# بسم الله الرحمن الرحيم

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بقسم التوثيق الإلكتروني بمركز الشبكات وتكنولوجيا المعلومات دون أدنى مسئولية عن محتوى هذه الرسالة.

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| **ملاحظات:**<br>.....<br>.....<br>.....<br>.....<br>..... |

# INTRODUCTION

Hepatitis C virus (HCV) infection is a significant cause of morbidity and mortality, infecting more than 180 million people worldwide **(Babiker et al.,2020).** Chronic viral infection is one of the most common causes of hepatic fibrosis, distortion of the hepatic architecture, and ultimate progression to cirrhosis of the liver. HCV infection inflames the liver which, if unmanaged, will inevitably result in fibrosis and then progress to cirrhosis which has many co-morbid complications such as hepatocellular carcinoma (HCC) **(Thrift et al., 2017).**

Accounting for 85–90% of all primary liver cancers hepatocellular carcinoma (HCC) is one of the most common malignancies. It is the sixth most common malignant tumor and the third highest cause of cancer-related death in the world **(Ozakyol,2017).**

HCC is an aggressive tumor usually involving late stage liver cancers when diagnosed. Although significant progress regarding the treatment options for HCC has been made in recent years, poor prognosis remains a problem because of late diagnosis and drug resistance, making recurrence almost inevitable **(Ogunwobi et al., 2019).**

Cancer stem cells (CSCs) are small populations of cells in tumor tissue with ‘stem cell-like’ characteristics. CSCs have the capacity to

self-renew and differentiate into heterogeneous tumor cells, which are responsible for the maintenance and propagation of the tumor **(Batlle and Clevers, 2017).**

Current surface markers of particular phenotype are used to identify CSCs. Several markers proposed in the literature to identify CSCs in liver cancer including CD44 and EpCAM **(Gu et al., 2020).**

The human genome consists of approximately 25000 genes, each one being responsible for the regulation and function of the different - even neoplastic – cells **(Mansoor & Ashraf, 2020)**.

It is estimated that more than 30- 60% of the human DNA is regulated by microRNAs **(Shifali et al., 2021).**

MicroRNAs have proven to be able to regulate different molecular pathways and checkpoints of the cell division cycle. Thus, microRNAs may play an important role in cell proliferation and apoptosis and possibly a key participatory role in cancer development **(Chen et al., 2020).**

In HCC, and its secondary conditions like chronic inflammation and cirrhosis, a wide spectrum of miRNAs become deregulated thus contributing to aberrant messaging in specific cancer pathways that regulate cell proliferation, apoptosis, angiogenesis, DNA repair, invasion and metastasis **(Morishita and Masaki., 2015).**

The inhibition of apoptosis in HCC is facilitated by the down regulation of miR-203 as a result of DNA methylation **(E. Callegari, et al 2015).**

In HCC tissue cells miR-203 inhibits cell proliferation via targeting survivin **(Simile et al., 2019).**

In HCC,Cyclin D1 is overexpressed in malignant tissue cells and Cyclin D1 silencing can suppress cell proliferation and promote cell apoptosis. For this reason, Cyclin D1 may be an effective biomarker in the detection of HCC, and silencing of cyclin D also could potentially be a new target in the treatment of HCC **(Chen et al., 2017).**

Therefore, this study is designed to Compare miRNA 203 and its target genes (Survivin, Cyclin-D) in circulating liver cancer stem cells in patients with chronic hepatitis C with and without H.C.C.

# AIM OF THE STUDY

Comparison Study of miRNA 203 and its target genes (Survivin, Cyclin-D) in circulating liver cancer stem cells in patients with chronic hepatitis C with and without H.C.C.

# Chapter 1

## Hepatits C virus and complications

### Hepatits C virus (HCV):

- **virology**

HCV, a member of Flaviviridae family, is a single stranded RNA virus with 9.6 kb genome size. HCV genome is processed into structural proteins core, E1, and E2, and non-structural proteins p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B **(Shlomai et al., 2014). Figure (1)**

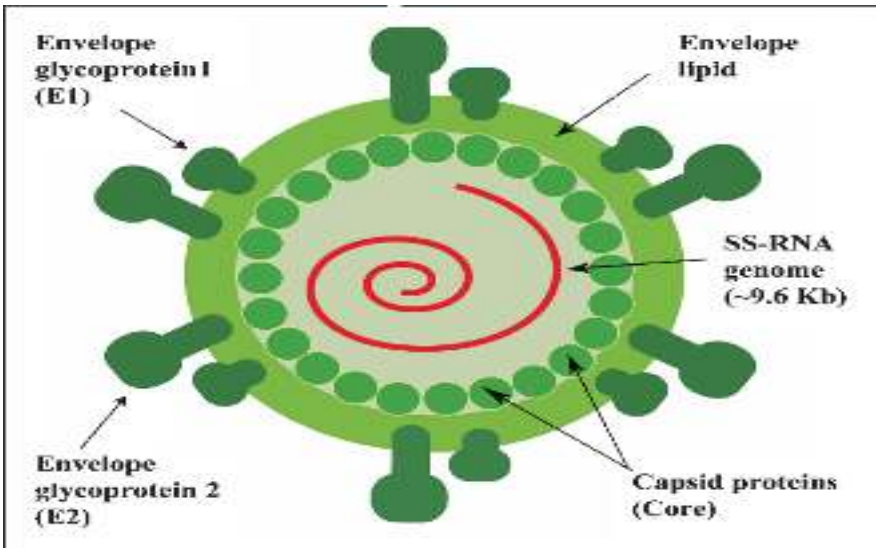


**Figure (**1**):** Hepatitis C virus particle structure **(Riva et al., 2019).**

HCV life cycle occurs mostly in the cytoplasm, with the viral replication complex enclosed within a membranous web structure that is closely associated with the ER membrane, mitochondrial outer membrane, and lipid droplets **(Oraby et al., 2019).**

## ❖ Transmission of HCV:

***The hepatitis C virus is a blood borne virus. It is most commonly transmitted through:***

- Injecting drug use through the sharing of injection equipment;
- The reuse or inadequate sterilization of medical equipment, especially syringes and needles in healthcare settings;
- The transfusion of unscreened blood and blood products;
- Sexual practices that lead to exposure to blood (for example, among men who have sex with men, particularly those with HIV infection) ***(Moosavy et al., 2017)***.
- HCV can also be transmitted sexually and can be passed from an infected mother to her baby; however, these modes of transmission are less common ***(Moosavy et al., 2017)***.

Hepatitis C shows no spread through breast milk, food, water or casual contact such as hugging, kissing and sharing food or drinks with an infected person ***(Moosavy et al., 2017).***

## ❖ Genotypes of HCV:

HCV is highly heterogeneous and can be classified into seven recognized genotypes (genotype 1 to 7) and multiple subtypes based on the differences of the whole viral genome. Genotypes and subtypes can be divided into quasispecies based on genetic diversity **(Smith et al., 2014).**

HCV genotypes have been associated with distinct pathological features, such as liver steatosis, insulin resistance, inflammation, and hepatitis reactivation **(Goossens&Negro., 2014).**

In regards on the association between HCV genotype and risk of developing HCC, the available evidences are quite inconsistent **(El-Serag., 2012).**

Early study showed that genotype 1b patients have a significantly higher risk of developing HCC **(Janiak et al., 2017).**

This early observation was supported by the result of a seventeen-year prospective cohort study, which showed 44 out of 104 genotype 1b followed-up patients developed HCC **(Axley et al., 2018).**

A meta-analysis study that calculated age-adjusted risk estimated genotype 1b patients had almost double the risk of developing HCC in comparison with patients infected with other genotypes **(Gadhia et al., 2021).**

In Egypt, genotype 4 is the predominant genotype and subtype 4a represents over 90% of cases, most of other cases are genotype 1**. (Gower et al., 2014).**

The rapidly evolving HCV treatment in the last decade has caused a decline in the incidence rate of viral infection **(Pawlotsky., 2014).**

## ❖ Diagnosis

Both serologic and nucleic acid-based molecular assays are available for the diagnosis of hepatitis C **(Wang et al., 2020).**

Serologic markers alone are not enough because HCV antibodies may develop late after transmission of the virus. In contrast, HCV RNA is detectable within a few days of infection, making nucleic acid-based is the test of choice in diagnosing acute hepatitis C (**Sarrazin et al., 2012).**

- **Hepatitis C Antibody Test:**

In current clinical practice, antibodies against multiple HCV epitopes are detected by commercially available 2nd and 3rd generation enzyme-linked immuno-assays (EIAs) (**Scott and Gretch., 2007).** HCV antibodies can be detected 10 weeks after infection with 2$^{nd}$ generation of EIAs ***(*Warkad *et al., 2019).***

To narrow the diagnostic window from infection to positive serological results, a 3$^{rd}$ generation EIA has been introduced that includes an antigen from the NS5 region and/or the substitution of a highly immunogenic NS3 epitope, which made it possible to detect HCV

antibodies about four to six weeks after infection with a sensitivity of more than 99% **(Kumar et al., 2018).**

False-negative results for the presence of HCV antibody can occur in immune-compromised patients, such as those with HIV type 1 infection, renal failure, or HCV-associated mixed cryoglobulinemia "Circulating immune complexes comprise *HCV* virions with low- or very low-density lipoproteins, immunoglobulin G -immunoglobulin M - Rheumatoid factor (IgG-IgM-RF) antibody complexes, and complement ***(*Warkad *et al., 2019).***

False-positive EIA results can occur; more in persons without risk factors and in those without signs of liver disease, as health care workers ***(*Warkad *et al., 2019).***

- ***Recombinant Immunoblot Assay*** (RIBA)

Supplemental testing using the RIBA helps to confirm positive results and may exclude the false-positive EIA results. RIBA is a qualitative test to detect HCV antibodies in human serum which is similar to EIA but using nitrocellulose, the recombinant immunoblot assay has limited use in clinical practice **(Infectólogo *et al., 2018).***

- **Quantitative HCV core antigen assay**

(Architect HCV Ag, Abbott Diagnostics) has been approved so far. It is highly specific (99.8%) and comprises 5 different antibodies **(Chang., 2018).**

False negative results are found in immunocompromised patients (**Mederacke et al., 2009).**

HCV core antigen in serum or plasma is a marker of HCV replication. Core antigen detection can be used instead of HCV RNA detection to diagnose acute or chronic HCV infection. HCV core antigen assays are less sensitive than HCV RNA assays (lower limit of detection equivalent to approximately 500 to 3,000 HCV RNA (IU)/ml, depending on the HCV genotype ***(EASL, 2018).***

As a result, the HCV core antigen becomes detectable in serum or plasma a few days after HCV RNA in patients with acute hepatitis C. In rare cases, the core antigen is undetectable in the presence of HCV RNA ***(EASL, 2018).***

- **Nucleic acid testing for HCV**

Because of the importance of presence of HCV RNA load for the therapeutic management, the World Health Organization (WHO) established the HCV RNA international standard based on international units (IU) which is used in all clinically applied HCV RNA tests. Currently, several HCV RNA assays are commercially available ***(*Tateishi et al., 2018).**

Historically, qualitative assays have been more sensitive than quantitative assays. Recently with the availability of real time polymerase chain reaction (PCR) with sensitivities of 10-50 IU/mL, there is no longer need for qualitative assays (**Scott &Gretch., 2007).**

It is a highly sensitive assay with lower limit of detection which is considered the most appropriate test for monitoring during therapy. All currently available assays have excellent specificity, in the range of 98% to 99% **(Shin et al., 2018).**

**- Genotyping Assays**

Genotyping is mandatory for proper choice of the optimal treatment regimen and duration of therapy, since many DAA agents are effective for only some HCV genotypes ***(Matthew et al., 2015).***

## NATURAL HISTORY OF HCV INFECTION:

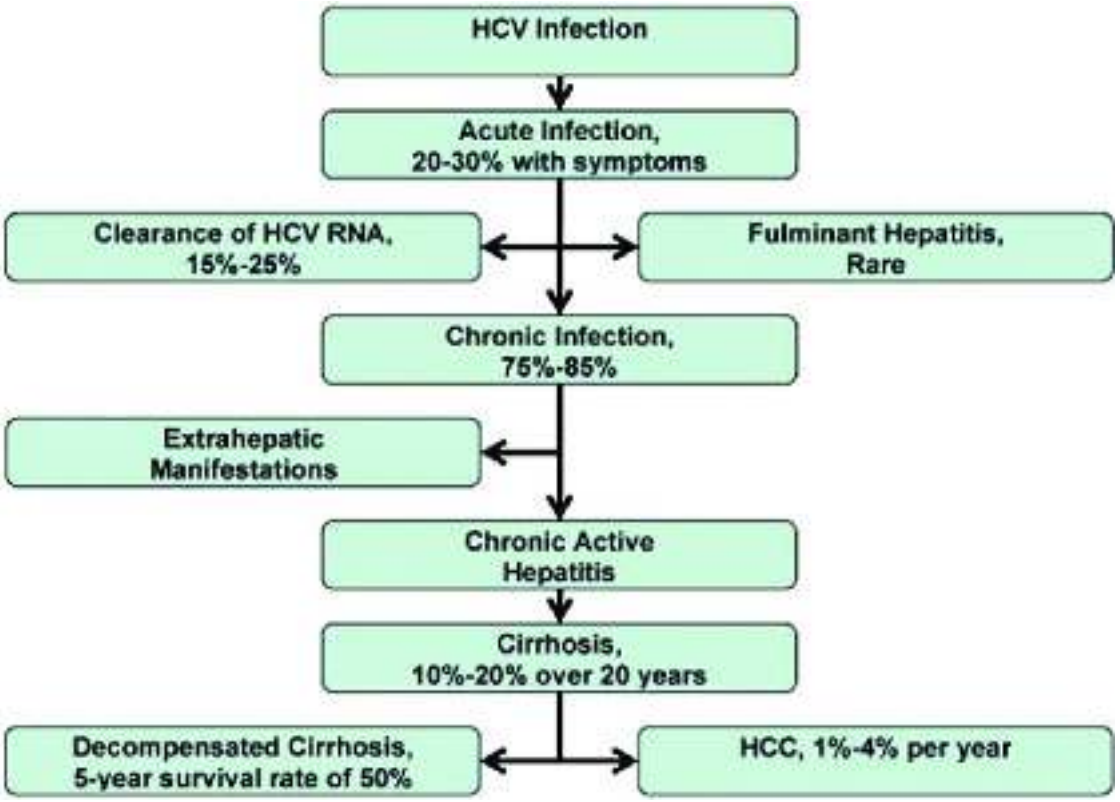


**Figure (2):** Natural history of HCV infection (**Axley et al., 2018).**

## CHRONIC HCV INFECTION

Chronic HCV infection affects approximately 170 million people worldwide, and may lead to development of liver fibrosis, cirrhosis, and HCC **(Messina et al., 2015).**

Chronic hepatitis is defined as the persistence of infection for at least 6 months after the onset of infection and is characterized by necroinflammation accompanied by a variable degree of fibrosis**,** end-stage liver disease and HCC (**Dhingra et al.,2016).**

The risk of chronic HCV infection is high. 75-100% of acute hepatitis C patients remain HCV RNA positive with persistently elevated liver enzymes **(Provazzi et al., 2019).**

Most patients with chronic hepatitis C are asymptomatic before the onset of advanced hepatic fibrosis. Patients who have been diagnosed with chronic infection, however, often complain of fatigue or depression, and they consistently score lower than HCV-negative persons in all aspects of health related quality of life (HRQOL) **(Provazzi et al., 2019).**

Most patients have only slight elevations of transaminases. Up to 30% of patients have normal serum Alanine transaminase (ALT) level. About 25% of patients have serum ALT between 2 and 5 folds above the upper limit of normal (ULN). Elevations of 10 folds the upper limit of normal are rarely seen **(Fried et al., 2012).**

There is a poor correlation between liver histology and levels of aminotransferases. In the majority of cases even patients with normal serum ALT show histologic evidence of chronic inflammation. The degree of injury is typically minimal or mild in these patients **(Durante et al., 2013).**

HCV infection may result in extra hepatic manifestations and metabolic disorder, including insulin resistance, type 2 diabetes and cardiovascular disease **(Westbroo& Dusheiko., 2014).**

## CIRRHOSIS AND HEPATIC DECOMPENSATION

Complications of HCV occur almost exclusively in patients with cirrhosis. Also the non-liver related mortality is higher in cirrhotic patients. However, cirrhosis is difficult to be diagnosed clinically, as most cirrhotic patients will be asymptomatic as long as the liver is not decompensated **(Matthew&Samuel., 2015).**

Findings that can be associated with cirrhosis are hepatomegaly "early" and splenomegaly on physical examination, elevated serum bilirubin concentration, hypoalbuminaemia, or thrombocytopenia. Other clinical findings associated with chronic liver disease may be found such as spider angioma, caput medusae, palmar erythema, testicular atrophy or gynecomastia **(Maesaka et al., 2021).**

Hepatic decompensation has many forms. Most common is portal hypertension (ascites and variceal bleeding), encephalopathy and

jaundice. As mentioned before, hepatic decompensation will develop only in cirrhotic patients. However, not all patients with cirrhosis will show signs of decompensation over time. ***(Van Keuren-Jensen et al., 2016)***.

The risk for decompensation is estimated to be close to 5% per year in cirrhotic patients.Once decompensation has developed the 5-year survival rate is roughly 50% **(Maesaka et al., 2021).**

HCC develops mostly in cirrhotic patients which is the most serious long-term complication of HCV infection. Annual incidence rates of HCC in patients with HCV-related cirrhosis range from 1% to 8% with an interval of 20-30 years between infection and development of HCC **(Delicque et al., 2019).**

**Assessment of the prognosis of cirrhosis**:

1. **Child–Pugh score**

The score, corresponding to the sum of individual points, allows to categorize patients in Child– Pugh grades A (5–6 points), B (7–9 points) and C (10–15 points). Importantly, the total range of points (5–15) is not equally distributed across grades A, B and C, probably in an attempt to mirror more efficiently the clinical impact of each grade in terms of prognosis. **(Wan et al., 2020).**