Gene expression profiling of HCV genotype 3a initial liver fibrosis and cirrhosis patients using microarray

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Abstract

**Background:** Hepatitis C virus (HCV) causes liver fibrosis that may lead to liver cirrhosis or hepatocellular carcinoma (HCC), and may partially depend on infecting viral genotype. HCV genotype 3a is being more common in Asian population, especially Pakistan; the detail mechanism of infection still needs to be explored. In this study, we investigated and compared the gene expression profile between initial fibrosis stage and cirrhotic 3a genotype patients.

**Methods:** Gene expression profiling of human liver tissues was performed containing more than 22000 known genes. Using Oparray protocol, preparation and hybridization of slides was carried out and followed by scanning with GeneTAC integrator 4.0 software. Normalization of the data was obtained using MiDAS software and Significant Microarray Analysis (SAM) was performed to obtain differentially expressed candidate genes.

**Results:** Out of 22000 genes studied, 219 differentially regulated genes found with \( P \leq 0.05 \) between both groups; 107 among those were up-regulated and 112 were down-regulated. These genes were classified into 31 categories according to their biological functions. The main categories included: apoptosis, immune response, cell signaling, kinase activity, lipid metabolism, protein metabolism, protein modulation, metabolism, vision, cell structure, cytoskeleton, nervous system, protein metabolism, protein modulation, signal transduction, transcriptional regulation and transport activity.

**Conclusion:** This is the first study on gene expression profiling in patients associated with genotype 3a using microarray analysis. These findings represent a broad portrait of genomic changes in early HCV associated fibrosis and cirrhosis. We hope that identified genes in this study will help in future to act as prognostic and diagnostic markers to differentiate fibrotic patients from cirrhotic ones.

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**Background**

Chronic hepatitis C is a major liver related health problem destroying liver architecture leading to cirrhosis and hepatocellular carcinoma. Almost 3% of the world population is infected with this deadly virus and in future, it is predicted that infection will rise to 3 fold of the present number [1-6]. HCV persist(s) beside the specific humoral responses and the mechanism of viral persistence and viral clearance is not fully understood. During HCV infection, initial fibrosis development is the method to overcome the damage caused by the virus. But the early events are the basis of disease outcome. Initial fibrosis is thought to be reversible, although many studies do not support this phenomenon. As extracellular matrix (ECM) tissues not only involve matrix production but also matrix degradation leading to ECM remodeling [7-9]. Fibrosis is caused by excessive deposition of ECM by histological and molecular reshuffling of various components like collagens, glycoproteins, proteoglycans, matrix proteins and matrix bound growth factors. Fibrosis stage information not only indicates treatment response but also reflect/indicate cirrhosis development disaster [4,10-16]. ECM metabolism is a balance between ECM deposition and removal influenced by cytokines and growth factors.
 Genome-wide analysis of abnormal gene expression showed transcripts deregulation differences among normal, mild and severe fibrosis during HCC development with identification of novel serum markers for its early stage. Recent studies suggest that genetic markers may be able to define exact stage of liver fibrosis. For this purpose, limited but functional studies have proposed quite a few genetic markers with individual genes or group of genes [18,19]. Advantage of genetic markers over liver biopsy is intrinsic and long-term while, liver biopsy represents only one time point [20]. Researchers found specific genes such as AZIN1, TLR4, CXCL9, CXCL10, CTGF, ITIH1, SERPINF2, TTR, PDGF, TGF-β1, collagens COL1-A1, TNFα, interleukin, ADAMTS, MMPs, TIMPs, LAMB1, LAMC1, Cadherin, CD44, ICAM1, ITGA, APO and CYP2C8 that showed deregulation during liver fibrosis and may be used to access liver fibrosis and cirrhosis [11-28]. Microarray is a powerful technique used for the identification of differentially expressed genes within control and experimental samples in different diseases and conditions like cancer development. Very few studies are available that use microarray for the identification of specific genes related to fibrosis [27,28]. In a recent study, Caillot et al. used microarray technique and found a significant association of ITIH1, SERPINF2 and TTR gene expression and their related proteins with all fibrosis stages [28]. Expression of these genes and related proteins gradually decreased during the fibrosis development to its end stage cirrhosis. Mostly, HCV expression based studies using microarray are carried out with genotype 1 and 2. Very few studies exploring the role of HCV genotype 3a are done during the fibrosis development to its end stage cirrhosis. In Pakistan, genotype 3a is the major contributor and has strong association with HCC. The aim of the present study was to examine gene expression profiles in the HCV associated liver disease progression. We have identified for the first time, those genes that are differentially regulated in initial fibrosis and advance stage liver cirrhosis 3a patients and identified potential targets that can be used as effective markers to differentiate between fibrotic and cirrhotic liver with genotype 3a. This data may also help to understand the disease stages between initial versus end stage cirrhosis, as there are limited studies concerning HCV genotype 3a disease progression.

Materials and methods

Patients

This study was conducted at Department of Pathology, Jinnah Hospital, Lahore, Mayo Hospital, Lahore and Liver Centre Faisalabad with collaboration of Applied and Functional Genomics Lab, National Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan. HCV RNA-positive patients were identified among HCV antibody (anti-HCV) positive patients. Patients who had received a previous course of INF or immunosuppressive therapy, or who had clinical evidence of HBV or HIV and any other type of liver cancer were excluded from the study. Patients who refused to have a liver biopsy or for whom it was contraindicated, i.e., because of a low platelet count, prolonged prothrombin time or decompensated cirrhosis were also excluded from the study. The liver biopsy procedure, its advantages and possible adverse effects were explained to the patients. Written informed consent for biopsy procedure was obtained from patients, also contained information about demographic data, possible transmission route of HCV infection, clinical, virological and biochemical data. The study was approved by institutional ethical committee.

Patients and liver biopsy

A group of patient was selected from previously described study with known fibrosis evaluation [29]. Two groups of samples consisted of early fibrosis (F1) and cirrhosis (F4) containing 9 samples each were made. Patient’s characteristics are given in Table 1.

RNA isolation, cDNA and aRNA preparation, and dye labeling for microarray experiments

RNA from liver biopsy samples were isolated using RNeasy mini elute kit (Qiagen, USA) and preparation of cDNA and aRNA was carried out using RNA pulse amplification and labeling kit (Kreatech, USA), according to manufacturer. aRNA from HCV infected patients and normal subjects were labeled with Cy3 and Cy5, respectively. A detailed protocol describing each step from start to microarray hybridization can be

| Table 1 Clinical Characteristics of the patients used in this study |
|-------------------|-----------------|-----------------|-----------------|
| Factor            | Fibrotic patients | Cirrhotic patients | P value         |
| Age               | 37.9 ± 9.5       | 48.4 ± 7.1       | < 0.05          |
| Sex (M/F)         | 5/4              | 6/3              | 0.247           |
| HAI score         | 605.1 ± 25.8     | 76 ± 2.9         | < 0.05          |
| Viral load        | 1.3 ± 10^7 ± 1.5 ± 10^7 | 2.9 ± 10^7 ± 2.9 ± 10^7 | < 0.05          |
| Hb level          | 126 ± 1.2        | 123 ± 2.1        | 0.328           |
| Bilirubin         | 8.8 ± 0.2        | 1.62 ± 0.31      | < 0.05          |
| ALT               | 1178 ± 55.3      | 1475 ± 61.2      | 0.091           |
| ALP               | 88.1 ± 47.5      | 323.8 ± 80.1     | < 0.05          |
| AST               | 107.1 ± 66.5     | 155.5 ± 90.6     | < 0.05          |
| Albumin           | 43 ± 0.16        | 3.6 ± 0.33       | < 0.05          |
| Platelet count    | 185 ± 21.2       | 81.6 ± 17.7      | < 0.05          |
downloaded from (http://www.operon.com/products/microarrays/OpArray%20Protocol.pdf).

Array hybridization and scanning
Biopsy samples were analyzed on cDNA microarrays (Oparray) containing > 22000 named genes with 37584 spots. Equal amount of Cy3 and Cy5 (55 pmol each) labeled targets were mixed with 45 μl of OpArray Hyb Buffer. Pre-washing, array hybridization and post-washing of microarray labeled slides were performed according to the manufacturer protocols at 42°C for 18 hours on fully automated workstation “GeneTAC™ HybStation”.

Microarray data analysis
GeneTAC™ UC4 × 4 scanner was used for scanning slides at 10 μm resolution for both Cy3 and Cy5 channels. GeneTAC Integrator 4.0 software was initially used for main data output as “csv” format file containing all necessary information. This “csv” file was converted to “mev” format for normalization by using software “ExpressConverter” (http://www.tm4.org/utilities.html). MIDAS (Microarray Data Analysis System) software was downloaded (http://www.tm4.org/midas.html) and used for normalization of data. Fold induction was determined by using formula log2(Cy5/Cy3). A rank-based permutation method SAM was used to identify significantly expressed genes among fibrosis stages (http://www-stat.stanford.edu/~tibs/SAM/). Gene expression patterns through k-means clustering were produced and viewed using freely available programs CLUSTER 3.0 (http://rana.lbl.gov/EisenSoftware.htm) and Tree View 1.45 (http://rana.lbl.gov/downloads/TreeView/), respectively. To identify biological themes among gene expression profiles, the Expression Analysis Systematic Explorer (EASE) was used (http://david.abcc.ncifcrf.gov/content.jsp?file=~/ease/ease1.htm&type=1) [30]. The microarray data have been deposited to the GEO accession database (http://www.ncbi.nlm.nih.gov/geo) with accession number GSE33258.

Real-time reverse transcriptase (RT)-PCR analysis
Genes with known function and significantly up-regulated or down-regulated were analyzed by real-time RT-PCR with RNA used for microarray analysis. Total RNA was converted to cDNA using MmLV (Moloney murine leukemia virus). Selected and tested oligonucleotide primer pairs for their specificity were used for real time RT-PCR using ABI 7500 real time PCR system using syber green chemistry. Each experiment was run in triplicate including GAPDH as endogenous control (Table 2). Each gene was quantified relative to the calibrator. Applied Biosystem Sequence Detection Software and calculations were made by instrument using the equation $2^{-\Delta \Delta C_{T}}$.

Results
Patient’s characteristics
Among 18 patients, equal number of patients belonged to F1 (9) and cirrhotic (9) group. Out of these, six best samples each with good RNA were used for microarray experiments. Normal liver biopsies were also obtained in triplicate. The serum viral load, bilirubin, albumin, and platelet count of cirrhotic patients were significantly low (P < 0.05), while, serum ALP and AST levels were high when compared to patients with F1 stage. There were no significant differences between serum ALT and Hb level in the patients with F1 or cirrhotic stage (Table 1).

Microarray analysis: expression behavior of significant genes
We found 219 differentially regulated genes in fibrosis versus cirrhotic groups (Figure 1). Among these, 107 genes were up-regulated (Figure 2) whereas, 112 genes were down-regulated (Figure 3). Significant genes with their symbols and functions are listed in Tables 3 and 4. Genes were classified into 31 categories according to their biological functions (Figure 4).

Significantly synchronized genes with known biological functions
The differentially regulated genes were grouped according to their biological functions by EASE program that uses information from Entrez Gene (http://jura.wi.mit.edu/entrez_gene/) and KEGG database (http://www.genome.jp/kegg/kegg1.html). Our results showed variation in gene regulation in both early fibrosis and cirrhosis stages (Figure 1). Out of 107 up-regulated genes, 65 belonged to early fibrosis stage, whereas, 42 genes belonged to the cirrhotic stage. Genes related to immune response, cell signaling, kinase activity, lipid metabolism, metabolism, vision and transcriptional regulation were up-regulated in both early fibrosis and cirrhotic samples (Table 2). We found that most genes related to apoptosis, cell structure, cytoskeleton, nervous system protein metabolism, protein modulation, signal transduction, transcriptional regulation and transport

| Gene name | Primer sequence | Annealing temp |
|-----------|-----------------|----------------|
| OAS       | s5'-ACTTTAAAACCCCATATTGAA-3' as5'-GGGAGGGGGCGGAGATATAT-3' | 58°C |
| FAM14B    | s5'-TCTCACCCCTCAAGCACCGGACACCG-3' as5'-CCTGAGATAGGAGAATTTG-3' | 60°C |
| CASPASE9  | s5'-ATGTCGCTCCGCGTCTCAAC-3' as5'-GGAACTGTTGAGCCGTCCTC-3' | 58°C |
| TGFBR     | s5'-TTCCCTGGGGATACAGAGACA-3' as5'-AGATTCGGCTGTTGAGTCC-3' | 58°C |

Table 2 Primer sequences used for Real time RT-PCR analysis

| Gene name | Primer sequence | Annealing temp |
|-----------|-----------------|----------------|
| OAS       | s5'-ACTTTAAAACCCCATATTGAA-3' as5'-GGGAGGGGGCGGAGATATAT-3' | 58°C |
| FAM14B    | s5'-TCTCACCCCTCAAGCACCGGACACCG-3' as5'-CCTGAGATAGGAGAATTTG-3' | 60°C |
| CASPASE9  | s5'-ATGTCGCTCCGCGTCTCAAC-3' as5'-GGAACTGTTGAGCCGTCCTC-3' | 58°C |
| TGFBR     | s5'-TTCCCTGGGGATACAGAGACA-3' as5'-AGATTCGGCTGTTGAGTCC-3' | 58°C |

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Figure 1 Significant host genes regulated by HCV infection
Clustering results for differentially expressed genes between HCV infected patients with initial fibrosis and cirrhosis. Clustering was performed by Cluster 3.0 software. The fold changes in mRNA expression are represented with green and red squares showing down- and up-regulation of genes in liver biopsy samples, respectively. Each vertical column represents an independent experiment, while color scale represents the fold change magnitude.

Figure 2 Heat map of up-regulated genes
Clustering results for differentially expressed genes between HCV infected patients with initial fibrosis and cirrhosis. Clustering was performed by Cluster 3.0 software. The fold changes in mRNA expression are represented with green and red squares showing down- and up-regulation of genes in liver biopsy samples, respectively. Each vertical column represents an independent experiment, while color scale represents the fold change magnitude.
were up-regulated in early fibrosis. Many uncharacterized genes were also found up-regulated in liver disease progression. We identified 112 genes (F1 = 92; F4 = 20) related to above mentioned pathways down-regulated when fibrosis lead to cirrhotic stage (Table 2 and Figure 2). Genes related to these pathways showed varied response and none of biological function was specifically related to any liver disease stage (Table 4 and Figure 3).

Independent validation of candidate genes using quantitative real-time RT-PCR
Total RNA extracted from infected liver biopsies was used for real time RT-PCR analysis to validate microarray data. Expression analysis of the genes involved in apoptosis, immune response and transcriptional regulation was performed. We randomly selected four genes, CASPASE9, FAM14B, OAS2 and TGFBR2 from our study. CASPASE9 is apoptosis related gene, FAM14B and OAS2 are immune responsive genes, whereas, TGFBR2 is multifunctional gene and found to be up-regulated in fibrosis.

Discussion
Liver fibrosis can progress to cirrhosis after an interval of 15-20 years in patients with HCV [31]. It is very important to identify such markers that can differentiate liver fibrosis from cirrhosis. Liver biopsy is a common tool for the detection of liver current situation but due to some limitations its use as diagnostic tool is denied. Microarray analysis is an emerging and novel approach to study gene expression in HCV associated fibrosis and cirrhosis. As liver gene expression in HCV patients is variable and it might be partially dependent on the corresponding genotype [32]. In this study, we specially focused on gene expression analysis in patients with genotype 3a that is most common in our region. We found that many genes associated with apoptosis, several cellular functions, immune response, metabolism including energy, liver, sulphur protein metabolism, transcriptional regulation, signal transduction, transport, DNA replication were dys-regulated both in early fibrosis and cirrhosis. In some cases, gene expression tends to be increased from initial fibrosis to cirrhosis. Induction of gene expression associated with proapoptotic, proinflammatory and proliferative activities is in accordance with previous studies [18,27,33-35]. Although, we found some dysregulation of genes related to vision and nervous system first time.

Differential expression of apoptosis related genes in HCV associated initial fibrosis and cirrhosis
In this study, host genes involved in apoptosis (Figure 5) such as BCL212 and PDCD1 showed down-regulation in initial fibrosis and significant up-regulation in
Table 3: Up-regulated genes in cirrhotic and non-cirrhotic HCV liver biopsy samples

| Function                  | Symbol      | Description                              | GeneBank               | t-test       |
|---------------------------|-------------|------------------------------------------|------------------------|--------------|
| Apoptosis                 | CASP9       | Caspase-9 precursor (EC 3.4.22)           | NM_001229.2            | 0.000207     |
| apoptosis                 | EMP1        | Epithelial membrane protein 1            | NM_001423.1            | 1.38E-06     |
| cell adhesion             | YIF1A       | Protein YIF1A                             | NM_020470.1            | 0.000115     |
| Cell Cycle                | CHES1       | Checkpoint suppressor 1                   | NM_005197.2            | 0.047852     |
| Cell Cycle                | CCNG1       | Cyclin-G1                                 | NM_004060.3            | 0.002296     |
| Cell singling             | LRRC41      | Leucine-rich repeat-containing protein 41 | NM_006369.4            | 0.000132     |
| cell singling             | SCG3        | Secretogranin-3 precursor                 | NM_013243.2            | 4.69E-05     |
| Cell singling             | FLRT1       | Leucine-rich repeat transmembrane protein FLRT1 precursor | NM_013280.4 | 0.022495 |
| cell singling             | SIGLEC8     | Sialic acid-binding Ig-like lectin 8 precursor | NM_014442.2 | 0.020819 |
| cell structure            | HYL1        | Hydrothalamus syndrome 1                  | NM_145014.1            | 7.28E-05     |
| cell structure            | TOR1AIP1    | Torsin-1A-interacting protein 1           | NM_015602.2            | 5.13E-05     |
| cytokine                  | CRLF3       | Cytokine receptor-like factor 3           | XM_001128008.1         | 3.25E-06     |
| cytokine                  | PLEKHG6     | pleckstrin homology domain containing, family G | NM_018173.1 | 0.004661 |
| Cytoskeleton              | KRTAP19-5   | Keratin-associated protein 19-5           | NM_181611.1            | 0.000296     |
| cytoskeleton              | COMMD5      | COMM domain-containing protein 5           | NM_014066.2            | 8.2E-07      |
| DNA replication           | CENPJ       | Centromere protein J                      | NM_018451.2            | 2.22E-05     |
| DNA replication           | TOP3B       | DNA topoisomerase 3-beta-1                | XM_001129880.1         | 1.79E-05     |
| DNA replication           | DDX54       | ATP-dependent RNA helicase                | NM_024072.3            | 0.080445     |
| Energy                    | QSVTUB      | ATP synthase,                            | NR_002162.1            | 9.93E-05     |
| Energy                    | COX6A1      | Cytochrome c oxidase polypeptide Vla-liver | NM_004373.2            | 0.077561     |
| Immune response           | CRYBB3      | Beta crystallin B3                        | NM_004076.3            | 2.89E-05     |
| Immune response           | FCR3        | Fc receptor-like 3 precursor              | NM_052939.3            | 9.14E-06     |
| Immune response           | IFITM2      | interferon induced transmembrane protein 2 | NM_006435.1 | 0.000197 |
| Immune response           | DEF8114     | Beta-defensin 114 precursor               | NM_001037499.1         | 0.030399     |
| Immune response           | IFNA21      | Interferon alpha-21 precursor             | NM_002175.1            | 0.019498     |
| Immune response           | KIR2DL1     | Killer cell immunoglobulin-like receptor 3DL2 precursor | NM_153443.2 | 0.014098 |
| ion transport             | CANX        | Calnexin precursor                        | NM_001024649.1         | 0.097857     |
| ion transport             | CLGN        | Calcmein precursor                        | NM_004362.1            | 0.002724     |
| ion transport             | HHLA3       | HERV-H LTR-associated 3 isoform 2         | NM_001036645.1         | 0.003147     |
| kinase activity           | PDXK        | Pyridoxal kinase                          | NM_003681.3            | 0.037672     |
| kinase activity           | PRKCB1      | Protein kinase C beta type                | NM_002738.5            | 0.009381     |
| Lipid Metabolism          | PPAPDC3     | Probable lipid phosphate phosphatase PPAPDC3 | NM_032728.2 | 6.06E-05 |
| Lipid Metabolism          | OSBPL2      | Oxysterol-binding protein-related protein 2 | NM_144498.1 | 0.016902 |
| lipid metabolism          | QSR387      | Novel protein                             | XM_372769.4            | 0.000376     |
| Liver functions           | LEPROT      | Leptin receptor precursor                 | NM_017526.2            | 0.107525     |
| Metabolism                | EMR1        | EGFl-like module-containing mucin-like hormone receptor-like 1 precursor | NM_001974.3 | 0.00022 |
| Metabolism                | UROD        | Uroporphyrinogen decarboxylase            | NM_000374.3            | 0.004853     |
| metabolism                | DCN         | Decorin precursor                         | NM_019203.2            | 0.000807     |
| Metabolism                | FADH2B      | Fumarylacetoacetate hydrolase domain containing 2B | XR_016023.1 | 4.16E-06 |
| Metabolism                | ACSBG2      | Prostatic acid phosphatase precursor      | NM_001099.2            | 1.55E-05     |
| Metabolism                | ANTXR2      | Anthrax toxin receptor 2 precursor        | NM_058172.3            | 1.92E-05     |
| Metabolism                | CDA         | Cytidine deaminase                        | NM_001785.2            | 0.005579     |
| Metabolism                | CTSD        | Cathepsin D precursor                     | NM_001909.3            | 0.028633     |
| Metabolism                | GOT2        | Aspartate aminotransferase, mitochondrial precursor | XR_016602.1 | 0.000258 |
| Metabolism                | NAT13       | Mak3 homolog                              | XR_018106.1            | 5.03E-06     |
| Metabolism                | TIGD5       | Tigger transposable element-derived protein 5 | NM_052862.2 | 0.057722 |
| Neuronal system           | NPSA3       | Neuronal PAS domain-containing protein 3   | NM_022123.1            | 0.00115      |
| Neuronal system           | GPR98       | G-protein coupled receptor 98 precursor   | NM_032119.3            | 0.000239     |
| Neuronal system           | NEUROD2     | Neurogenin differentiation factor 2        | NM_006160.3            | 2.17E-05     |
| Neuronal system           | LAMB2       | Laminin subunit beta-2 precursor          | NM_002292.3            | 0.007081     |
| Protein Metabolism        | CSDE1       | GTPase Nras precursor                     | NM_002524.2            | 0.017096     |
| Protein Metabolism        | ENPP7       | Ectonucleotide pyrophosphatase            | NM_178543.3            | 0.000321     |
| Protein Metabolism        | KIAA1147    | KIAA1147 (KIAA1147), mRNA                 | NM_001080392.1         | 0.000106     |
Table 3 Up-regulated genes in cirrhotic and non-cirrhotic HCV liver biopsy samples (Continued)

| Protein Function                        | Gene Symbol | Description                                      | P-value |
|----------------------------------------|-------------|--------------------------------------------------|---------|
| Protein Metabolism                     | KIAA2013    | KIAA2013 (KIAA2013), mRNA                       | NM_138346.1 2.86E-05 |
| Protein Metabolism                     | KNG1        | Kininogen-1 precursor                           | NM_000893.2 0.002086 |
| Protein Metabolism                     | APOOL       | Protein FAM121A precursor                       | NM_198450.3 0.019311 |
| Protein modulation                     | HAT1        | Histone acetyltransferase type B catalytic subunit | NM_001033085.1 0.000447 |
| Protein modulation                     | RIMS2       | Regulating synaptic membrane exocytosis protein 2 | NM_014677.2 0.000572 |
| Protein modulation                     | UBL4B       | Ubiquitin-like protein 4B                       | NM_203412.1 2.33E-05 |
| Protein modulation                     | UBE1L       | Ubiquitin-activating enzyme E1 homolog          | NM_003353.2 0.021531 |
| Protein modulation                     | USP54       | Ubiquitin specific protease 54                  | NM_152586.2 0.005541 |
| Protein synthesis                      | RNPEP       | Aminopeptidase                                  | NM_002016.3 0.0218 |
| PTMs                                    | SNF1LK2     | Serine/threonine-protein kinase SNF1-like kinase 2 | NM_015191.1 0.00037 |
| RNA modelling and synthesis            | IMP3        | U3 small nuclear ribonucleoprotein protein IMP3 | NM_018285.2 7.13E-05 |
| RNA modelling and synthesis            | SF3A2       | Splicing factor 3A subunit 2                     | NM_007165.4 0.123287 |
| Signal Transduction                    | CACNB3      | Voltage-dependent L-type calcium channel subunit beta-3 | NM_000725.2 0.00248 |
| Signal Transduction                    | PCSK5       | Proprotein convertase subtilisin/kexin type 5 precursor | NM_006200.2 6.62E-06 |
| Signal Transduction                    | VDAC3       | Voltage-dependent anion-selective channel protein 3 | XR_019103.1 0.000231 |
| Signal Transduction                    | ITGB6       | Integrin beta-6 precursor                       | NM_000888.3 0.089005 |
| Sulphur metabolism                     | FAM119B     | family with sequence similarity 119            | NM_015433.2 0.018357 |
| Transcriptional regulation             | LYSMD3      | LysM and putative peptidoglycan-binding domain-containing protein 3 | NM_198273.1 0.004237 |
| Transcriptional regulation             | FOX1        | Forkhead box protein II                          | NM_012188.3 1.84E-05 |
| Transcriptional regulation             | MYCL1       | L-my-1 proto-oncogene protein                    | NM_001033081.1 4.97E-05 |
| Transcriptional regulation             | MYOD1       | Myoblast determination protein 1                | NM_002478.4 1.24E-05 |
| Transcriptional regulation             | PRDM5       | PR domain zinc finger protein 5                  | NM_006200.2 6.62E-06 |
| Transcriptional regulation             | YBX1        | Nuclease sensitive element-binding protein 1     | XR_019103.1 0.000231 |
| Transcriptional regulation             | ANKHD1      | Eukaryotic translation initiation factor 4E-binding protein 3 | NM_020600.4 0.041134 |
| Transcriptional regulation             | RUNX2       | Runt-related transcription factor 2              | NM_001024630.2 0.064668 |
| Transcriptional regulation             | SUSD4       | Sushi domain-containing protein 4 precursor     | NM_017982.2 0.004775 |
| Transport                              | CLPB        | Caseinolytic peptidase B protein homolog         | NM_030813.3 0.002308 |
| Transport                              | K1024       | UFF0258 protein KIAA1024                        | NM_015260.1 0.001564 |
| Transport                              | NOS2A       | Nitric oxide synthase 2, inducible 1             | NM_000625 0.017057 |
| Transport                              | SCGN        | Secretagogin                                    | NM_006998.3 2E-06 |
| Transport                              | FBXO32      | F-box only protein 32                           | NM_148177.1 0.043284 |
| Uncharacterized                        | C12orf41    | CDNA FLJ2670                                    | NM_017822.2 0.001604 |
| Uncharacterized                        | C17orf56    | CDNA FLJ1528                                    | NM_144679.1 1.11E-06 |
| Uncharacterized                        | C21orf59    | Uncharacterized protein                         | NM_021254.1 0.001335 |
| Uncharacterized                        | C4orf20     | CDNA FLJ1200                                    | NM_018359.1 0.000365 |
| Uncharacterized                        | C9orf7      | Uncharacterized protein                         | NM_017586.1 0.002015 |
| Uncharacterized                        | C9orf91     | C9orf91 protein                                 | NM_153045.2 2.21E-06 |
| Uncharacterized                        | KIAA0562    | Glycine-, glutamate-, thienylcyclohexyloxyperidine-binding protein | NM_014704.2 5.09E-06 |
| Uncharacterized                        | KLHL30      | Kelch-like 30                                   | NM_198582.1 5.06E-06 |
| Uncharacterized                        | LOC728660   |                                                     | XM_001128340.1 0.000153 |
| Uncharacterized                        | Q71MF4      |                                                     | 7.89E-05 |
| Uncharacterized                        | Q8TCQ8      | CDNA FLJ90801 fis, clone Y79AA1000207            | XM_001134000.1 0.028312 |
| Uncharacterized                        | Q8WY63      |                                                     | 0.017959 |
| Uncharacterized                        | ST8SIA6     | Alpha-2,8-sialyltransferase 8F                   | NM_001004470.1 0.008458 |
| Uncharacterized                        | C10orf6     | Uncharacterized protein C10orf6                  | NM_018121.2 0.000673 |
| Uncharacterized                        | Q75264      |                                                     | XM_209196.5 0.01282 |
| Uncharacterized                        | Q9NW32      | CDNA FLJ10346                                   | 0.038301 |
| Uncharacterized                        | S11Y        | Putative S100 calcium-binding protein            | XM_001126330.1 0.000254 |
| Vision                                 | ST13        | Hsc70-interacting protein                       | XR_018201.1 0.012074 |
| Vision                                 | DUPD1       | Dual specificity phosphatase and pro isomerase domain containing 1 | NM_001003892.1 4.5E-06 |
| Vision                                 | OR6P1       | Olfactory receptor 6P1                          | 1.78E-05 |
| Vision                                 | ARSH        | Arylsulfatase H                                 | NM_00101719.1 0.000229 |
| Vision                                 | OR5F2       | Olfactory receptor 5F2                          | NM_001004753.1 0.018938 |
| Vision                                 | OR7G3       | Olfactory receptor 7G3                          | NM_001001958.1 0.077667 |
| Function                        | Symbol     | Description                          | GeneBank                  | t-test  |
|--------------------------------|------------|--------------------------------------|---------------------------|---------|
| Apoptosis                      | BCL2L12    | Bcl-2-related proline-rich protein   | NM_001040668.1            | 0.00335 |
| Apoptosis                      | PDCD1      | Programmed cell death protein 1      | NM_005018.1               | 7.94E-06|
| carbohydrate metabolism        | OGDHL      | oxoglutarate dehydrogenase-like      | NM_018245.1               | 0.000909|
| cell adhesion                  | THUMPD1    | THUMP domain-containing protein 1    | NM_017736.3               | 0.044141|
| Cell Cycle                     | AKAP4      | A-kinase anchor protein 11            | NM_016248.2               | 0.000598|
| cell cycle                     | TINF2      | TERF1-interacting nuclear factor 2    | NM_012461                 | 0.045359|
| cell cycle                     | VEGFB      | vascular endothelial growth factor B  | NM_003377                 | 0.017886|
| cell singling                  | GRIN3A     | Glutamate [NMDA] receptor subunit 3A | NM_133445.1               | 1.17E-06|
| cell singling                  | Q8N9G6     | similar to nuclear pore membrane protein 121 | XM_498333.2 | 7.46E-05|
| Cell Structure                 | ENO3       | Beta-enolase                          | NM_001976.2               | 0.050309|
| cell structure                 | MAPK6D1    | MAP6 domain-containing protein 1      | NM_02487.1                | 0.034981|
| cytokine                       | IL13RA2    | Interleukin-13 receptor alpha-2 chain precursor | NM_000640.2          | 0.024814|
| Cytoskeleton                   | LLGL1      | Lethal(2) giant larvae protein homolog 1 | NM_004140.3         | 0.008177|
| cytokoskeleton                 | SNX17      | Sorting nexin-17                      | NM_014748.2               | 5.41E-06|
| DNA binding proteins           | ZNF236     | Zinc finger protein 236               | NM_007345.2               | 1.54E-07|
| DNA binding proteins           | ZBED4      | Zinc finger BED domain-containing protein 4 | NM_014838.1        | 0.003728|
| DNA replication                | WRB        | Tryptophan-rich protein               | NM_004627.2               | 0.000578|
| Energy                         | ABHD2      | ATP-binding cassette sub-family F member 2 | NM_005692.3            | 0.000193|
| Energy                         | ATAD2      | ATPase family AAA domain-containing protein 2 | NM_014109.2          | 8.95E-06|
| Energy                         | PSMD11     | 26S proteasome non-ATPase regulatory subunit 11 | NM_002815.2          | 0.000415|
| Energy                         | PSMD4      | 26S proteasome non-ATPase regulatory subunit 4 | NM_002810.2          | 0.003878|
| Energy                         | SYDE1      | Synapse defective 1                   | NM_033025.4               | 0.000222|
| Immune response                | ATG16L2    | ATG16 autophagy related 16-like 2     | NM_033388.1               | 1.31E-05|
| Immune response                | IL8RB      | High affinity interleukin-8 receptor B | NM_001557.2             | 0.00539 |
| immune response                | PTGS2      | prostaglandin-endoperoxide synthase 2 | NM_000963                 | 0.00504 |
| immune response                | FAM14B     | Interferon alpha-inducible protein 27-like protein 1 | NM_145249             | 0.006008|
| immune response                | OAS2       | 2′-5′-oligoadenylate synthase 2       | NM_016817                 | 0.009299|
| ion transport                  | DSG4       | Desmoglein-4 precursor                | NM_177986.2               | 1.23E-05|
| ion transport                  | SLC10A5    | Sodium/bile acid cotransporter 5      | NM_00101893.2             | 6.5E-08 |
| ion transport                  | CAPN7      | Calpain-7                             | NM_014296.2               | 0.003785|
| ion transport                  | MT1E       | Metallothionein-1E                    | NM_175617.3               | 0.001767|
| Lipid Metabolism               | DEGS2      | sphingolipid C4-hydroxylase/delta 4-desaturase | NM_206918.1        | 0.008105|
| lipid metabolism               | CHKA       | Choline kinase alpha                   | NM_001277.2               | 0.003609|
| lipid metabolism               | ADA        | bubblegum related protein             | NM_030924.3               | 6.6E-05 |
| Metabolism                     | HMGCL      | Hydroxymethylglutaryl-CoA lyase, mitochondrial precursor | NM_000191.2         | 0.010122|
| metabolism                     | HMGCS1     | Hydroxymethylglutaryl-CoA synthase, cytoplasmic | NM_002310.4       | 1.2E-05 |
| Metabolism                     | SH3BGR3    | SH3 domain-binding glutamic acid-rich-like protein 3 | NM_031286.3      | 0.000157|
| Metabolism                     | ARHGAPS    | Rho GTPase-activating protein 5        | NM_001173.2               | 0.010513|
| Metabolism                     | CKM        | Creatine kinase M-type                | NM_001824.2               | 0.000689|
| Metabolism                     | CPT1A      | Carnitine O-palmitoyltransferase 1, liver isofom | NM_01031847.1     | 0.008256|
| Metabolism                     | USP53      | Inactive ubiquitin carboxyl-terminal hydrolase 53 | NM_019050.1         | 2.46E-06|
| morphogenesis                  | SLC33A1    | Acetyl-coenzyme A transporter 1        | NM_004733.2               | 8.12E-06|
| morphogenesis                  | PDYN       | Beta-neoendorphin-dynorphin precursor  | NM_024411.2               | 0.055803|
| nervous system                 | NINJ2      | Ninjin-2 (Nerve injury-induced protein 2) | NM_016533.4           | 0.004163|
| protein Metabolism             | GON4L      | GON-4-like protein                    | NM_00137533.1             | 0.00137 |
| protein Metabolism             | PHACTR4    | phosphatase and actin regulator 4     | NM_00104813.1             | 0.000139|
| protein Metabolism             | OTUD7A     | OTU domain-containing protein 7A       | XM_001127986.1            | 0.00394 |
| protein Metabolism             | Q96NT9     | GR AF-1 specific protein phosphatase  | XM_497354.1               | 7.5E-05 |
| protein Metabolism             | WDFC13     | Protein WDFC13 precursor              | NM_172005.1               | 0.080737|
| Protein modulation             | SMAP1      | Stromal membrane-associated protein 1  | NM_00144305.1             | 8.97E-08|
| Protein modulation             | DYSRK1B    | Dual specificity tyrosine-phosphorylation-regulated kinase 1B | NM_004714.1         | 0.001009|

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| Protein modulation | COQ5 | Ubiquinone biosynthesis methyltransferase COQ5 | NM_032314.3 | 2.07E-05 |
|--------------------|------|---------------------------------------------|------------|---------|
| Protein modulation | MTIF2 | Translation initiation factor IF-2 | NM_01005369.1 | 0.002917 |
| Protein synthesis | MRPL46 | 39S ribosomal protein L46, mitochondrial precursor | NM_022163.2 | 2.83E-06 |
| Protein synthesis | MRPS35 | 28S ribosomal protein S35, mitochondrial precursor | NM_021821.2 | 0.000245 |
| protein synthesis | PLAT | Tissue-type plasminogen activator precursor | NM_000930.2 | 0.014382 |
| protein synthesis | SENP1 | Sentrin-specific protease 1 | NM_014554.2 | 1.63E-06 |
| protein synthesis | ELL | RNA polymerase II elongation factor ELL | NM_006532.2 | 0.003242 |
| Protein synthesis | PACS1 | Phosphofurin acidic cluster sorting protein 1 | NM_018026.2 | 0.005118 |
| protein synthesis | FTP4A1 | Protein tyrosine phosphatase type 1A protein 1 | NM_003463.3 | 0.001949 |
| PTMs | SNF1LK | Serine/threonine-protein kinase SNF1-like kinase 1 | NM_173354.3 | 0.000169 |
| Reproduction | LOC283116 | similar to Tripartite motif protein 49 | XR_016154.1 | 5.32E-07 |
| Reproduction | QSVYG3 | OTTHUMP0000018545 | - | 2.51E-06 |
| RNA modelling and synthesis | EXOSC2 | Exosome complex exonuclease RRP4 | NM_014285.4 | 0.080373 |
| RNA modelling and synthesis | RBM41 | RNA-binding protein 41 | NM_018301.2 | 0.002623 |
| RNA modelling and synthesis | ADCY2 | Double-stranded RNA-specific adenosine deaminase | NM_001111.3 | 6.64E-05 |
| Signal Transduction | FGF17 | Fibroblast growth factor 17 precursor | NM_003867.2 | 0.000254 |
| Signal Transduction | ADH1A | Adenylate cyclase type 2 | NM_020546.2 | 0.00139 |
| Signal Transduction | HOMER1 | Homer protein homolog 1 | NM_004272.3 | 0.001951 |
| Signal Transduction | TMEM100 | Transmembrane protein 100 | NM_018286.1 | 3.31E-05 |
| sulphur metabolism | FAN62B | family with sequence similarity 62 | NM_020728.1 | 1.65E-06 |
| transcriptional regulation | CRAMP1L | Protein cramped-like | NM_020825.2 | 0.006587 |
| transcriptional regulation | FOXK2 | Forkhead box protein K2 | XM_001134364.1 | 0.00156 |
| transcriptional regulation | HMGN2 | Nonhistone chromosomal protein HMV-17 | XM_001133530.1 | 0.01162 |
| Transcriptional regulation | NANO Q8 | Homeobox protein NANO | Q8 | 0.003264 |
| Transcriptional regulation | NFYL1 | nuclear transcription factor | NM_152995.4 | 8.53E-05 |
| transcriptional regulation | NR113 | Orphan nuclear receptor NR113 | NM_01077470.1 | 0.00247 |
| Transcriptional regulation | SNORA32 | Protein JOSD3 | NR_003023.1 | 0.107321 |
| Transcriptional regulation | GTF2B | Transcription initiation factor IIB | NM_001514.3 | 0.002357 |
| Transcriptional regulation | PAX8 | Paired box protein Pax-8 | NM_004663.3 | 4.89E-05 |
| Transcriptional regulation | CTCFL | Transcriptional repressor CTCFL | NM_080618.2 | 0.003129 |
| Transcriptional regulation | EEF1AL3 | Eukaryotic translation elongation factor 1 alpha 1 | - | 0.000917 |
| Transcriptional regulation | INTU | PDZ domain-containing protein 6 | NM_015693.2 | 0.003842 |
| transcriptional regulation | TGFB2 | TGF-beta receptor type-2 precursor | NM_01024847.1 | 0.007651 |
| Transport | KIF1A | Kinesin-like protein KIF1A | NM_004321.4 | 0.002119 |
| transport | NUP160 | Nuclear pore complex protein Nup160 | NM_015231.1 | 3.11E-06 |
| transport | SLIT3 | Slit homolog 3 protein precursor | NM_003062.1 | 0.000577 |
| Transport | AMICA1 | Junctional adhesion molecule-like precursor | NM_153206.1 | 3.86E-06 |
| Transport | KIF17 | Kinesin-like protein KIF17 | NM_020816.1 | 0.007279 |
| Transport | SCAMP4 | secretory carrier membrane protein 4 | NM_079834.2 | 0.026864 |
| transport | MUC6 | Mucin-6 protein (Gastric mucin-6) | XM_290540.7 | 0.054436 |
| Transport | SNF8 | Vacular sorting protein SNF8 | XR_019363.1 | 0.000425 |
| Uncharacterized | C14orf101 | Uncharacterized protein C14orf101 | NM_017799.3 | 0.02931 |
| Uncharacterized | C16orf57 | C16orf57 protein | NM_024498.2 | 0.004056 |
| Uncharacterized | Q6PDA4 | - | - | 2.68E-05 |
| Uncharacterized | Q6ZMS0 | - | - | 0.002171 |
| Uncharacterized | Q6ZRH2 | - | - | 1.15E-06 |
| Uncharacterized | Q8BN05 | - | - | 0.000459 |
| Uncharacterized | SEC14LS | - | - | 0.002171 |
| Uncharacterized | CD164L2 | CD164 sialomucin-like 2 protein precursor | NM_207397.2 | 0.000576 |
| Uncharacterized | CNO T6 | CCR4-NOT transcription complex subunit | NM_015455.3 | 0.000838 |
| Uncharacterized | Q6Y355 | - | - | 0.00218 |
| Uncharacterized | Q8N279 | CDNA: FLJ21438 | XM_029084.8 | 0.007508 |
citruline, whereas, expression levels for CASP9 and EMP1 genes were high at initial stage and were downregulated in cirrhosis stage. Regulation of apoptotic inducer and program cell death genes, BCL212 and PDCD1 in cirrhosis is according to previous observations where pro-apoptotic gene signaling has been observed in infection with HCV [36,37]. CASP9 is known as apoptosis initiator [38] and EMP1 is also found to induce apoptosis [39,40]. Expression of caspases is higher in early and moderate HCV infection, and enhanced apoptosis occur through the intrinsic apoptotic pathway via mitochondria [41,42].

**Cellular functions, cell cycle, signaling and cytoskeleton associated genes**

Genes related to various cellular functions showed different expression patterns (Figure 6). The cytoskeleton (COMMD5, KRTAP19-5, LLGL1 and SNX17) related genes were down-regulated in cirrhosis (F4). Most cell structure related genes were up-regulated in initial fibrosis (HYLS1, MAP6D1 and TOR1AIP1) and genes related to cell adhesion, cell cycle and signaling showed differential expression in both initial fibrosis and cirrhosis. It has been observed that HCV RNA synthesis may require an intact cytoskeleton [43]; our data indicated that many genes related to cytoskeleton were regulated by HCV infection.

**Genes associated with Immune response and cytokines**

A number of genes related to immune response and cytokines were identified (Figure 7). ATG16L2, DEFB114, FAM14B, IFNA21, IL8RB and KIR2DL genes were up-regulated in cirrhosis, whereas, FCR3L3, IFITM2 and OAS2 genes were up-regulated in initial fibrosis. Genes related to cytokine regulation, IL13RA2, PLEKHG6 and XCL2 were down-regulated in initial fibrosis except CRLF3 gene. Interleukin related gene expression has been found to be increased at pathology stage 3 and 4 which is concurrent with the present study and is associated with metastasis, cell proliferation or angiogenesis [37,44]. An increased expression of immune responsive genes and cytokines as fibrosis progress is in agreement with previous evidence that liver inflammation may enhance with increase in infected hepatocytes [45]. FCR3L3, a genetically conserved gene family encodes orphan cell surface receptors bearing high structural homology to classical Fc receptors, with multiple extracellular Ig domains and either ITAMs, ITIMs, or both in the intracellular domains. The natural ligands of these family members are still unknown but due to their signaling domains and expression on multiple immune cell types, these members likely modulate immune cell functions by affecting signaling pathways [46]. FCR3L3 is expressed predominantly in B lymphocytes in lymph nodes and germinal centers [47-49].

Previous studies revealed that IFITM2 and IFITM3 (two structurally related cell plasma membrane proteins) interrupt early steps entry and/or uncoating of the viral infection. Interferon-induced transmembrane (IFITM) genes are transcribed in most tissues with the exception of IFITM5 interferon inducible gene. IFITM genes are involved in early development, cell adhesion, and control of cell growth. Elevated gene expression triggered by past or chronic inflammation can prevent spreading of pathogens by limiting host cell proliferation. Low level of expression is sufficient to capture the growth of cells, whereas, the loss of expression causes tumor growth. This gene is termed as tumor suppressor. However, in many cancers it is observed that despite high level of IFITM, it represents tumor progression stage especially where the one of anti-proliferative interferon pathway is shut down. The role of ATG protein in membrane trafficking is mostly not clear. ATGL16 is thought to play role in autophagosome formation in association with RAB33B. It is also considered an active player in HCV replication and assembly [50,51].

Natural killer cells are the important player of innate immune response. KIRDL gene expression is found to be high in chronic HCV patients [52]. We found the KIR2DL1 gene expression high in patients with cirrhosis as compared to initial fibrosis stage. OAS synthesized in response to IFN-alpha stimulation. In infected cells, OAS enzymatic activity is induced by double-stranded

### Table 4 Down-regulated genes in cirrhotic and non-cirrhotic HCV liver biopsy samples (Continued)

| Gene                  | CDNA      | P-value | Fold Change |
|-----------------------|-----------|---------|-------------|
| Q66NM1                | FLJ30594  |         |             |
| C22orf30              |           | NM_173566.1 | 0.000611   |
| SBDS                  |           | NM_016038.2 | 0.006543   |
| AR5J                  |           | NM_0245903 | 0.000249   |
| OR5T1T                |           | NM_001004759.1 | 0.000408   |
| OR6C1                 |           | NM_001005182.1 | 0.000136   |
| DUSP5                 |           | NM_0044193 | 0.000125   |
| OR5K1                 |           | NM_001004736.2 | 0.000169   |
| RPGR                  |           | NM_001023582.1 | 0.007569   |

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RNAs, such as the intermediates of replication of RNA viruses or folded single stranded RNAs. OAS catalyzes polymerization of adenosine triphosphate into oligoadenylate that, in turn, activates a cellular endoribonuclease, RNase L, at subnanomolar concentrations. RNase L degrades cellular and viral single-stranded RNAs. Thus, viral replication is inhibited as a result of protein synthesis inhibition in a totally non-virus specific way [53]. We found high expression of OAS2 gene in fibrotic samples as compared to the last stage cirrhosis. This may be a way to stop viral replication but as the disease steps forward, virus overcome the host immune response to replicate itself.

Genes associated with different metabolic processes
A number of genes associated with different metabolism (processes/pathways) like energy, kinases, lipid and sulphur metabolism were identified among significantly expressed arrays (Figure 8). Several studies observed that HCV induces alterations in lipid metabolism that can lead to oxidative stress [54,55]. Consistent with these observations, we found six genes, ADA, CHKA, DEGS2, OSBP2, PPAPDC3, and Q5R387; which are involved in lipid biosynthesis, tumor cell growth by phosphatidylinositol biosynthesis, negative regulation of myoblast differentiation and hydrolyzation of phospholipids into fatty acids etc. This finding is in agreement with Diamond et al.; that host cell lipid metabolism may represent an area for future HCV antiviral therapies [56]. We found two genes FAM119B and FAM62B associated with sulphur metabolism which were up-regulated in cirrhotic samples. A number of genes related to energy mechanism such as PSMD4, PSMD11, ABHD2, ATAD2 and COX6A1 were up-regulated while, SYDE1 and Q5VTUB genes were down-regulated in cirrhotic samples. Two genes PDXK and PRKCB1 with kinase activity, and one gene, OGDHL linked to carbohydrate metabolism were also identified. Role of PRKCB1 (also known as PKC) in cell growth and differentiation control is known. It has been also found elevated in breast and pituitary tumors and malignant gliomas [57-59]. PKC was also found up regulated in hepatocellular carcinoma which can lead to hyper proliferation of the HCV infected tissues [60].
Genes associated with protein synthesis, modulation and metabolism

Many genes involved in protein synthesis, modulation and metabolism have increased or decreased expression in patients with HCV (Figure 9). Genes representing protein synthesis were down-regulated in initial fibrosis and showed significant increased expression in cirrhotic samples. Two genes associated with protein post-translational modifications (PTMs) were also identified that showed increased expression in cirrhosis. Some genes linked with protein metabolism like GON4L, OTUD7A, PHACTR4, Q96NT9 and WFDCl3 showed low expression in initial fibrosis, while CSDE1, ENPP7, KIAA1147, KIAA2013 and KNG1 were up-regulated in early fibrosis. It was interesting to know that previous studies have not shown the regulation of PTMs and protein synthesis with respect to HCV, although other viruses such as HIV have shown
these trends. However, our findings were in agreement with Blackham et al. who showed these types of regulations in HCV infected hepatocytes [61].

Transcriptional regulation and signal transduction related genes
Several genes associated with transcriptional regulation and signal transductions were identified (Figure 10). Most genes were down-regulated both in HCV initial fibrosis and cirrhosis. However, ANKHD1, CRAMP1L, FOXX2, GTF2B, HMGN2, NR1I3, PAX8, RUNX2 and SUSD4 genes showed increased trend in cirrhotic samples. Xu et al. also reported up-regulation of liver enriched transcriptional factors in infected HCV tissues [62]. A comprehensive study is needed to address the exact role of these genes. Some genes associated with signal transduction like CACNB3, PCSK5, TMEM100 and VDAC3 were up-regulated in initial fibrosis. Up-regulation of signal transduction related genes in HCC due to HCV and HBV is previously reported [63,64]. This can lead to the hypothesis that cirrhosis due to HCV genotype 3a may lead to HCC in future.

Transport and ion channel transport related genes
A number of genes encoding cellular and ion transport functions were also recognized (Figure 11). AMICA1, HHLA3, KIF17, KIF1A and SLC10A5 showed significant high expression, while, CLPB, K1024, MUC6, SCGN and MT1E expression was down in cirrhotic arrays. Previous studies related to HCV infection and entry has shown that HCV replication needs regulations in cellular trafficking [65-67]. High expression of SLC10A5, also known as putative bile acid transporter gene, it may indicate dysregulation of liver as well as pancreas in patients infected with HCV. Up-regulation of kinesin family members KIF17 or KIF2B may upset inner segment and synaptic terminal and consequently results in cell death [68].

Others significant genes
Irrespective of above mentioned genes; we have also found several genes related to DNA binding proteins, DNA replication, morphogenesis, reproduction and liver function (Figure 12). The expression of DNA binding protein and replication genes change from initial fibrosis to cirrhosis. The high expression in early fibrosis may underlie a repair mechanism, whereas, reduced gene expression in cirrhosis stage may indicate that virus has overcome the repair mechanism for its replication resulting in total deterioration of liver cells and
It is interesting to note that some genes associated with nervous system and vision pathways were also identified. A lot of uncharacterized genes were also recognized. The link of expression of vision related genes with HCV is not clear.

Real time RT-PCR validation of results

Analysis with real time RT-PCR confirmed that the selected genes were significantly differentially expressed in initial fibrosis and cirrhotic samples (Figure 13). Although, we observed higher fold induction values with real time RT-PCR, however, the trend was same between both analysis indicating reproducible gene expression patterns. CASPASE9, OAS2 and TGFBR2 genes showed up-regulation, whereas, FAM14B gene expression was down-regulated in early fibrosis. These findings open a new spectrum of genetic markers to differentiate fibrosis from cirrhosis.
A comprehensive review of literature revealed that very few studies related to HCV expression based studies leading to initial to final stage cirrhosis have been carried out in association to genotype. Walters et al. used J6/JFH (genotype 2a) infected Huh-7.5 cells for the expression analysis of host in response to virus at different time points of infection. They observed that TGF-beta signaling genes were up-regulated 72 hrs post infection, it induces ROS activity. Liver injury during chronic HCV infection is immune mediated [37]. Hagist et al. compared differentially expressed genes in patients with mild and severe iron depleted HCV genotype 1a liver samples with hereditary hemochromatosis. They found many ISG genes dysregulated in HCV infection and related to RNA processing and carcinogenesis [69]. We also found up-regulation of ISG genes in initial fibrosis stage as host defense system try to limit the viral pathogenesis. A study conducted by Blackham et al. in JFH1 infected huh-7 cells by microarray identified genes mainly apoptosis, proliferation, intracellular transport and cellular mechanism [61]. A few studies to explore the role of individual genes of HCV in pathogenesis have been studied in association to genotype. Shah et al. compared the expression of oxidative stress related genes in blood samples and found that the expression of COX-2, iNOS and VEGF was high in 3a in comparison to 1a [70]. We found the expression is high in initial fibrosis stage and down regulation at the advance stage of liver cirrhosis.

**Conclusion**

There are limited studies available dealing with gene expression profiling in cirrhotic and non-cirrhotic (initial fibrosis) patients infected with HCV. In this study, we have observed that HCV infection due to genotype 3a has widespread effects on host gene expression involved in apoptosis, metabolism, transport, transcriptional regulation and immune response. This gives comprehensive information about the pathogenesis caused by HCV genotype 3a leading from initial to end stage liver cirrhosis. Although, HCV genotype 3a showed same pathways activation caused by other genotypes, further studies are required to understand the mechanism by which different genotypes can affect various pathways. Meanwhile, we found that expression of these genes was significantly changed within initial and final stage of fibrosis. A study describing the progression of these genes in mild and severe fibrosis stages (F2 and F3) will be required for future perspectives.

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**Authors' contributions**

WA and BI contributed equally to this work. They analyzed the data and wrote paper. All work was performed under supervision of SH. We all authors read and approved the final manuscript.

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**Competing interests**

The authors declare that they have no competing interests.

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