Hepatitis C virus and HIV type 1 co-infection

Priyanka Gupta
Retroviral Genetics Division, Centre for Virus Research, Westmead Millennium Institute, Sydney, Australia

Abstract

Around 33 million people worldwide are living with Human Immunodeficiency Virus (HIV) infection, and approximately 20-30% of HIV-infected individuals are also infected with Hepatitis C virus (HCV). The main form of HCV transmission is via the blood borne route; high rates of co-infection are found in intravenous drug users with HCV prevalence rates as high as 90%. Introduction of effective antiretroviral therapy (ART) has led to a significant decline in HIV-related morbidity, but at the same time the incidence of HCV related liver disease is increasing in the co-infected population. Meta analysis has revealed that individuals who are co-infected with HIV/HCV harbor three times greater risk of progression to liver disease than those infected with HCV alone. Increased risk of progression to Acquired Immunodeficiency Syndrome (AIDS) and AIDS-related deaths is shown among the co-infected patients by some studies, suggesting that HCV infection may accelerate the clinical course of HIV infection. HCV may also affect the incidence of liver toxicity associated with ART, affecting the management of HIV infection. There is a lack of optimal therapeutic approaches to treat HCV infection in HIV co-infected patients. This review discusses recent literature pertaining HIV/HCV co-infection, in addition to providing a snapshot of impact of co-infection on human genome at the level of gene expression and its regulation by microRNAs (miRNAs).

Epidemiology of Hepatitis C virus/HIV co-infection

Globally, an estimated 170 million people are infected with Hepatitis C virus (HCV) and 33 million people are infected with HIV. About 20-30% of HIV infected patients in USA are infected with HCV, owing to the shared modes of transmission. HCV is spread 10 times more efficiently than HIV through percutaneous blood exposures; although co-transmission of both viruses can happen, co-infected individuals are usually infected first with HCV. Primary mode of acquisition of HCV is through injection drug abuse in various settings. Transfusion of contaminated blood and blood products was once an important mode of transmission of HCV and HIV but is now rare because of effective screening procedures. HCV is spread less efficiently than sexual transmission than HIV. In hemophiliacs co-infected with HIV and HCV, HIV infection was detected in 13% and HCV in only 3% of 162 female sexual partners of hemophiliacs. However, multiple outbreaks of HCV infection have been seen in HIV-infected men who have sex with men (MSM), and have no percutaneous risk factors. These are attributed to the high-risk sexual practices such as unprotected receptive anal intercourse and recreational drug use has also been reported in these men. Vertical transmission from a HCV infected mother to her newborn child is uncommon; the reported risk of transmission ranges between 2-5%, with the risk three fold higher if the mother is also HIV seropositive.

Clinical picture of Hepatitis C virus/HIV co-infection

Influence of Hepatitis C virus infection on natural course of HIV infection

The effect of HCV infection on HIV disease is not clear. Early studies suggested that there was no association between HIV-HCV co-infection and poor disease outcome. These studies suggested that although the co-infected patients had higher rate of liver-related mortality, there was no increased risk of AIDS and mortality rates as compared to HIV mono-infected individuals. Kaufmann et al. demonstrated that HCV seropositive patients had smaller increase in CD4+ T lymphocytes than HCV seronegative patients. But this disappeared during the duration of 4 years of the follow up. Rockstroh et al. did not find any effect on HCV serological status on HIV disease progression, but there was an increased risk of liver-related deaths among the co-infected patients. However, more recent studies have suggested that co-infection is associated with greater risk of progression to AIDS or death, despite similar use of antiretroviral therapy (ART) within the mono-infected and co-infected groups. Greub et al., in the Swiss Cohort study, demonstrated that HCV infection, in combination with intravenous drug abuse are significant risk factors in the morbidity and mortality between HIV infected subjects. The authors concluded that following antiretroviral therapy and during a mean follow up of 42 months; there was a negative effect on the magnitude of CD4+ T cell reconstitution among patients with co-infection. Similarly in an Italian cohort study, De Luca et al. showed a shorter time to the clinical progression of HIV disease in co-infected patients after starting ART during a follow up of 36 months. A review in the favor of co-infection impairing HIV-specific responses comes from a meta-analysis of 8 different trials involving 6216 patients, which clearly showed that the magnitude of immune system recovery in HIV-HCV co-infected individuals was slower than the HIV-mono-infected individuals alone.

High levels of T cell activation were also seen in co-infected individuals as compared to HCV non-infected individuals even following ART. This activation of T cells can cause immune dysfunction and cytokine production leading to increased replication rates of HCV and HIV and lower CD4+ T cell counts. High levels of activated CD8+ T cells were associated with HCV viremic but not HCV seronegative women, and were also associated with incident AIDS among these groups. However, the impact of HCV infection on CD4+ T cell recovery following ART is conflicting; some reports note a poorer CD4+ T cell response in co-infected compared to mono-infected patients, and others do not. Co-infection with HCV may cause CD4+ T cell
apoptosis in HIV-untreated patients and more rapid progression to severe immunodeficiency however this effect is rapidly lost with effective ART. Sulkowski et al. showed that there was no difference in the increase of CD4+ T count during ART in HCV infected individuals as compared to HCV un-infected individuals.

**Influence of HIV infection on natural course of Hepatitis C virus infection**

While there has been a significant decline in HIV related morbidity due to more effective therapy, incidence of HCV related liver disease is still increasing in the co-infected population. As early as 1993, it was reported that HCV RNA levels were higher in people with hemophilia who were co-infected with HIV and HCV, than in those who remained HIV negative, and liver failure was shown exclusively in co-infected patients. While approximately 20% of HCV mono-infected patients clear the virus but HCV clearance occurs in only 5-10% of co-infected individuals, especially in patients with lower CD4+ T cell counts. Infection with HIV has been associated with higher HCV RNA viral load and more rapid progression of cirrhosis, liver failure, and hepatocellular carcinoma. In a meta-analysis of 8 studies, the average risk of progression to cirrhosis or decompensated liver disease is 3 times higher in HCV-HIV co-infection than HCV mono-infection. Similarly, a recent meta-analysis of 17 studies showed that 21% of co-infected individuals progress to cirrhosis after 20 years of infection and 49% after 30 years.

Studies analyzing the effect of ART on the natural history of Hepatitis C have been contradictory. Some studies suggest that there is a lower risk of liver mortality in patients who have received effective ART, while other studies suggest that among persons with effectively controlled HIV infection, the progression of fibrosis was similar in persons with or without HIV infection. In a meta-analysis involving 27 studies, Thein et al. did not detect any significant association between ART and the risk of cirrhosis. Increased risk of cirrhosis in patients with co-infection relative to those with mono-infection was similar in person taking ART and those not taking ART.

However, despite the positive impact of ART, as shown by some studies, some studies do not show any benefit of ART. Increased risk of hepatitis/liver-related deaths were seen in a 20-year prospective study despite the use of ART among co-infected drug users (DUs) compared to HCV mono-infected DUs, providing further evidence that HIV accelerates liver disease.

Furthermore, some studies have shown an association of ART with increased hepatic injury as suggested by elevated liver transaminases or frank liver decompensation.

**Medical management of co-infected patients**

**Before initiation of antiretroviral therapy**

HIV infected patients who are also HCV seropositive should be tested for the presence of HCV RNA using a qualitative or quantitative assay to confirm the presence of active infection. Co-infected patients should be advised to abstain from alcohol, hepatotoxic drugs and vaccinated against Hepatitis A and Hepatitis B viruses. The treatment for HCV follows standard guidelines.

**The CD4+ T cell count is an important determinant for the treatment response in the co-infected patients; the higher it is the greater the chances of response to anti-HCV therapy.** Patients with CD4+ T cell counts >500 cells/mm³ are good candidates for treatment of HCV, while in patients with lower CD4+ T cell counts (e.g. <200 cells/mm³), it may be preferable to initiate ART and delay HCV therapy until CD4+ T cell counts increase as a result of HIV treatment.

**Antiretroviral therapy in HIV/Hepatitis C virus co-infection**

Patients with lower CD4+ T cell counts (e.g. <200 cells/mm³) should be given ART as a priority and these patients can be considered as candidates for anti-HCV therapy once the CD4+ T cell counts rise above 200 cells/mm³. If both HIV and HCV treatments are indicated, the choice of ART regimen should be guided by the HCV treatment regimen selected with careful consideration of potential drug-drug interactions and overlapping toxicities. Additionally, certain ART drugs should be avoided because they have been associated with higher incidence of serious liver-associated adverse effects, such as fatty liver disease with nucleoside reverse transcriptase inhibitors (NRTIs) such as Zidovudine or Didanosine. Clinical and laboratory monitoring for adverse effects should be done with the help of laboratory tests such as hemoglobin, amylase, lipase and serum transaminase levels. The use of ARTs may be maintained as long as the serum transaminase levels do not go above 3.5 fold of the normal values. If serum transaminase concentrations increase above this threshold, then different decisions may be taken e.g. start anti-HCV therapy (if indicated), switch to an alternative regimen, or discontinue the therapy immediately if a hypersensitivity reaction occurs.

**Gene expression profiling in HIV/Hepatitis C virus co-infection**

Genomic analysis of liver biopsies and PBMC (Peripheral Blood Mononuclear Cells) with the help of techniques like microarray analysis and PCR (Polymerase chain reaction) provide a unique opportunity to study the HCV-host interaction in the presence and absence of HIV co-infection. The use of the gene expression profiling may allow the identification of novel biomarkers by comparing the differences in gene expression in PBMCs and liver biopsies of individuals with different stages of fibrosis.

Co-infected patients had decreased intrahepatic inflammatory cytokine mRNA levels, with a notable decrease in IFN-γ compared with mono-infected patients consistent with other studies.

Gonzalez et al. assessed cytokine levels in liver biopsies on liver samples from HCV/HIV co-infected and HCV mono-infected patients. They measured IFN-γ, Tumor Necrosis Factor (TNF)-α, Transforming Growth Factor (TGF)-β1, interleukin (IL)-4, IL-10, IL-12p35 and IL-12p40 mRNA levels by real-time PCR performed on liver biopsies of HCV mono-infected (n=19) and HCV/HIV co-infected (n=24) patients (Table 1). The results showed that the co-infected patients had decreased intrahepatic mRNA levels of IFN-γ, IL-4 and IL-12p35 compared with mono-infected patients while IL-10 was increased. Intrahepatic IFN-γ levels were also significantly reduced in patients with advanced immunodeficiency. IFN-γ mRNA levels increased linearly with increasing peripheral CD4+ T cell counts by 1.23 times for every 100 CD4+ T cells/mm² increase. Decreased numbers of intrahepatic CD4+ T cells in HCV/HIV co-infected patients, shown in this study, may suggest that patients with very low CD4+ T cell numbers may have decreased IFN-γ secretion that may be permissive for accelerated fibrogenesis.

Flynn et al. looked at the effect of HIV infection on natural interferon production with HCV by directly comparing HCV specific T-cell responses and cytokine profiles of 40 Australian Trial in Acute Hepatitis C (ATAHC) participants with acute hepatitis C, 20 of them were HIV/HCV co-infected and 20 of them HIV seronegative. The study showed that HIV incurs detrimental effect on HCV-specific cytokine production in people with acute hepatitis C, with notable decrease in Interferon (IFN-γ). The response to this interferon was significantly lower in magnitude and smaller in breadth uniquely in the HIV/HCV group. The reduced interferon production was associated with lower peripheral CD4+ T-cell counts, but not with detectable HIV viral load.

Abbate et al., analyzed mRNA levels for IFN-α, IFN-γ, IFNAR-1 and protein kinase R (PKR) in liver biopsies of HCV/HIV co-infected patients to compare their levels with those found in singly HCV-infected individuals. In HCV/HIV-co-inf-
ed patients intra-hepatic IFN-γ mRNA levels were up-regulated compared with HCV-mono-infected patients, whereas IFN-α, interferon-α receptor-1 (IFNAR-1) and PKR were profoundly down-regulated. IFN-γ up-regulates IFNAR-1 and it is likely that the absence of mRNA for both factors in the liver of co-infected patients may be causally related. IFN-α is shown to be up-regulated, presumably driven by HIV, but the absence of the IFNAR-1 suggests that there is an impaired ability to respond to IFN-α. PKR is also virtually absent supporting the above fact (Table 1). Blackard et al., retrospectively examined cytokine expression in HCV mono-infected and HCV/HIV co-infected individuals to determine how HIV specifically alters the intrahepatic environment observed during chronic HCV infection. They used real-time PCR to quantify cytokines that contribute to innate and adaptive immune responses, summarized in Table 1. The results showed that the detection rates of cytokine mRNAs were lower for HCV/HIV co-infected group compared to the HCV mono-infected group; the detection rates for TNF-α, IL-8, and IL-10 were statistically significant. Overall, cytokine mRNA quantities were lower for HCV/HIV co-infected compared to HCV mono-infected persons, with the exception of TGFβ1 (Table 1). TGFβ1 is a potent inducer of fibrogenesis in the effector cells of hepatic fibrosis and HIV co-infection may further contribute to liver fibrosis in HCV-positive persons by inducing TGFβ1 expression. TGFβ1 production has been shown to reduce the IFN-γ response of CD8+ T cells to viral infection. This may suggest an important mechanism by which HIV, through increased TGFβ1 and decreased IFN-γ expression, may promote HCV persistence. This data suggests that defects in cytokine activation may occur in HCV/HIV co-infected persons that limit efficient clearance of HCV from the liver. Kottilil et al. demonstrated higher expression of Interferon inducible genes (IFIG) in HCV patients co-infected with HIV suggesting that HIV infection is a major driving factor in turning the type-I IFN signature expression. Moreover, the IFIG expression, which is highly predictive of the therapeutic response to IFN-γ based therapy, is expressed at a higher level in HCV-infected individuals. This reiterates a mechanistic role for type-I IFN γ baseline hyperexpression in vivo in HCV/HIV co-infected individuals who fail to clear HCV with IFN γ therapy. HCV-mono-infected and HIV/HCV individuals had increased serum levels of markers of liver fibrosis compared to seronegative HIV individuals and HIV-infected individuals without liver disease. Some immune markers such as Natural killer cell p30-related protein (NKP30) and Insulin-like Growth Factor 1 (IGF-1) Receptor (IGF-R1) up-regulated among patients with HIV or without HIV co-infection, whereas some others selectively up-regulated in HCV mono-infected subjects (Table 1). In contrast to above mentioned studies, Walters et al. showed that global gene expression does not differ between HCV mono-infected and HCV/HIV co-infected individuals. However, a subset of patients showed a specific pattern of gene expression known as enhanced gene expression patterns (EGE+). This group contained two HCV- and four HCV/HIV-infected individuals. Specifically, the EGE (+) patients show decreased expression of multiple genes associated with the FAS apoptosis pathway and increased expression of lymphocyte adhesion molecules and lymphocyte-specific genes. Patients with EGE (+) also had partially

Table 1. Gene expression studies showing identified genes known to be altered during Hepatitis C Virus/HIV co-infection.

| Study               | Sample/technique                                      | Number of patients | Important findings |
|---------------------|-------------------------------------------------------|--------------------|--------------------|
| Abbate et al. 200462 | mRNA extraction and RT-PCR on liver biopses           | 24 HCV infected patients and 20 HIV/HCV co-infected patients | ↓ Intrahepatic mRNA levels of IFN-γ, IFNAR-1, PKR & ↑ IFN-α levels in co-infected patients compared to mono-infected patients |
| Blackard et al. 200660 | mRNA extraction and RT-PCR on liver biopses           | 12 HCV infected and 14 HIV/HCV co-infected patients | ↓ Intrahepatic mRNA levels of TNF-α, IL-8, and IL-10 & ↑ TGFβ1 levels in co-infected patients compared to mono-infected patients |
| Walters et al. 200665 | mRNA extraction and microarray analysis on liver biopsies | 12 HCV infected and 16 HIV/HCV co-infected patients | No difference in global gene expression between mono-infected and co-infected individuals |
| Zhao et al. 201262  | mRNA extraction and microarray analysis on CD8+ T cells | 24 HCV infected, 24 HIV/HCV co-infected patients | 16 genes up-regulated in co-infection 56 genes down-regulated in co-infection & ↓ IFN-γ, IL-2 |
| Flynn et al. 201260 | HCV peptide enzyme-linked immunospot and multiplex in vitro cytokine production assays | 20 HCV infected and 20 HIV/HCV co-infected patients | ↓ IFN-γ production showing good correlation with CD4+ T cell count |
| Gonzalez et al. 200864 | mRNA extraction and RT-PCR on liver biopses           | 19 chronic HCV infected and 24 HCV/HIV co-infected patients | ↓ Intrahepatic mRNA levels of IFN-γ, IL-4, IL-12p35 & ↑ IL-10 levels in co-infected patients compared to mono-infected patients |
| Kottilil et al. 200963 | mRNA extraction and microarray analysis on PBMC. Validation of selected genes by microarray flow cytometry and ELISA | 7 HCV infected, 5 HIV/HCV co-infected patients | NKP30 and IGF-R1 among patients with HIV with or without HIV co-infection ↑ CD10, CD80, CCL7, CCL20, and CXCL1 in HCV infection |
| Rasmussen et al. 201266 | mRNA extraction and microarray analysis on liver biopsies and PBMC | 10 HCV infected and 13 HIV/HCV co-infected | Liver: 250 genes related to infectious disease and immune responses were up-regulated PBMC: 271 genes associated with inflammatory responses, antigen presentation, and humoral immune responses, as well as hematological, immunological, and inflammatory diseases up-regulated |
| Kunzen et al. 200967  | mRNA extraction and RT-PCR on liver biopsies           | 33 HCV infected and 40 HIV/HCV co-infected patients | IFNγ, RANTES, MIP-1α and IP-10 in HIV/HCV co-infected patients compared with HCV-mono-infected patients |

mRNA, messenger RNA; RT-PCR, real-time polymerase chain reaction; ELISA, enzyme linked immunosorbent assay; HCV, Hepatitis C virus; IFN, Interferon; IL, Interleukin; IFNAR, Interferon alpha receptor 1; PKR, protein kinase R; TNF, tumor necrosis factor; TGF, transforming growth factor; NKP30, natural killer cell p30-related protein; IGF-R1, insulin-like growth factor 1 receptor; CD, cluster of differentiation; CCL, chemokine (C-C motif) ligand; CXCL1, Chemokine (C-X-C motif) ligand 1; RANTES, regulated and normal T cell expressed and secreted; MIP-1α, macrophage inflammatory protein-1α; IP10, interferon inducible protein 10.

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impaired Type I and II IFN-mediated antiviral responses with decreased antifibrogenic cytokine IFN-γ, supporting the above studies (Table 1).  

Rasmussen et al. did gene expression analysis on paired liver biopsy samples and Peripheral Blood Mononuclear Cells (PBMCs). They identified a gene expression signature associated with increased inflammation and immune activation that was present only in liver and PBMC samples from co-infected patients.  

The researchers also identified liver- and PBMC-specific signatures enriched with fibrogenic/hepatocellular stellate activation and pro-inflammatory genes respectively (Table 1).  

Zhao et al. did microarray analysis on CD8+ T cells from HIV/HCV-co-infected or mono-infected treatment-naive individuals. They identified 72 transcript IDs to be differentially expressed (>2-fold and P<0.05) in comparisons between treatment-naive individuals. They identified a gene expression signature in Hepatitis C virus/HIV-1 co-infected groups (Table 1).  

Kuntzen et al. studied intrahepatic messenger RNA levels of cytokines and cellular markers defining distinct subsets of inflammatory cells in liver biopsies from 33 HIV-mono-infected and 40 HIV/HCV-co-infected patients. Increased levels of IFN-γ, Regulated upon Activation, Normal T-cell Expressed and Secreted proteins (RANTES) and interferon-inducible proteins were significantly higher in co-infected patients as compared to HIV mono-infected patients. Many of the down-regulated genes correlated with the immune response, few examples of genes being regulated IL-2 and genes responding to IFN-γ down-regulated in the HIV/HCV co-infected groups (Table 1).  

Enhanced expression of the genes was probably related to the effector function of CD8+ T cells as suggested by correlations between these cytokines and CD8. In contrast to the previous studies, Kuntzen et al. showed increased expression of IFN-γ. The plausible explanation is that HCV-specific cytokine production is influenced by progressive immunodeficiency, but the CD4+ T cell count was well preserved in the study done by Kuntzen et al.  

The results of the gene expression studies done on HIV-HCV co-infection are summarized in Table 1.

**MicroRNA expression profiling in Hepatitis C virus/HIV-1 co-infection**

A microRNA (miRNA) is a small RNA molecule (17-22 nucleotides) that functions in the post-transcriptional regulation of gene expression. They are transcribed from the nuclear DNA and function via base-pairing with complementary sequences within mRNA molecules, usually resulting in gene silencing via translational repression or target degradation. miRNA expression analysis will provide valuable insights in physiological and pathological processes regarding co-infection.  

There has been a lot of research done regarding the importance of various microRNAs in HIV-1 and HCV infection independently. No report has characterized the alterations in microRNA expression profiles upon HIV-1/HCV co-infection in vitro or in patients. But several miRNAs have been identified with the help of the literature search that can play role in HCV/HIV-1 co-infection.  

miRNA-122, a liver specific miRNA has been shown to play a critical role in HCV life cycle according to various studies. Of note, miR-122 is undetectable in uninfected, quiescent T cells, but infection of T cell lines significantly up-regulated the expression of miR-122. These findings can lead us to wonder if the overexpression of miR-122 expression upon HIV-1 infection of CD4+ T cells, monocytes and macrophages can explain the ability of HCV to infect cells beyond hepatocytes in co-infected patients.  

As previously mentioned, miR-29 (miR 29a, b and c) has been shown to be down-regulated in hepatocytes upon HIV-1 co-infection, and miR-29 over-expression significantly reduced HCV replication. miR-29a has also been reported to bind HIV-1 3'UTR by base-pair complementarities and target the viral RNA to P-bodies for degradation. Another independent report characterized that ectopic expression of miR-29a inhibits HIV-1 protein Nef, in addition to reducing viral infectivity. Thus the role of miR-29 in HCV-HIV co-infection needs to be further explored and efforts should be done to further define if the overexpression of miR29 could have dual inhibitory activity in HVI/HCV co-infected cells.  

miR-199a-3p has been associated with progression to liver fibrosis. Interestingly, while over-expression of miR-199a was shown to inhibit HCV replication by binding to the viral 5'UTR, it was also reported that HIV-1 infection resulted in greater than two-fold increase in miR-199a levels. Based on these data, it would be interesting to explore potential role of miR-199a in HCV/HCV co-infection.  

miR-223 and miR-150 were shown to inhibit HIV replication by binding to HIV-1 3'UTR enriched in resting CD4+ T cells as compared to activated CD4+ T cells claiming their role as anti-HIV miRNAs. miR-223 was also down-regulated by about 4.8-fold in HCV-induced hepatocellular carcinoma from HCV infected patients. However, it has not been investigated whether miR-223 expression is down-regulated in the liver of HCV/HIV-1 co-infected patients with HCC, and if so, whether it would correlate with higher HIV-1 viral load. Up-regulation of miR-150 was seen in chronic hepatitis C patients, this is interesting, because miR-150 was reported to negatively regulate hepatic stellate cells (HSC).  

Overexpression of miR-150 in human hepatic stellate cell lines LX-2 reduced proliferation of the cells (by ~25%), increased apoptosis, and reduced α-Smooth Muscle Actin (α-SMA) and collagen I levels.  

let-7b and let-7g microRNAs were significantly decreased in PBMCs and CD4+ T cells of HIV-1 infected patients as compared to healthy controls or patients, who can naturally control HIV-1 infection e.g. Long-Term Non Progressors (LTNP). Very recently, let-7b has been shown to bind to HCV protein NS5B and to the 5'UTR, and to significantly suppress HCV infection. HCV-1 replication can suppress let-7b levels and it is plausible that a decrease in let-7b miRNA in co-infection could potentially augment HCV replication.  

The summary of key findings discussed in this review is shown in Figure 1.

**Conclusions**

Although studies have been done on the clinical consequences of HIV/HCV co-infection and the influence of HCV on HIV, and vice versa, the mechanisms by which these viruses interact at the cellular level remain unexplored. Genomic and gene regulation studies have opened a new path towards the understanding of early programming of cancer related events during HCV mono and co-infection. A clear understanding of genes involved in this programming in blood and liver are sorely needed, along with a clear understanding of its regulation by miRNA. Together, such studies will be able to point out early therapeutic intervention needed in management of HIV/HCV co-infection.

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