests by SPSS v.22 software, considering $p > 0.05$

Results: A total of 180 CT subjects were included; whereas 90 patients with CHC with different grades of fibrosis: F0=20, F1=15, F2=15, F3=16 and F4=24, were enrolled. As has been studied, Hepatitis C Virus inhibits the activity of IFN-γ, despite of this, its concentration has been controversial in different populations. In our population, the levels were low with the exception for F2 ($p<0.01$). Another important factor of inflammation is TNF-α, which was found increased in all stages: F0-F4 and in F2 up to 6 times more than the CT and F4 groups ($p<0.001$). When analyzing the results of anti-inflammatory cytokines; the highest concentration of IL-10 was observed in F2 (increased up to 7 times) ($p<0.001$), while for IL-1RA in F2 it was found to be increased up to 10 times more than in F1 and F3 ($p<0.05$); what it shows that in F2 the inflammatory/anti-inflammatory microenvironment at the peripheral level has more activity. On the other hand, the highest concentration of IL-1RA was observed in F4, which may be actively participating in the repair due to decreased mediators of inflammation.

Conclusions: The F2 stage is an important turning point, indicating a shift in the microenvironment due to the accumulation of the extracellular matrix, making it clear the importance of measuring inflammation at intermediate stages of fibrosis, which would allow for impacting the limitation of the hepatic fibrogenic process.

Ethics statement: The protocol was approved by the Ethics and Research commissions of the General Hospital of Mexico "Dr. Eduardo Liceaga" (HG/DI/16/107/03/082), and from the Medicine Faculty UNAM (FMD/DI/15/2015)

Declaration of interest: None.

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Nopal and Pirfenidone Ameliorate Epididymal Fat Weight and Anthropometric Parameters in a Mouse Obesity Model with Diethylnitrosamine

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Introduction and Objectives: Obesity is associated with liver diseases. Mexico has a high prevalence of obesity and metabolic