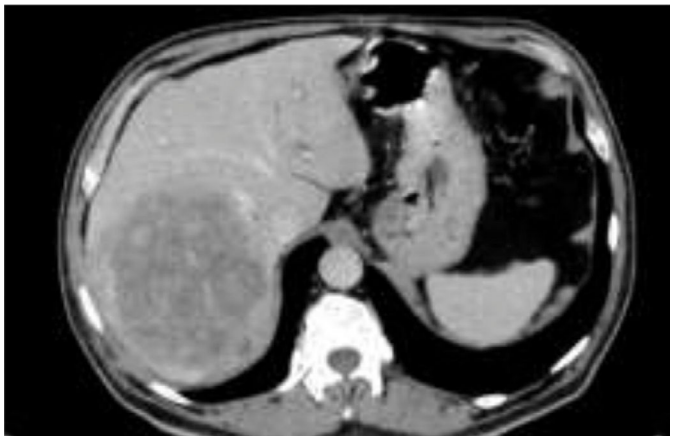

Figure 1.
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Complete genome sequence of Hepatitis C Virus isolated in Mexico

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Introduction and Objective: Death due to liver damage caused by the hepatitis C virus (HCV) represents one of the most frequent health threats in Mexico. However, the complete genome of HCV has not yet been sequenced. The aim of this study was to obtain the complete genome sequence of HCV isolated from patients in Mexico.

Materials and Methods: We evaluated patients with hepatitis C who sought medical care at the "Liver Unit" that belongs to the "Hospital Universitario Dr. José Eleuterio" in Monterrey, Mexico from May 2016 to August 2019. We extracted RNA from five samples and amplified the whole genome of HCV with tiled-PCR. Amplicons were sequenced with MinION, a third-generation sequencer technology. Obtained sequences were assembled with the Genome Detective program and posteriorly analyzed with IQtree platform.

Results: We obtained four partial and one complete VHC genome that corresponded to genotype 1b. The average coverage of the complete genome was 600X. The phylogenetic analysis of the complete genome showed that this sequence from Mexico was related to viruses isolated in the United States of America, Indonesia, and Japan. Because there is not a full HCV complete genome sequenced before in our country, we used the partial viral genomes reported before in Mexico to compare NS3 and NS5A genes with our reported sequences. The NS3 gene alignment showed that the newly sequenced viruses grouped in a clade different from the previously sequenced viruses. When NS5A gene was used, the newly obtained sequences grouped with the previously sequenced viruses in Mexico.

Conclusion: We were able to obtain the first complete and four partial HCV genomes from Mexican patients. This newly sequenced virus will improve the molecular epidemiology of HCV in Mexico.

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Identification of resistance mutations to DAA's against Hepatitis C Virus in infected subjects in Mexico

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Introduction and Objectives: Death due to liver damage caused by hepatitis C virus (HCV), this agent represents one of the most frequent health threats in Mexico. Now direct-acting antiviral agents (DAA's) are available to treat HCV infections. Nevertheless, HCV has gained mutations that hinder the antiviral effect. The presence of these mutations in Mexico is unknown. The aim of this study was to identify resistance-associated substitutions (RAS) in subjects infected with HCV from Mexico.

Materials and Methods: We evaluated patients with hepatitis C who sought medical care at the "Liver Unit" that belongs to the "Hospital Universitario Dr. José Eleuterio" in Monterrey, Mexico from May 2016 to August 2019. We extracted RNA from five samples and amplified the whole genome of HCV with tiled-PCR. Amplicons were sequenced with MinION, a third-generation sequencer. Obtained sequences were assembled with the Genome Detective program and resistance-associated substitutions were identified with HCV-Glue software.

Results: We obtained four partial and one complete VHC genome. According to HCV-glue algorithm, we detected one virus with resistance to daclatasvir, and probable resistance to ledipasvir and velpatasvir. Another HCV with probable resistance to daclatasvir and ombitasvir and possible resistance to grazoprevir, peritaprevir and ledipasvir. Two HCV isolated had probable resistance to daclatasvir and possible resistance to grazoprevir and peritaprevir. Only one HCV had probable resistance to daclatasvir.

Conclusion: We detected one HCV with resistance to daclatasvir and four other viruses with probable antiviral resistance mutations. These findings are crucial to effectively managing the patient's treatment.

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Hepatitis C virus NS5A and core proteins regulate epithelial-mesenchymal transition biomarkers in hepatoma cells

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Introduction and Objectives: Hepatitis C virus (HCV) NS5A and Core proteins play a key role in carcinogenesis development. Epithelial-mesenchymal transition (EMT) induced by HCV has been related to Snail and TGFβ1 upregulation and E-cadherin downregulation. This study aimed to evaluate the effect of HCV NS5A and Core proteins in the regulation of Snail, TGFβ1 and E-cadherin by transient transfection in the hepatoma cell system.

Materials and Methodology: Huh 7 cells were transfected with an empty vector, and 0.5 - 1.0μg of pNluc-NS5A-HCV or pCore-HCV plasmids for 24 - 48h and then proteins and RNA were extracted. NS5A protein expression was measured by NanoLuc activity. Core, Snail, TGFβ1 and E-cadherin protein levels were evaluated by Western Blot. NS5A and Core transcripts were quantified by RT-qPCR. Relative expression of SNAI, TGFβ1 and CDH1 was calculated in relation to ACTB and GAPDH endogenous expression.

Results: Viral NS5A and Core proteins were expressed in transfected Huh 7 cells. Transient transfection with pNluc-NS5A-HCV upregulated snail expression (4-fold) but downregulated E-cadherin expression (0.6-fold) at 24h. In addition, upregulated TGFβ1 expression (4-fold) and downregulates E-cadherin expression (0.7-fold) at 48h compared to the control. Meanwhile, transfection of pCore-HCV for 24h upregulated snail expression (2-fold); in contrast, it downregulated E-cadherin expression (0.3-fold) compared to control at 48h.

Discussion: Snail and TGFβ1 upregulation and E-cadherin downregulation had been associated with HCV infection in other studies. Our results suggest that HCV NS5A and Core have a direct role in EMT.

Conclusion: Hepatitis C virus regulates epithelial-mesenchymal transition biomarkers by NS5A and Core proteins expression in hepatoma cells.

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Diethylnitrosamine and 2-acetylaminofluorene chronic administration leads to biochemical and histologic changes related to hepatocellular carcinoma in Wistar rats

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Introduction and Objectives: This study aimed to analyze the biochemical and histological alterations produced in a model of chemical hepatocarcinogenesis by the chronic administration of diethylnitrosamine (DEN) and 2-acetylaminofluorene (2-AAF) in Wistar rats.

Materials and methods: Twelve Wistar rats weighing 180 to 200 g were divided into control and damage groups: rats were treated with DEN (50 mg/kg/wk) i.p and an intragastric dose of 2-AAF (25 mg/kg/wk) for 18 weeks. Serum clinical biochemistry was performed on VITROS Chemistry System 350® equipment. Masson's trichrome and Hematoxylin-Eosin stains were performed on the liver tissue. The trial was approved by the research ethics committee.

Results: The damage group had significant increases in total cholesterol, HDL-C, AST, ALT, ALKP, and GGT. Furthermore, histological analysis showed the loss of normal liver architecture with nuclear pleomorphism in the hepatocytes, atypical mitosis, and fibrous septa distributed between portal triads and collagen fibers through the hepatic sinusoids.

Discussion: Hepatocellular carcinoma models are a valuable tool to identify alterations during the progression of the disease. The Fischer-344 strain is frequently used in chemical hepatocarcinogenesis models since this strain shows greater susceptibility to the development of liver tumors. The damage induction model used in this work causes advanced hepatocellular carcinoma in Wistar rats, in spite of being a strain with intermediate susceptibility to hepatocarcinogenesis. The damage was evidenced by the presence of hepatomegaly, fibrosis, abundant nodules, histological changes, and biochemical alterations.

Conclusion: Chronic administration of DEN and 2-AAF induces characteristic alterations of hepatocellular carcinoma in Wistar rats.

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In vitro evaluation of the antifibrogenic effect of tamsulosin during its interaction with activated stellate cells

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Introduction and Objective: Liver cirrhosis is a chronic disease that affects one-fifth of the world; in Mexico, it is the third cause of mortality. It is caused by the uncontrolled production of the extracellular matrix. Attempts have been made to develop treatments that can reverse this disease, attention has been paid to the stimuli responsible for the activation of hepatic stellate cells (HSC), and the presence of adrenoreceptors in these cells has also been demonstrated. This study aimed to demonstrate in the present work a possible treatment with a neuroimmune activity that decreases the fibrogenic capacity of HSC.

Material and Methods: Rat's HSC in a quiescent state and activated by primary culture were used. Cells in a quiescent state contain retinol and lose it by activation. The degree of activation was assessed by immunofluorescence for α-SMA and cytochemistry with Oil Red. Cell proliferation was assessed by the MTT reduction technique. Norepinephrine was used to activate adrenergic signaling and tamsulosin was used as an antagonist of this pathway.

Results: Initially, we standardized the primary culture of HSC, identified by the α-SMA marker at seven days of culture. Subsequently, it was demonstrated that Noradrenaline treatment activated stellate cells due to the progressive increase of α-SMA and its proliferation. Moreover, tamsulosin treatment was shown to decrease retinol loss by preventing its activation and reducing proliferation.

Conclusion: Tamsulosin has a direct effect on decreasing the activity of quiescent and activated HSCs.