

CAUSES AND CURRENT TRENDS IN THE DIAGNOSIS AND MANAGEMENT OF HEPATITIS C VIRUS INFECTION: A REVIEW

By

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Abstract

A global principle of eliminating Viral Hepatitis by 2030 was adopted by the United Nations during the 59th World Health Assembly in May 2016. Hepatitis C Virus (HCV) is now a major concern as it affects an estimated 170 million people worldwide. Nigeria is a major significant contributor to the burden of chronic viral hepatitis infection globally corresponding to above 20 million people living with viral hepatitis B and/or C in a population of 177 million individuals that are not aware and are at risk of developing chronic complications of liver cirrhosis and primary liver cell cancers. This paper therefore looked into the causes of HCV in individuals, the current trends that are used in the diagnosis of the disease and how HCV infection can be managed in patients found with the disease. It causes disease in two general ways: by infecting cells and by provoking an immune response. HCV can be transmitted through needles tick injuries, splashes/mucocutaneous exposures, saliva, sexual exposure etc. Direct Acting Antiviral Drugs and Interferon based Regimen are the current treatment used. Health system strengthening is recommended for the control of HCV in Nigeria.

Keywords: Current trend, Diagnosis, Management, Hepatitis C, Virus

Introduction

The knowledge of Viral Hepatitis is still low in Nigeria despite being a leading infectious cause of death each year. About 20 to 24 million Nigerians are living with the disease and most of them are undiagnosed thereby increasing future transmission to others and placing many people at risk of getting infected with the disease (Federal Ministry of Health, 2016).

Viral Hepatitis is inflammation of the liver caused by one or more of five main hepatic viruses: A, B, C, D and E. These Viruses have the potential to cause liver disease to varying degrees and have similar symptoms, but they can differ in terms of epidemiology, prevention, diagnosis and care and treatment (Federal Ministry of Health, 2016).

Hepatitis C virus (HCV) is a genetically diverse positive-stranded RNA virus of the *Flaviviridae* family infecting an estimated 170 million people worldwide (Shepard et al., 2005; Lavanchy, 2009). Based on genetic

diversity, HCV is divided into six major genotypes (genotypes 1–6) and numerous subtypes with different geographic distributions; genotypes 1 and 3 are the most prevalent worldwide. HCV infection is the leading cause of chronic liver disease that persists for decades and eventually progresses to cirrhosis, liver failure, or liver cancer (Liang and Heller, 2004). HCV was first identified in 1989 as a principal agent in post-transfusion and sporadic acute hepatitis (Choo et al., 1989; Kuo et al., 1989).

Hepatitis C virus (HCV) infection has been called the silent epidemic — the majority of infections are asymptomatic, but in up to 80% of cases the virus persists, so many individuals infected with HCV are not aware of their infection. For this reason, infected individuals do not receive medical care even though they have a high risk of developing serious liver disease, including fibrosis and liver cirrhosis, which is often a prelude to hepatocellular carcinoma. (Bartenschlager et al., 2013).

With an estimated 170 million chronically infected individuals worldwide, ~27% of all cases of liver cirrhosis and ~25% of hepatocellular carcinomas might be related to HCV infection, explaining why chronic hepatitis C has become one of the main indications for liver transplantation (Perz *et.al.*, 2006).

Ways by Which Hepatitis C Virus Causes Disease

According to current understanding, the hepatitis C virus (HCV) causes disease in two general ways. The first is by infecting cells. Once inside the cell, the virus directly damages or kills the cell. This mechanism is called *cytopathic* damage. The second way the hepatitis C virus causes damage is by provoking an immune response. The *immune system* is your body's way of protecting itself from invading agents such as viruses and bacteria. An overactive or misdirected immune response can damage infected cells and the normal surrounding tissues. This mechanism is called *immunopathic* damage. When HCV was first discovered, experts thought the virus infected only liver cells. However, research has revealed that HCV also infects parts of the immune system, specifically the *lymphatic system* and *peripheral blood mononuclear cells*. Experts now understand that hepatitis C is not just a liver disease, but is a systemic disease, meaning it can affect nearly any organ of the body.

HCV contains a positive stranded RNA genome of around 10,000 nucleotides with an internal ribosome entry site which encodes a large polyprotein of over 3000 amino acid which is cleaved co- and post-translationally into virion structural proteins (nucleocapsid (C) and envelope glycoproteins gpE1 and gpE2 and p7), a plethora of non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) involved in viral replication and assembly (Paul *et al.* 2014). Notable features of HCV replication include its ability to induce a membranous web within the endoplasmic reticulum (Moradpour *et al.*, 2003) in which virus is replicated (Romero-

Brey *et al.* 2015), assembled on lipid droplets (Barba *et al.*, 1997; Miyanari *et al.*, 2007) and secreted using the low-density lipoprotein secretory pathway resulting in the production of apolipoprotein-associated viral particles of very light density (Huang *et al.*, 2007).

HCV induces the recruitment of nuclear pore proteins into the membranous web possibly to facilitate a protective subcellular environment from the cell's innate immune response and to create a custom-made virus factory (Neufeldt *et al.*, 2013). Meanwhile, the innate immune response is down-regulated via cleavage of MAVS and TRIF by the viral serine protease (Foy *et al.*, 2005; Li *et al.*, 2005).

Because it is an RNA virus that replicate via its own RNA-dependent RNA polymerase that lacks proof-reading activity, mutations are common in every replication cycle leading to a highly fluid and heterogenous viral genome currently consisting of at least seven basic genotypes, whose distribution varies around the world with many subtypes (Smith *et al.*, 2014).

Signs and Symptoms

- i. **Generalised:** Arthralgia, fatigue, fever chills and night sweat, fluid retention, flu-like syndrome, lymphadenopathy, myalgia, pruritis, sleep disturbances, spider nevi, and weakness (Fauci, 1998).
- ii. **Abdominal and Digestive signs and symptoms:** Abdominal pain, appetite changes, weight loss, bloating, diarrhoea, heart burn, jaundice and nausea.
- iii. **Cognitive, mood and nervous system signs and symptoms:** Cognitive change, depression, dizziness, headache, mood swings, numbness or tingling and visual change. Another symptom you may experience is dryness of the eye or feeling like there is something in the eyes. Other signs and symptoms include blood sugar abnormalities (either high or low), chest pain, menstrual and menopausal changes, sexual changes and palpitations (Fauci, 1998).

Ways of Transmission of HCV

HCV is transmitted by blood and currently occurs primarily through injecting drug use, and less frequently through sex with an infected partner, occupational exposure, and maternal-foetal transmission. In some cases, no risk factors can be identified (Lauer, 2001). Transfusion-related HCV infection is rare now since the introduction of routine screening of blood for HCV antibodies in the early 1990s.

Transmission risks

i. Needlestick injuries: It has been reported that a wide range of estimates for the risk of transmission of HCV after a needlestick or sharps injury from a source patient – between 0 and 10% (Mitsul *et al.*, 1992). The estimated risk from a needlestick injury from a source with detectable HCV RNA is 6.1%. The risk of developing HCV is greater following an injury with a hollow-bore needle, or deep injuries, compared with other injuries. A study showed an 11-fold increase in transmission of HCV from source patients with viral load $>6 \log_{10}$ copies/ml, compared with source patients with viral load $\leq 4 \log_{10}$ copies/ml following percutaneous exposure (Yazdanpanah *et al.*, 2005). The risk of transmission depends on whether the source is co-infected with HIV.

HCV can survive in cold temperatures in syringes for many days in laboratory studies (Painstsil *et al.*, 2012). The clinical implications of this are unknown, but the risk of becoming infected with HCV from an abandoned syringe depends on the prevalence of HCV in the local community. There are case reports of HCV transmission from needlestick injuries in the community (Haber *et al.*, 2007), but as the exact incidence of injuries in the community is not known, the risk of transmission from such injuries cannot be quantified accurately.

ii. Other percutaneous exposures: The risk of acquiring HCV during an operation performed by an infected surgeon is reported to be between 0 and 3.7% (Olsen

et al., 2010). Generally, the risk of contracting HCV following an injury from an unknown source is negligible. Sharps in the workplace, other than in the healthcare setting, such as razors and meat slicers have also been implicated in the transmission of HCV (Bocket, 2011).

Those who have tattoo have an increased incidence of HCV, with a pooled odds ratio of 2.73 (95% CI 2.38-3.15). Large tattoos, and those received in non-professional locations are associated with the greatest risk (Jafari, 2010).

iii. Splashes/mucocutaneous exposures:

Some reports have been published describing the transmission of HCV following a splash of blood into the eye of the recipient (Hosoglu *et al.*, 2003). Also, transmission of HCV has occurred following splashes of infected blood onto broken skin (Toda *et al.*, 2009). The exact risk associated with these exposures is unknown.

iv. Exposure to saliva (including injuries caused by human bites):

HCV RNA has been demonstrated in saliva (Lock *et al.*, 2006). Case reports describe transmission of HCV following human bites, but precise details of the nature of the bites, and whether blood was present in the mouth of the biter, or whether skin was broken at the time of the bite, are not known. 39 Inoculation with saliva has caused transmission of the virus in experimental studies (Abe *et al.*, 1987; Abe and Hchauspe, 1991).

Studies in dentists indicate a low incidence of nosocomial transmission of HCV. It is also thought, however, that HCV can be transmitted via sharing a toothbrush with an index case (Akhtar *et al.*, 2002).

v. Sexual exposures: Generally, transmission of HCV via sexual contact is inefficient in stable monogamous heterosexual couples (Tahan *et al.*, 2005). However, there is a low rate of transmission of HCV between discordant heterosexual couples and a higher prevalence of anti-HCV has been observed in those with multiple sexual partners.

vi. **Exposure to other body fluids:** HCV RNA has been identified in blood, saliva, sweat, semen and cervicovaginal secretions. The infective potential of cervicovaginal secretions is questioned, but may increase during menstruation (Wang *et al.*, 2011).

Tests Carried Out to Determine Hepatitis C Infection

The laboratory tests for hepatitis C can be divided into four general categories:

1. **Screening:** This is done with a serologic test for the HCV antibody (Ab).
2. **Confirmatory:** To diagnose chronic hepatitis C requires the presence of HCV RNA, most often called hepatitis C viral load.
3. **Genotype:** If HCV RNA is present, the specific genotype and subtype of the virus can then be determined with a genotype test.
4. **Drug resistance:** Some protein mutations in HCV will allow the virus to have resistance to direct-acting antivirals (DAAs), mostly referred to as resistance-associated variants (RAVs) or resistance-associated polymorphisms (RAPs) (Carrazin, 2016).

Treatment of HCV Infection

Once the viral genome is identified, it was possible to quickly develop blood tests to capture and detect circulating HCV-specific antibody in infected individuals (Kuo *et al.*, 1989). In addition, with the advent of PCR- and TMA-nucleic acid amplification technologies, these tests were quickly approved around the world and have effectively eliminated transfusion-associated HCV transmission where blood screening is performed. Successful therapeutic approaches were initiated prior to the identification of HCV with the seminal discovery of alpha interferon able to cure a small minority of non-A, non-B hepatitis (NANBH) patients (Hoofnagle *et al.*, 1986). Stabilizing the interferon by conjugation with polyethylene glycol led to better potency as did the addition of ribavirin, guanosine analogue that works to

prevent relapse using an unknown mechanism (Manns *et al.*, 2001). This combination was the mainstay of therapy for many years although it was limited by high toxicity and by only partial potency (McHutchison & Fried 2003).

Surprisingly, it took more than 20 years to develop approvable drugs targeting the HCV protease and polymerase, despite these targets being evident from the initial HCV genomic sequence (Choo *et al.*, 1991). This long delay was caused primarily by many pre-clinical and clinical failures as a result of drug toxicity rather than from lack of antiviral potency. In addition, unlike the HIV protease, the serine protease encoded by HCV has a rather shallow active site rendering it inherently difficult to design specific drugs with high affinity of binding and of high specificity. In addition, it has proven difficult to develop nucleoside inhibitors specific for the active site of the HCV polymerase. However, this was finally achieved recently and the drug Sofosbuvir now represents one major cornerstone in effective therapy (Alqahtani *et al.*, 2015).

Using the HCV replicon systems, outstanding drug development work identified the symmetrical drug Daclatasvir as the most potent antiviral drug ever developed for any virus (Gao *et al.*, 2010; Belema *et al.*, 2014). Targeting non-structural protein5a (NS5a), this drug could not have been discovered without use of the replicon system and it has a unique mode-of-binding (Belema *et al.* 2014; Barakat *et al.*, 2015).

Various combinations of Sofosbuvir, non-nucleoside polymerase inhibitors, Daclatasvir or Daclatasvir-like drugs along with second and third generation protease inhibitors have now transformed the therapy of HCV such that it has become the only curable chronic viral infection, with nearly all patients now curable after short regimen treatments and with little resistance reported in combination therapy (Nyalakonda & Utay, 2015).

Conclusion

It has been found out that HCV also infects parts of the immune system, specifically the lymphatic system and peripheral blood mononuclear cells. Therefore, Hepatitis C is not just a liver disease but a systemic disease because it can affect nearly any organ of the body. It is therefore very important to treat the disease as it is the most significant cause of infectious death worldwide.

Recommendations

The followings are recommended for control, prevention and cure:

1. Medical personnel and experts should educate and ensure effective application of Combination therapy as the most recommended treatment for the disease because nearly all patients are cured with little resistance.
2. Government should put in place a Health System Strengthening, shifting of tasks, as well as decentralization of services.
3. Monitoring and evaluation mechanisms, Logistics Management and an operational Research should also be enhanced and sustained in our health facilities and services for the control of Viral Hepatitis in Nigeria.
4. Public should be educated and be encouraged to embrace Infant Vaccination and catch-up vaccination for prevention.

References

- Abe, K. and Hchauspe, G. (1991). Transmission of Hepatitis C by Saliva. *The Lancet* 337(8735):248.
- Akhtar, S., Moatter, T., Azam, S.I., Rahbar, M.H., Adil, S. (2002). Prevalence and risk factors for intrafamilial transmission of hepatitis C virus in Karachi, Pakistan. *J Viral Hepat* ;9(4):309-14.
- Alqahtani, S. A., Afdhal, N., Zeuzem, S., Gordon, S. C., Mangia, A., Kwo, P. Fried, M., Yang, J.C., Ding, X., Pang, P.S., and McHutchison, J. G. (2015). Safety and Tolerability of Ledipasvir/Sofosbuvir with and without Ribavirin in Patients with Chronic Hepatitis C Virus Genotype 1 Infection: Analysis of Phase III ION trials. *Hepatology*, 62(1): 25-30.
- Barba, G., Harper, F., Harada, T., Kohara, M., Goulinet, S., Matsuura, Y., Eder, G., Schaff, Z., Chapman, M.J., Miyamura, T., Bre'chot, C. (1997). Hepatitis C Virus Core Protein Shows a Cytoplasmic Localization and Associates to Cellular Lipid Storage Droplets. *Proceedings of the National Academy of Sciences*, 94(4):1200 – 1205.
- Barakat, K. H., Anwar-Mohamed, A., Tuszynski, J. A., Robins, M. J., Tyrrell, D. L., & Houghton, M. (2014). A Refined Model of the HCV NS5A Protein Bound to Daclatasvir explains Drug-resistant Mutations and activity against Divergent Genotypes. *Journal of chemical information and modelling*, 55(2): 362-373.
- Bartenschlager, R., Lohmann, V. and Penin, F. (2013). The Molecular and Structural Basis of Advanced Antiviral Therapy for Hepatitis C Virus Infection, *Nature Reviews Microbiology*, 11(7):482.
- Belema, M., Nguyen, V.N., Bachand, C., Deon, D.H., Goodrich, J.T., James, C.A., Lavoie, R., Lopez, O.D., Martel, A., Romine, J.L., Ruediger, E.H., Snyder, L.B., St Laurent, D.R., Yang, F., Zhu, J., Wong, H.S., Langley, D.R., Adams, S.P., Cantor, G.H., Chimalakonda, A., Fura, A., Johnson, B.M., Knipe, J.O., Parker, D.D., Santone, K.S., Fridell, R.A., Lemm, J.A., O'Boyle, D.R. 2nd, Colonno, R.J., Gao, M., Meanwell, N.A. and Hamann, L.G. (2014). Hepatitis C Virus NS5A Replication Complex Inhibitors: The Discovery of Daclatasvir. *Journal of Medical Chemistry*, 57(5): 2013–2032.
- Bocket, L., Chevaliez, S., Talbodec, N., Sobaszek, A., Pawlotsky, J. M., & Yazdanpanah, Y. (2011). Occupational transmission of hepatitis C virus resulting from use of the same supermarket meat slicer. *Clinical Microbiology and Infection*, 17(2), 238-241.
- Carrazin, C. (2016). The Importance of Resistance to Direct Acting Antiviral Drugs in HCV Infection in Clinical

- Practice. *Journal of Hepatology*. 64(2):486-504.
- Choo, Q.L., Richman, K.H., Han, J.H., Berger, K., Lee, C., Dong, C., Gallegos, C., Coit, D., Medina-Selby, R., Barr, P.J. (1991). Genetic Organization and Diversity of the Hepatitis C Virus. *Proceedings of the National Academy of Sciences*, 88(6):2451–2455.
- Foy, E., Li, K., Sumpter, R., Loo, Y.M., Johnson, C.L., Wang, C., Fish, P.M., Yoneyama, M., Fujita, T., Lemon, S.M. and Gale, M. (2005). Control of Antiviral Defences Through Hepatitis C Virus Disruption of Retinoic Acid-Inducible Gene-I Signalling. *Proceedings of the National Academy of Sciences*, 102(8):2986-2991.
- Gao, M., Nettles, R.E., Belema, M., Snyder, L.B., Nguyen, V.N., Fridell, R.A., Serrano-Wu, M.H., Langley, D.R., Sun, J.H., O'Boyle DR 2nd, Lemm, J.A., Wang, C., Knipe, J.O., Chien, C., Colonno, R.J., Grasela, D.M., Meanwell, N.A., Hamann, L.G. (2010) Chemical Genetics Strategy Identifies an HCV NS5A Inhibitor with a Potent Clinical Effect. *Nature*, 465(7294): 96 – 100.
- Haber, P. S., Young, M. M., Dorrington, L., Jones, A., Kaldor, J., De Kanzow, S., and Rawlinson, W. D. (2007). Transmission of Hepatitis C virus by Needle-Stick Injury in Community Settings. *Journal of Gastroenterology and Hepatology*, 22(11): 1882-1885.
- Hoofnagle, J.H., Mullen, K.D., Jones, D.B., Rustgi, V., Di Bisceglie, A., Peters, M., Waggoner, J.G., Park, Y. and Jones, E.A. (1986). Treatment of Chronic Non-A, Non-B Hepatitis with Recombinant Human Alpha Interferon. A preliminary report. *New England Journal of Medicine*, 315(25):1575–1578.
- Hosoglu, S., Celen, M.K., Akalin, S., Geyik, M.F., Soyoral, Y., Kara, I.H. (2003). Transmission of Hepatitis C by Blood Splash into Conjunctiva in a Nurse. *American Journal of Infection Control* 31(8):502-504.
- Huang, H., Sun, F., Owen, D. M., Li, W., Chen, Y., Gale, M., & Ye, J. (2007). Hepatitis C virus Production by Human Hepatocytes Dependent on Assembly and Secretion of Very Low-density lipoproteins. *Proceedings of the National Academy of Sciences*, 104(14):5848-5853.
- Jafari, S., Copes, R., Baharlou, S., Etminan, M., Buxton, J. (2010). Tattooing and the Risk of Transmission of Hepatitis C: Systematic Review and Meta-Analysis. *International Journal of Infectious Diseases*, 14(11): e928-e940.
- Kuo, G., Choo, Q.L., Alter, H.J., Gitnick, G.L., Redeker, A.G., Purcell, R.H., Miyamura, T.E., Dienstag, J.L., Alter, M.J. and Stevens, C.E. (1989). An Assay for Circulating Antibodies to a Major Etiologic virus of Human Non-A, non-B Hepatitis. *Science*, 244(4902):362-364.
- Lavanchy, D. (2009). The Global Burden of Hepatitis C. *Liver International* 29: 74–81
- Liang, T.J. and Heller, T. (2004). Pathogenesis of Hepatitis C-associated Hepatocellular Carcinoma. *Gastroenterology*, 127: S62–S71.
- Li, K., Foy, E., Ferreon, J.C., Nakamura, M., Ferreon, A.C., Ikeda, M., Ray, S.C., Gale, M. and Lemon, S.M. (2005). Immune evasion by hepatitis C virus NS3/4A protease-mediated cleavage of the Toll-like receptor 3 adaptor protein TRIF. *Proceedings of the National Academy of Sciences*, 102(8): 2992-2997.
- Lock, G., Dirscherl, M., Obermeier, F., Gelbmann, C.M., Hellerbrand, C., Knöll, A., Schölmerich, J. and Jilg, W. (2006). Hepatitis C–contamination of Toothbrushes: Myth or Reality? *Journal of viral hepatitis*, 13(9):571-573.
- Manns, M.P., McHutchison, J.G., Gordon, S.C., Rustgi, V.K., Shiffman, M., Reindollar, R., Goodman, Z.D., Koury, K., Ling, M., Albrecht, J.K. (2001) Peginterferon Alfa-2b Plus Ribavirin Compared with Interferon Alfa-2b plus Ribavirin for Initial Treatment of Chronic Hepatitis C: A Randomised Trial. *The Lancet* 358(9286):958–965.

- McHutchison, J.G. and Fried M.W. (2003). Current Therapy for Hepatitis C: Pegylated Interferon and Ribavirin. *Clinics in Liver Disease*, 7(1):149–161.
- Mitsui, T., Iwano, K., Masuko, K., Yamazaki, C., Okamoto, H., Tsuda, F., Tanaka, T. and Mishiro, S. (1992). Hepatitis C Virus Infection in Medical Personnel after Needlestick Accident. *Hepatology*, 16(5):1109–1114.
- Miyanari, Y., Atsuzawa, K., Usuda, N., Watashi, K., Hishiki, T., Zayas, M., Bartenschlager, R., Wakita, T., Hijikata, M., Shimotohno, K. (2007). The lipid droplet is an important organelle for Hepatitis C Virus Production. *Nature Cell Biology*, 9(9):1089–1097.
- Moradpour, D., Gosert, R., Egger, D., Penin, F., Blum, H.E. and Bienz, K. (2003). Membrane association of hepatitis C virus non-structural proteins and identification of the membrane alteration that harbours the viral replication complex. *Antiviral research*, 60(2):103–109
- Federal Ministry of Health (2016) National Guidelines for the Prevention, Treatment and Care of Viral Hepatitis in Nigeria, Federal Ministry of Health Abuja-Nigeria.
- Neufeldt, C.J., Joyce, M.A., Levin, A., Steenbergen, R.H., Pang, D., Shields, J., Tyrrell, D.L.J. and Wozniak, R.W. (2013). Hepatitis C virus-induced cytoplasmic organelles use the nuclear transport machinery to establish an environment conducive to virus replication. *PLoS pathogens*, 9(10): e1003744.
- Nyalakonda H, Utay NS (2015). A New Era of Therapy for Hepatitis C Virus Infection. *Current opinion infectious diseases*, 28(5): 471–478
- Olsen, K., Dahl, P. E., Paulssen, E. J., Husebekk, A., Widell, A. and Busund, R. (2010). Increased risk of transmission of hepatitis C in open heart surgery compared with vascular and pulmonary surgery. *The Annals of thoracic surgery*, 90(5): 1425–1431.
- Paintsil, E., He, H., Peters, C., Lindenbach, B.D. and Heimer, R. (2012). Survival of Hepatitis C Virus in Syringes: Implication for Transmission among Injection Drug Users. *Journal of Infectious Diseases*, 202(7):984–90.
- Paul, D., Madan, V. and Bartenschlager, R. (2014). Hepatitis C virus RNA Replication and Assembly: living on the Fat of the Land. *Cell Host Microbe*, 16(5):569–579.
- Perz, J. F., Armstrong, G. L., Farrington, L. A., Hutin, Y. J. and Bell, B. P (2006). The Contributions of Hepatitis B Virus and Hepatitis C Virus Infections to Cirrhosis and Primary Liver Cancer Worldwide. *Journal of Hepatology*, 45(4):529–538.
- Puro, V., Petrosillo, N., Ippolito, G. (1995). Risk of Hepatitis C Seroconversion after Occupational Exposures in Health Care Workers. Italian Study Group on Occupational Risk of HIV and other Bloodborne Infections. *American Journal of Infectious Control*, 23(5):273–277.
- Romero-Brey, I., Berger, C., Kallis, S., Kolovou, A., Paul, D., Lohmann, V., Bartenschlager, R. (2015). NS5A Domain 1 and Polyprotein Cleavage Kinetics are Critical for Induction of Double-Membrane Vesicles Associated with Hepatitis C Virus Replication. *Mol Biol* 6(4): e00759–15.
- Shepard, CW., Finelli, L. and Alter, M.J. (2005). Global Epidemiology of Hepatitis C Virus Infection. *The Lancet Infectious Diseases*, 5(9): 558–567.
- Smith, D.B., Bukh, J., Kuiken, C., Muerhoff, A.S., Rice, C.M., Stapleton, J.T. and Simmonds, P. (2014). Expanded Classification of Hepatitis C Virus into 7 Genotypes and 67 subtypes: Updated Criteria and Genotype Assignment web resource. *Hepatology* 59(1):318–327.
- Tahan, V., Karaca, C., Yildirim, B., Bozbas, A., Ozaras, R., Demir, K., Avsar, E., Mert, A., Besisik, F., Kaymakoglu, S. and Senturk, H. (2005). Sexual Transmission of HCV Between Spouses. *The American Journal of gastroenterology*, 100(4):821.
- Toda, T., Mitsui, T., Tsukamoto, Y., Ebara, T., Hirose, A., Masuko, K., Nagashima, S., Takahashi, M. and Okamoto, H. (2009). Molecular Analysis of Transmission of Hepatitis C Virus in a Nurse who Acquired

- Acute Hepatitis C after Caring for a Viremic Patient with Epistaxis. *Journal of medical virology*, 81(8):1363-1370.
- Wang, C.C., Cook, L., Tapia, K.A., Holte, S., Krows, M., Bagabag, A., Santos, A., Corey, L. and Jerome, K.R. (2011). Cervicovaginal Shedding of Hepatitis C viral RNA is Associated with the Presence of Menstrual or other Blood in Cervicovaginal Fluids. *Journal of Clinical Virology*, 50(1):4-7.
- Yazdanpanah, Y., De Carli, G., Miguere, B., Lot, F., Campins, M., Colombo, C., Thomas, T., Deuffic-Burban, S., Prevot, M.H., Domart, M. and Tarantola, A. (2005). Risk Factors for Hepatitis C Virus Transmission to Health Care Workers after Occupational Exposure: a European