Computational Modeling and Analysis of Hepatitis C Pathway using Static Analysis Approach to Identify Critical Biomarkers and Drug Targets for Effective Diagnosis

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Declaration

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This thesis is dedicated to *my beloved parents*

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Abstract

Hepatitis C Virus is the vital etiological agent of one of the fatal diseases Hepatitis, leading towards hepatic infiltration with fats known as steatosis followed by fibrosis, cirrhosis, HCC hepatocellular carcinoma and ultimately functional collapsing of the liver. Hepatitis C Virus infection is one of the world-wide indispensable health burdens primarily, in the developing countries like Pakistan where 10 million cases account for HCV. Based on nucleotide transpositions, HCV genome tends to have high mutation rate, resulting in its classification to no less than six genotypes and further subtypes. This categorization impacts the clinical profiles of the patients along with intensity of the liver disease and treatment response to interferon alpha- ribavirin therapy. In Pakistan, genotype 3a is the most prevalent. The past decade has witnessed tremendous breakthrough in comprehending HCV biology and its allied disease hepatitis. Computational methodologies have greatly reduced both the escalating research and development (R&D) costs and raising development times, by sighting up to date research models. Here in this research by deploying the tractability of Process hitting over large complex biological system of Hepatitis C Virus infection pathway, we have implemented the software Pint that works under the parasol of Process Hitting framework. Numerous findings have been enlightened by this phenomenal computational technique, including an elaborated list of novel Bio-markers specifically inclusive of AKT with the onset of enhanced proliferation along with steatosis that marks down the presence of HCV infection. It also acquainted us with the proof that how applicability of cut set on AKT enables restoring the homeostatic balance of apoptosis in the diseased state. The significant drug targets are having been identified inclusive of PDPK1, PIP3, LXR-alpha, PA28-gamma and PI3K along with others that ameliorates the basis for therapeutic strategies to combat HCV disease. This technique has promising attributes of trend setting in the field of computational systems biology by undertaking the colossal biological automata networks and enabling

interpretable models.

Keywords: *Hepatocellular carcinoma, Modeling, Process Hitting, Biomarkers, Automata*

Chapter 1

Introduction

1.1 Background

The foremost cause of long-term hepatitis, cirrhosis followed by hepatocellular carcinoma is the Hepatitis C virus whose molecular cloning was revealed in1989 [2]. It is one of the major health burdens worldwide but strikingly in developing countries like Pakistan where 10 million individuals are infected with HCV [3–5].

1.2 Epidemiology

Globally the occurrence for detection of positive HCV RNA is estimated at nearly 1%, fluctuating from 0.8 to 1.14%, making it closely 71 million, with range of 62 to 79 million individuals that are infected with HCV [6]. Accessibility of robust data around the globe is one the primary limitations that consequences in 29% of low-income countries along with 60% of high-income countries stating the prevalence of HCV contagion. Among all these, the western countries inclusive of China, Pakistan, India, Egypt and Russia, over all contribute to a small percentage of HCV infections globally [7].

1.3 Risk Features

Primarily, HCV infection is transmitted through percutaneous revelation to blood because of the medical related processes or sharing of contaminated machines for injecting drugs or by mother to infant acquisition of HCV infection takes place [8]. In the early

1990s, iatrogenic infections inclusive of transfusion of blood as well as dispensation of clotting factors used to place owing to the absence of screening related prior to the blood transmission. Similarly, long procedures of hemodialysis along with injecting many persons with the same single syringe and reuse of glass made syringes for other medical procedures may result in the onset of HCV infection. Principally, this reuse of syringes for the sake of medical practices still prevails in some regions, that is the key risk factor for spreading of infections in Pakistan.

1.4 Morbidity and Infection Rate

Nearly 200 million people are agonized by HCV infection, making it 3.3% around the globe with rest of as either not verified or are not treated fully [9] .In Pakistan, owing to the low standards of health and education along with lack of practice of international standards concerning transfusion of blood, usage of already used syringes and needles, poverty-stricken decontamination practices by doctors along with dentists and barbers and principally the lack of apprehension, the HCV endemic is rising and is generally 4.95% with 57% in injecting drug use population of Pakistan [10].

1.5 Molecular Biology of HCV

With the use of host cellular machinery and the ability of virus to modulate the immune response to combat host anti-inflammatory defense mechanism to avoid the apoptotic normal cell death of HCV infected cells, almost 50 to 80% of the infected cases fall under the category of chronic infection [11]. Being genetically heterogenous with nucleotide substitutions that enables its genome to have diversifications, HCV has been characterized into seven genotypes with more than 67 confirmed subtypes [12] that play a decisive role in the clinical management, treatment dosage and prognosis of sustained virologic response (SVR) during the treatment of chronic infection [13–16] .In Pakistan, due to lack of data regarding HCV mediated steatosis, HCV genotype 3a induced steatosis is endemic as genotype 3a is predominant in Pakistan. Immoderate assembling of lipid within the hepatocytes termed as hepatic steatosis plays a crucial role in progression to cirrhosis [4, 16].

1.6 Available Drugs and Treatment Resistance

Evolution of target-based drug development has resulted in identification of viral proteins and enzymes blocking direct acting antivirals (DAA) as in NS3/4A protease inhibitors, NS5A and NS5B polymerase inhibitors [17, 18]. Above all, because of drug resistance mutations, resistance to the drugs exists as a significant challenge owing to genetic alterations in NS3/4A, NS5A and NS5B [19].At present the drugs that could target the HCV proteins-based enzymes in a defined manner are still in the long run to reach the market. While, the current treatment is inclusive of unified pegylated interferon-ribavirin that brings along notable side effects and tends to have restricted efficacy [20].

1.7 HCV Morphology

Hepatitis C virus is a small, coated with envelop glycoprotein having positive single-stranded RNA genome which encodes an open reading frame having non-translated regions (NTR). Among the NTRs, the 5' NTR possesses an internal ribosome entry site (IRES) that is required for ORF translation, while, 3' NTR plays role in viral replication [1], HCV is classified under Hepacivirus genus within Flaviviridae family [21].

1.8 Lifecycle of HCV

The biological life cycle of HCV comprises step wise viral attachment, entry and fusion its RNA translation and replication and ultimately assembly synthesized virions and release [22].Identification and attachment to specific set of cellular receptors initiates the course of viral infection. It is then followed up by cellular ingestion with little pH mediated HCV-plasma membrane fusion and then emancipation of nucleocapsid in the cytosol. A single long polyprotein of around 3000 amino acids is generated through cellular ribosome liaised translation of 9.6 kb RNA.

1.9 Genome Organization of HCV

The host along with viral proteases then split the polyprotein into ten defined gene end products namely core that forms nucleic capsid, E1 and E2 are the envelop glycoproteins and these three proteins serve as structural proteins that compose s viral components with p7 as channel protein. The remaining make up the non-structural proteins inclusive of NS2 that together with NS3 amino terminus forms viral protease, NS3 functions as helicase and NTPase, NS4A is the viral protease cofactor, NS4B generates the membranous web, NS5A adjusts the cellular pathways and viral replication while, NS5B develops RNA-dependent RNA polymerase (RdRp) and these non-structural proteins carry out cytoplasmic replication of HCV genome [23–25].Prior to the spread of infection to other healthy cells, at intracellular plasma membrane, finally composed virions sprout and emerge out through the cell's secretory pathway.

1.10 Current Need

The current need is to come up with such quantifiable traits as in the tissue specific biomarkers which circulate in the blood or urine, that could differentiate normal biological condition from pathologically disturbed processes [26]. Determination of such biomarkers that enables tumor diagnosis at its early stage to combat against it has been recently enlightened in scientific as well as clinical perspective. With the interlinking of scientific data to clinical information, Bioinformatics being a developing technology can revolutionize the discovery of Biomarkers in the case of fatal diseases like HCV.

1.11 Problem Statement

Advancements in the field of biology made it possible to obtain comprehensive map of genomes of many living organisms. Similarly, development of DNA micro array technology enabled to access data of expression of thousands of genes. One of the main challenges is to integrate this high-throughput data to infer genetic networks using this previously inaccessible vast biological data. In addition, the biological data driven from experiments is generally quite noisy, thus needs to be filtered efficiently. Henceforth, discrete modeling methodology has been proven efficient in tackling such qualitative

biological questions including understanding how hepatitis C pathway has evolved or determining the reachability of states in the pathway of Hepatitis C infection. The computational analysis of qualitative dynamics of biological networks faces the state space explosion problem, limiting the tractability of detailed models. Many studies must use reduced models which often lose important properties and may lead to approximative results. This problem is addressed through formal and scalable analysis for the transient discrete dynamics (traces/trajectories) of automata network.

1.12 Challenges in the Progress

In view of fact, it was not possible to consider the complete behavior set exhibited by the colossal HCV system due to limited size and that quantitative data needs further filtration and removal of noisy data, it may face the obstacle of deletion of important data as well. Hepatitis C Virus infection pathway is quite an extensive and multiplex on scale due to which, the complete dynamics of this complex pathway has not yet been undertaken owing to the constraints of the previous implemented techniques.

1.13 Theme and Structure of the Study

Computation modeling techniques in this regard play significant role in dynamically combating the over whelming issue that is faced while dealing with all possible states of the system. Integration of systems biology approaches with immunology and virology-based data allows the understanding of highly interconnected immune regulatory signaling cascades within single view of the complete system. The software Pint supported under the framework of Process Hitting contributes its role by undertaking the complex pathway of HCV in terms of its tractability and generation of the required stable states or disease states from such multiplex Automata Network.

Chapter 2

Literature Review

2.1 HCV Appendage to Host Cell

As discussed earlier, multiplex arrangement of receptors upon cell surface allows specific HCV attachment. This includes series of receptors as low-density lipoprotein receptor (LDL), cluster of differentiation tetraspanin receptor (CD81) [27], scavenger receptor class B type 1 receptor (SR-B1) [28] and claudins and occludin tight junction proteins [29, 30]. Followed by clathrin mediated endocytosis, viral polyprotein gets translated into series of structural and nonstructural proteins which halt the cellular immune system in one way or another [31].

2.1.1 Host Anti-Viral Immune Response Against HCV

The first line of defence in fighting against viral pathogens is comprised of innate antiviral mechanisms which mediates further the adaptive immune responses [32]. The pathogen associated molecular patterns (PAMPs) receptors detect the viral entities and triggers an antiviral state by activating immune reactions. The RNA of hepatitis C virus replicates in a well-ordered manner using dsRNA as intermediate. During this process, it enables cell to produce of IFN and antiviral response against upon recognition of viral epitomes by the PAMPs receptors. Infected liver tends to have high proportion of ISGs. Initial expression of ISGs within the hepatocytes exponentially reduces the HCV RNA level and viral spread through ISGs mediated antiviral response [33–37].

2.1.2 RNaseL Mediated Immune Response to HCV

In response to the manifestation of an external RNA particle, RNaseL gets activated and results in degradation of HCV RNA by inducing IFN response [38]. The HCV core protein NS5A inhibits this antiviral activity of RNaseL by inhibiting 2'-5'-oligoadenylate synthetase gene promoter within the hepatocytes and regulates the replication process [39, 40].

2.1.3 TLR3 and RIG-1 Mediated Apprehension of HCV

Cellular sensors like TLR3 and RIG-1 apprehend the viral associated PAMPs and commence the innate immune responses against them. Activation of IRF3-IRF7 dependent immune response and devising NF-kB mediated proinflammatory process is at its pinnacle through TLR3 and RIG-1 signaling pathways [41]. RIG-1 undergoes conformational changes by E3 ubiquitinated ligases when it encounters viral RNA. The ubiquitinated RIG-1 forms a homo-oligomer that has binding with caspase recruitment domain (CARD) and activates IPS-1 (MAVS) [42]. Once activated, prion like aggregates formed because of activated IPS-1 recruit further E3 ubiquitin ligases namely TRAF2, TRAF3, TRAF5 AND TRAF6 [43] that subsequently conscript TBK1 (TANK binding kinase 1) and IKK-E (IKBKE) kinases. This follow-up the IRF3-IRF7 phosphorylation, impelling IFN beta mRNA transcription in the nucleus [43–45]. Apparently, recognition of HCV dsRNA replication intermediate brings forth the antiviral state developed by TLR3 within the endosomal compartments. Through adaptor TRIF, TLR3 activates the transcription of interferon. The activated adaptor TRIF together with ubiquitin modified TRAF3 activates TBK1, resulting in the activation of IRF3. On the other hand, TRIF also recruits TRAF6 and RIP-1 that leads to activation of NF-kB [46, 47]. These ubiquitin ligases drive the production of enzymes that initiate downstream signaling pathways of IRF3 and NF-kb for induction interferons [48]. Infected hepatocytes tend to have cleaved IPS-1 [49, 50]. Supporting the fact that the NS3 and NS4A complex protease of hepatitis C virus cleaves IPS-1 at its transmembrane domain thereby impeding IPS-1 mediated interferon production [51, 52]. Primarily, TLR3 signaling gets disabled by HCV NS3-4A protease mediated cleavage of TRIF adaptor [46, 51].

2.1.4 Instigation of PKR Induced Antiviral Strategy Against HCV

One of the additional PAMPs is the internal ribosome entry site that gets recognized by PKR early right after the viral infection [53]. This stress signal initiates a cascade of signaling pathways that induce IFN-Beta and ISGs mediated antiviral response [54]. Auto-phosphorylation of PKR along with its physiological target, the eukaryotic translation initiation factor 2 alpha was observed in the tumor tissue of HCV induced hepatocellular carcinoma patient [55]. Despite of inhibition of host cellular translation of antiviral ISGs by PKR mediated phosphorylation and halting of the translation initiation factor 2 alpha, HCV retains its own replication of RNA and virion particles through internal ribosome entry site dependent translation process [53, 56]. In addition to cellular response to dsRNA, PKR also coordinates IRF-1, p38 and NF-kB regulatory pathways through phosphorylation. Phosphorylation of NFkB1A by PKR mediates inhibition of NF-kB [57]. This antiviral state induced by PKR gets inhibited by the direct interaction of viral envelop glycoprotein E2 and nonstructural protein NS5A [58, 59] that hinders the PKR dimerization and its activation [58, 60] leading to chronic infection . Enhanced auto-phosphorylation of PKR and its substantial target eIF2-alpha along with direct association between core protein of HCV and PKR has been manifested in HCV viral infected tumorous tissues [55]. PKR counterbalances its dual role in terms of its first line of defence mechanism against HCV as well as a growth regulator [61]. Infected hepatocytes have hampered interferon action that was analogous to over expression of proinflammatory chemokine IL-8. This was attributed to the viral NS5A [62, 63] Mitochondrial localization was displayed in hepatic cells by HCV NS5A, inciting an enhance in reactive oxygen species (ROS) levels and thus, contributes to HCV pathogenesis [64]. Upregulation of NF-kB through Tumor necrosis factor-alpha serves its role to incite the expression of cytokine IL-6, subsequently triggering the STAT3. Thus, engendered NF-kB mediates STAT3 initialization follow up by IL-6 cytokine [65].

2.1.5 Type-1 Interferon Antiviral Response

Now, viral NS5A activates NF-kB and STAT3 mediated IFN based ISGs expression through this reactive oxygen species based oxidative stress driven by perturbed intracellular calcium levels [66] IFNAR1 and IFNAR2 are the two distinct chains of single receptor to which the IFN-alpha/beta proteins exhibit binding. Out of the above chains,

IFNAR2 anchors with Jak1 while, the IFNAR1 intracellular domain affix with Jak kinase family member Tyk2 [67].This serves as the purpose of phosphorylation of proteins STAT1 and STAT2, which upon activation dimerizes and corresponds to interferon regulatory factor 9 (IRF9) to form a complex namely IFN- stimulated gene factor 3 (ISGF3). Which in turn binds to its IFN stimulated response elements (ISRE) and initiates ISGs mediated gene transcription [68–70]. PP2A is known to impart its role in terms of establishing chronic viral infection. The expression of Hepatitis C virus polyprotein instigates this major serine/threonine phosphatase 2A (PP2A) which interconnects with and inhibits JAK1/Tyk2 mediated phosphorylation of STAT1, resultantly undermines IFN-alpha stimulated antiviral response [71]. Furthermore, overly expressed PP2A inhibits the activity of protein arginine N-methyltransferase 1 enzyme, thereby mitigating the methylation of STAT1 protein [72, 73]. Hypomethylated STAT1 is prompted to bind with the protein inhibitor of activated signal transducer and activator of transcription 1 (PIAS1), followed by diminished antiviral immune response due to inefficacy of binding of STAT1 with DNA [74].

2.1.6 Core Protein Mediated Perturbation of Interferon Anti-Viral Response

Perturbation of IFN-alpha mediated immune response has been linked precisely to the core protein of HCV .Interferon-alpha intervened expression of STAT1 was retarted directly through overly expressed viral core protein [75]. Moreover, STAT1 inhibition was also analogous to the HCV core protein mediated upregulation of SOCS3. Thereby, SOCS3 occludes JAK/STAT signaling pathway through its breakdown within the proteasome or by hampering its kinase related activity through its SH2 binding domain and mitigates ISGs expression [76, 77].

2.1.7 Role of EGFR Pathway in Cellular Proliferation

Extracellular ligands for instance epidermal growth factor EGF tends to activate receptor bound tyrosine kinases namely epidermal growth factor receptor by phosphorylation and thereby initiating its kinase activity at its cytoplasmic domain. Phosphorylated tyrosine residues intimate binding with the docking protein GRB2 [78], which then coheres with guanine nucleotide exchange factor SOS. Docking of this GRB2-SOS complex

with activated phosphorylated EGFR yields activated SOS [79]. While, this triggered SOS enables Ras activation by mediating its binding to GTP after removal of GDP. Followed by auto-phosphorylation of receptor, a serine kinase Raf gets enkindled by the activated Ras. Which eventually promotes pairwise selective protein kinases and stimulation of MAPKs. Series of serine threonine mitogen- activated protein kinase MAPKs include ERK, c-Jun N- terminal kinase (JNK) and p38 MAPK [80]. Extracellular signal-regulated kinases 1 and 2 (ERK1 and ERK2) upon activation and phosphorylation imposes a negative regulation on its own activators inclusive of suppression of SOS-Ras-Raf and thus, regulates different gene expression along with apoptosis and cellular proliferation [81, 82]. PKR mediated explicit antiviral immune response of interferon-alpha has been suggested through first hand activation of P38 MAPK signaling pathway, causing halted translation of viral genome [83].

2.1.8 Viral NS5A Interaction with EGFR Pathway

Epidermal growth factor interceded stimulation of Ras/Raf- Extracellular signal-regulated kinases 1 and 2 (ERK1/2) signaling pathway has been exhibited to be hampered by hepatitis C virus NS5A. Viral NS5A correspondence with adaptor protein GRB2 [84] is one of the approaches by which hepatitis C virus deprecates the signaling pathway of MAPKs [85, 86]. Elevated gene expression of several cellular stimulators including NF-kB along with Ras/Raf phosphorylation induced MAPK signaling components c-JNK and ERK has been exhibited by hepatitis C virus core protein [87]. Augmented downstream signaling transduction of PI3K-Akt pathway has been explained in cancer cells by epidermal growth factor (EGF) mediated phosphorylation of EGFR [88] HCV non-structural protein 5A (NS5A) has been demonstrated to modify one of the central cell survival pathways namely PI3K/Akt pathway. Modulating the survival mechanism of infected host cell, NS5A provides tenacity to the viral infection by altering the PI3K-Akt pathway, that is linked with increased cell proliferation [89].

2.1.9 Viral NS5A Mediated Inhibition of Apoptosis through Akt Pathway

Through enhanced phosphorylation, viral polyprotein enhances biological functioning of Akt attributed pathways which resultantly imposes inhibitory phosphorylation of

downstream targeted proteins inclusive of the apoptosis related functioning BAD protein, GSK-3 beta and p21 protein [89].

2.1.10 Role of Tumor Suppressor Protein p53

Enhanced cellular proliferation is exhibited by subdued expression of regulatory gene of cell cycle, p21 protein by viral NS5A Serving as major cellular guardian of genome, the signal transduction pathway of p53 gets triggered in response to number of stress factors that influence the homeostasis related mechanisms including replication of DNA, cell differentiation [90]. DNA damage occurs in response to signals including ultra violet irradiation, cross-joining of DNA bases along with modification of deoxyribose sugar structure and so more. p53 protein gets triggered because of above input stress signals and mediates transcription of genes through its enhanced concentration [91]. Administered by proteolytic turn over, p53 protein tends to have small half-life interposed by ubiquitin ligase MDM2 protein. Resultantly after DNA related damage, ubiquitination of MDM2 protein occurs and makes itself inactivated while, p53 concentration gets enhanced [92, 93]. Correspondingly, disordered oncogenes such as mutated Ras induces the production of P14 alternative reading frame (P14ARF)) protein, whose ARF serves its role of directly impeding E3 ligase mediated activity of MDM2, subsequent inhibition downregulates p53 protein. p53 protein gets assembled and induced at cellular level through deregulated aspect of E2F1 [94]. In addition to ability of Stimulated E2F1 to upregulate process of apoptosis in a p53 dependent manner [95–97] it also promotes G1-S cell cycle progression [97, 98]. p53 mediated apoptosis is primarily induced through P14ARF by E2F1 [99, 100]. Restraining the expression of E2F1, P14ARF establishes a negative mediated feedback loop with E2F1 [101].

2.1.11 Inhibition of p53 Tumor Suppressor Protein

Henceforth, the complex interrelationship of P14ARF-MDM2-P53 serves as a fundamental part of mitosis based cellular networks [102, 103]. In this way, MDM2 protein plays its role as negative regulator of p53 [104]. This hampered expression of p21 protein through HCV NS5A is also mediated by tumor suppressor gene p53 dependent manner [105]. Repression of p53 gene transcriptional activity has been exhibited by its direct interaction with viral NS5A [106], which mitigates the p21 gene expression [107]. Thus,

virus impedes the cellular apoptotic process primarily either by hampering p21 or by PKR related molecular mechanisms [60]. Through halted regulation of cellular caspases along with downstream apoptosis related processes [108] viral NS5A agonized tumor necrosis factor-alpha liaised brought about of NFKB1A, that inhibits NF-kB, primarily by direct collaboration and suppression of the entities namely TRADD and TRAF2 [109].Cytokines mediated modulation of signaling pathway has been elucidates through profound interaction between tumor necrosis factor-alpha as well as TNF-receptor and viral core protein [110, 111].

2.1.12 Induction of Steatosis by Viral Core Protein

Regulation of ERK1-ERK2 along with phosphorylation of p38 related MAPK pathway has been manifested to exhibit indirectly replication of PPAR-alpha gene [112] through viral core protein [113, 114]. PA28-gamma supplements the Viral core protein expression [115] which exhibits magnified steatosis through LXR-alpha and RXR-alpha complex. Retinoid X receptor forms dimers with PPARs and LXRs [116]. PPAR-alpha plays a significant role in hepatitis C virus mediated steatosis. Since, PPAR-alpha acts its own ligand and enhances its own production by increased influx of fatty acids [112, 117]. Similarly, LXR resided in liver [118] regulates homeostasis of lipid metabolism [119]. In viral infected cells, LXR-alpha has been manifested to elevate the synthesis of fatty acids including saturated as well as unsaturated along with other triglycerides, thus, exhibits augmented steatosis [115].

2.2 Computational Modelling

2.2.1 Computational Modeling of Innate Immune Response Against HCV

Cell culture-based limitations is one of the reasons that hampers the interpretation of highly interconnected biological networks such as hepatitis C virus infection pathway [120]. A systematic and computation approach of hybrid Petri net (HPN) has been employed to model dynamics of HCV infectious pathway within a unit system, mediating 23 entities as a whole comprising 23 places, 18 interactions and 52 vertices. The pathways enlightened in the study is inclusive of ribonuclease L, toll-like receptor 3, retinoic acid

inducible gene 1 and protein kinase R. While, considering all the behaviors exhibited by the system, the issue of complexity of system and outburst of state space problem arises [121] with enhance number of entities [122].

2.2.2 Mathematical Modeling of Jak/Stat Pathway

In depth explanation of molecular dynamics of signaling pathways is obtained by computational models. Based on ordinary differential equations, mathematical kinetic modeling of non-linear Jak/STAT pathway has been developed comprising of 11 kinetic parameters and 9 species variables [123].

2.2.3 Hybrid Functional Petri Net model of ERK-MAPK, PI3K-Akt pathway

In biological systems, owing to the lack of complete knowledge regarding the defined parameters, quantitative modeling is difficult to implement as such. Since that Hybrid Functional Petri Net has stable fundamental mathematics basis for its components [124, 125], the pathway has been modeled to using this technique work out the systematic perspective of cellular functions. Two pathways have been modeled including PI3K-Akt pathway and ERK-MAPK pathway.

2.2.4 Mathematical Modeling of p53 gene pathway

They applied mathematical and computational modeling approaches in combination with mouse challenge studies to study the mechanisms underlying the interactions between PPAR-gamma activity and miRNA-146b to regulate colitis during C. difficile infection. an ordinary differential equation-based computational model was developed describing the molecular dynamics of some key cytokines and transcription factors involved in C. difficile infection. Although modeling approaches cannot replace traditional experimentation, the construction of such computational model synthesized, organized and integrated all the concepts and mechanisms studied, facilitating a more systematic hypothesis generation process. They constructed a network model with five dynamic variables representing miR-146b, NCOA4, PPAR-gamma, interleukin 17 (IL-17) and IL-10, plus an external input: the infectious dose of C. difficile[109].

2.3 Aims and Objectives

The primary aim and objectives of current study are:

• Construction and curation of Hepatitis C pathway through extensive literature review.

• Modeling of the HCV pathway through PINT approach for static analysis, under the framework of Process Hitting.

• Computation of the stable states in hepatitis C pathway through abstract analysis.

• Computation of the cut sets and mutations for defining reachability in the pathway of HCV.

• Computation of reachable state graph towards all possible states in the complex pathway.

• Identification of potential Biomarkers for effective diagnosis of Hepatitis C.

• Identification of Drug targets after the analysis of the computed results.

Chapter 3

Methodology

3.1 Answer Set Programing

Answer Set Programing is a declarative computational technique based on stable model semantics of logic-based programing in such a manner that its professed answer sets serve as elucidation to the problem [126]. ASP tends undertakes integration of various computer science domains inclusive of cognitive, feigned intelligence, representation of knowledge along with constraint complacency and combinatory optimization [127]. It enables solutions for various strenuous combinatorial problems of systems biology behind the specification of program's computational logic with no necessitation of the programmer to define the precise approach for its execution. It personifies the encryption of its input data with detailed background of the problem, its allied limitations as well as the optimization protocol under the parasol of Answer Set Programing. Potsdam Answer Set Solving Collection, Potassco tends to have two major sections comprising of ASP grounder-gringo that embodies all the rational commands and an up to date ASP solver-clasp for decryption of those logical rules by reinforcing diverse cognitive means for evaluating myriad of answer sets inclusive of systematic protractile, interchanged multi-objective optimization.

ASP also tends to play paramount role in the foundation of BioASP proficient by Potsdam Answer Set Solving Collection (Potassco) [1]. One of the foremost acknowledged qualitative modeling techniques implemented by biologists to facsimile the gene regulatory systems is the discrete dynamical complex systems based on Boolean networks as can be viewed in the exemplary works of [128, 129] where nodes constitute the enti-

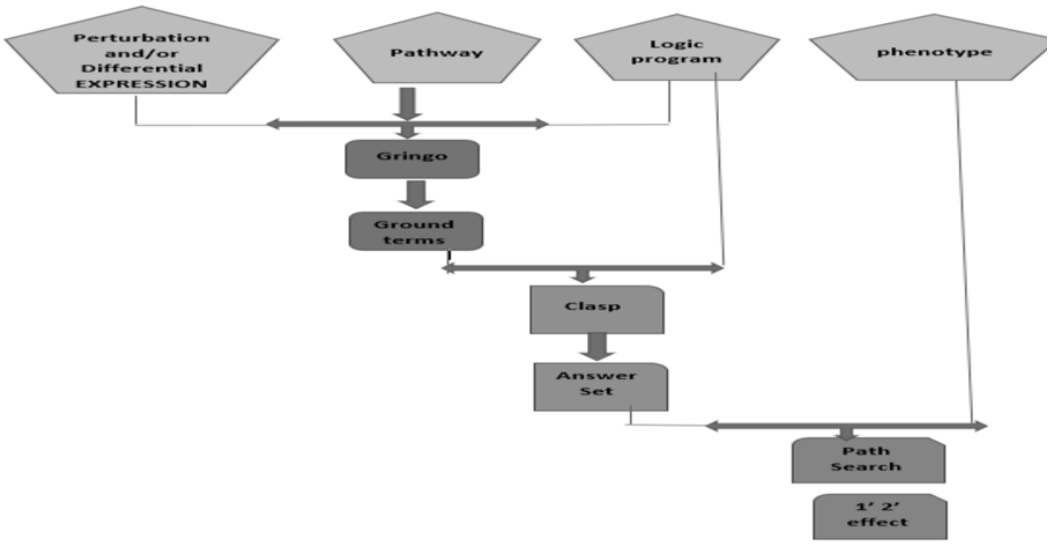

Figure 3.1: General scheme for methods and types of data [1]. It provides solution to the complex combinatorial problems by its component gringo that encompasses the required commands and the solver clasp that comes up with the answer sets of the combinatorial problem

ties as proteins or genes and directed vertices entitle the interactions such as activation and inhibition. ASP recapitulates the Boolean networks that in accordance with the experimental data fulfills the prerequisites of the given dynamics [130] and exhaustively characterize all these logic-based biological networks by considering the complete search space, giving quantifiable and accurate results as an output with little [131, 132]. It renders plentiful and versatile toolbox that enables to broaden the biological Boolean networks framework with the desired attributes. Answer Set Programing has ample of significance in systems biology. Implementation of cognitive depiction and intellectual methods to the dilemma of signaling networks logical execution has been proposed by [133]. several biological networks have been addressed using ASP. The issue of remodeling of all probable networks in collaboration with empirical time lined data has been suggested by [134]. Discernment of irregularity along with other improvements in complex biological networks has been evinced by [135, 136].ASP has been symbolized for the dynamics of BNs along with perceiving their attractors in the work. Integration of logic-based analytical data so that to obtain coherent states of biological regulatory system underneath variegated restraints has been enlightened in the work of [137]. Besides that, Ray have also devised a system of Answer Set Programing to proffer emendations to the

biological metabolic networks [138]. ASP has also been executed in the findings of [1] suggesting the consolidation of expression of RNA in accordance with the apprehension of signaling networks and to deduce in what manner the genetic alterations impact the phenomenon of aging [1]. Apart from that, Schaub and Thiele are the pioneers to analyze issue of exhaustivity upon augmentation of metabolic networks with the applicability of ASP [139]. Lately, their work was outstretched by Collet et al. and administered it on a factual case study [140]. overall, this concatenated list of contributions exemplifies perspectives of Answer Set Programing to direct the combinatorial search along with the issues related to optimization in the discipline of systems biology. Despite that in the future prospective, enthralling challenges exist owing to its attribute of being discrete in nature in hybrid cognitive system in both qualitative as well as quantitative modeling [126].

3.2 Process Hitting

One of the latterly established chassis to model the parallel processes where subdivision of tasks takes place for simultaneous performance on multiple processors, is the Process Hitting [141]. Owing to the concern of state space paroxysm resulting in the intractability of complex BRNs, Process Hitting allows the formal assessment of complex systems as complaisant by one of its attributes of definite r eduction o n t he a ctions causality. Based on elucidation of indulgent nature of dynamics according to parameter related curtailments, PH models the Biological Regulatory Networks assigned with either complete or partial learning of liaison among the regulators as positive or negative interactive actions linking the components. Primarily, Rene Thomas' Formalism in1973 forms the groundwork of the discrete qualitative modeling aspect of BRNs since it excludes the need for identification of definite concentrations along with other kinetic reaction parameters in context of quantitative data [142, 143]. Within Process Hitting model, the first t hing r eckoned i s e lemental i nteraction g raph a llying t he c omponents inclusive of dependency graph, graph of local causality as well as reachability state graph.

After that, the presumption of congruous Rene Thomas' models is furnished based on Answer Set Programing that permits principally an effectual listing of consistent parametrizations. Henceforth, to orate the formal halting of dynamical attributes of BRNs, Process Hitting is commenced to model parallel systems with handful of quali-

tative levels [144], that deals with one component at a time and in an atomic conduct delineates the viable evolution of a process that is prompted by the hit of no more than one other process in the framework.

It must be acknowledged that based on abstract explications, very efficacious static analysis approaches have been flourished that allows analysis of a program with no need of it to be executed [145], to make the formal interpretation of Biological Regulatory Networks with hundreds of components as compliant by the over- and under- proximation of reachability properties owing to the distinct assembly of interactions within the Process Hitting framework [141].

3.3 Pint

The software PINT accessible at http://loicpauleve.name/ pint, renders the ascendible static evaluation of trajectories of Boolean, multiplex asynchronous biological automata networks for their transitory attributes of reachability inclusive of transmutation prognosis and cutdown of the model, through causality of series of adjacent vertices. In this regard, asynchronicity being close to biological perspective is challengeable since distinct genes undergo alterations of their expression levels at diverse time levels, making it laborious to model and interpret [146]. The enclosure of PINT is within Jupyer IPython notebook that is a customary substructure for data-driven tools of bioinformatics [147–149]. It permits an easy handling of complex biological systems by providing a suitable nature for retaining, correcting, emulating along with distributing the workflows of model-based evaluations. PINT fulfills the need of aptness and reproducibility for computational biological systems. Its main attributes are the prognostication of mutations to manage the reachability features, the discernment of bifurcating transitions leading to distinct processes and the cutdown of the model to the requisite needs. For either of the instance, the outputs have authorized assurance of being correct in nature in way that the under-approximations fulfill the required conditions while, absoluteness with over-approximations meet the imperative conditions [141].

3.3.1 Directed Graph

In graph theory, a directed graph well known as digraph is a set of vertices linked with edges having direction integrated with them. It is a tuple of two components such that D (Vertices, Edges) or D (V, E) while, V designates a set whereby its elements are termed as vertices or nodes and E is an ordered pair of vertices termed as directed arcs or directed edges. It implies that an edge is inevitably directed from one entity to the other, that is between nodes to nodes. While, in directed graph, D - (x) and D+ (x) refers to antecedent and descendent nodes with respect to a particular node [150].

3.3.2 Automata Networks

"An automata network is defined by a tuple (Îč, S,L, T) where;
Îč is the finite set of automata identifiers.
S is the finite set of global states.
L is the finite set of transition labels.
T is the mapping from automata to their finite set of local transitions [151]."

Automata Networks frame the explicit sets of finite-state machines that is enacted to constitute the qualitative dynamics of interconnected biological signaling systems. It encompasses biological entities that could be proteins, genes, RNA etc. while, it embraces Boolean and discrete networks with implying transitions of the form synchronous or either asynchronous [152, 153]. Since it comprises of local transformations that are constrained by the state of other automaton entities within the system. These local states together with non-deterministic transitions constitute the global state space of the biological system. Accordingly, the file tag terminates with an. Considering the example below, here each entity constitutes a single automaton that is linked with other automatons by the local transitions within the system Pint [146].

3.3.3 Dependency Graph

The term Dependency Graph yields a directed graph within a biological automata network where the reliance for the respective vertex might be a common Boolean function of further vertices. In the given example, the trajectory from Y to X signifies that rela-

tively a single transition of X is determined by the local state of Y and vice versa. The obtained Dependency Graph can be additionally modified or specifically exhibited on the web interface of Jupyter notebook [141]. Dependency graph of 3 automata in their network has been shown in Figure 3.2. It indicates how each state depends on the other in terms of its interactions.

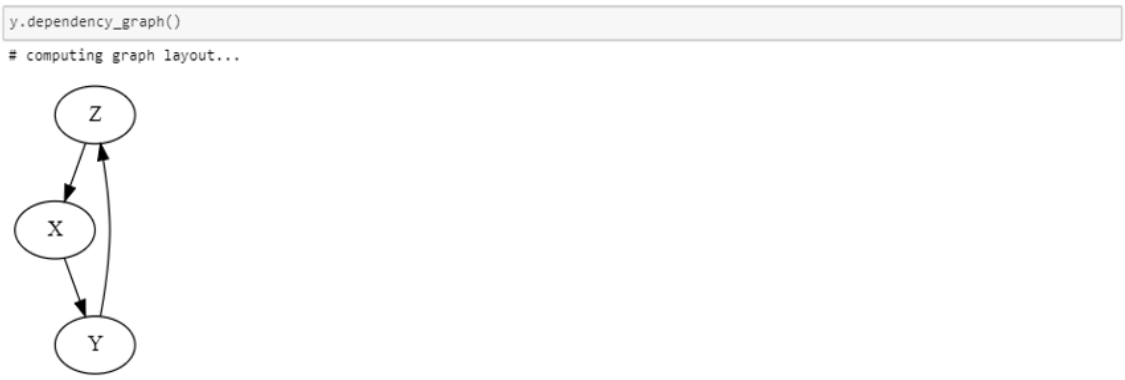

Figure 3.2: Dependency graph represents the reliance of each vertex on the other. It shows how the entity X depends on other entities Y and Z in terms of their interactions and vice versa.

3.3.4 Graph of Local Causality

Based on the semantics of the definite model, graph of local causality allies the local states whose preliminary occurrence is imperative to the reachability of local state of interest. That within a biological automata network, based on the assessment of local causality, the reachability of unique local state resulting from some global states is illustrated by an objective that extracts the requisite footprints whose well-ordered execution is crucial to outreach the local state of interest [146]. Graph of local causality outlines the basics of static analysis and abstracts various dynamical curtailments from the elemental discrete model and means as an input for enumerating the reachability, cut sets as well as goal-driven reduction of the model. In the figure below, the graph of local causality among the automata network of 3 entities has been shown in Figure 3.3. Based on the causality, it indicates the expression level of each entity for activation of the specified state [154].The general system for types of methods along with the data types implemented within ASP through its components grounder and solver is shown in the Figure 3.3

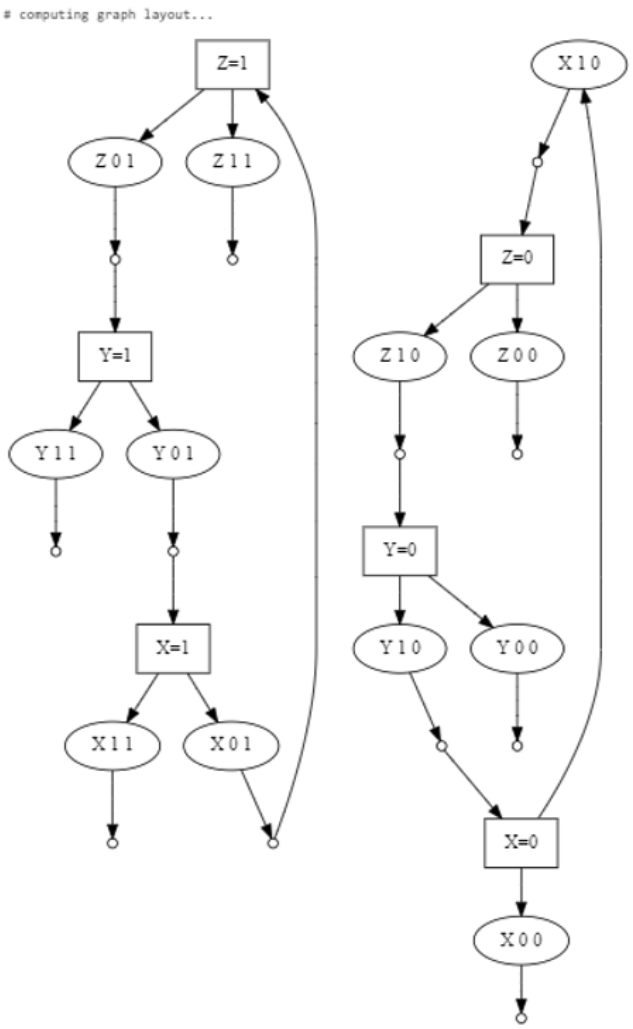

Figure 3.3: Local Causality Graph indicates if for specific state of interest Z requires the occurrence of other states X and Y prior for the reachability towards state Z

3.3.5 Fix Points

Based on the diverse types of data-structures and the particular approach, the pypint python module pledges function of stable state generation that makes the use of input model of the complementary tool Pint and yields a checklist of Python lexicons explicating the specified states through static analysis, mapping apiece node of the automata network to a value in terms of fixpoint. Within a biological network, Pint implements Boolean requisite abstinences to compute the fixed-points which is Python catalogue of states [155, 156]. Through intricated analysis of the interaction graph, definite stable states or disease states can be characterized [157, 158]. Being in accordance with the

interaction graph, these precise states tend to have fixed trajectories, or no further transitions can take place through them. For example, in case of automata network of 3 entities, we obtain the following fix points as shown in Figure 3.4.

```
fps = y.fixpoints()
import pandas as pd # for pretty display of fixpoints
pd.DataFrame(fps)
```

	X	Y	Z
0	0	0	0
1	1	1	1

Figure 3.4: In the Fixpoint Analysis, the given stable states encompasses the upregulation of all those states that lead to the disease state. Those upregulated entities X,Y and Z indicates the diversion from their homeostatic balance thus, causing the disease occurrence

3.3.6 Mutations

One of the foremost enactments of static reachability analysis by Pint is the assessment of certain commotions that would impact the reachability towards goal besides being tractable on large biological systems. Pint model appraisal culminates with the prognostication of listing of those mutations that impede to reach the disease state by obstructing the reachability of the concerned state. While, taking into account numerous elementary values for a single vertex, it relates formal methodologies which deduce novel sets of alterations that pledge to restraint from viable initial state, any trajectory to outstretch the definite stable state, laying foundation to draft innovative therapeutic strategies [146].In Figure 3.5 it is shown that the altered expression levels of A and D as 0 expression level impedes its path towards disease state.

```
y.oneshot_mutations_for_cut("Z=1")
```

This computation is an *under-approximation*: returned mutations are all valid, but they may be non-minimal, and some solutions may be missed.

Limiting solutions to mutations of at most 5 automata. Use `maxsize` argument to change.

```
[{'X': 0}, {'Y': 0}]
```

Figure 3.5: Mutations Analysis indicates the change of expression level of all those entities that set the path towards stable state. Here decreasing the expression level of X and Y to zero will directly impede the reachability towards diseased state of interest Z

3.3.7 Bifurcations

Based on the designation of an inceptive state and a goal intended reachability in terms of stable state, Pint undertakes huge biological systems and executes its abstract-based analysis to declare those trajectories which serve as the local state transitions within the network but caters an influential role during differentiation step. The discerned bifurcation transitions hence, barricade the reachability towards disease state by diverting the track of states towards trajectories other than disease state [146, 159]. Here, in order to avoid the trajectories towards stable state, bifurcating paths have been provided in result of bifurcation trajectories for each of the states involved in the automata network as shown in Figure 3.6.

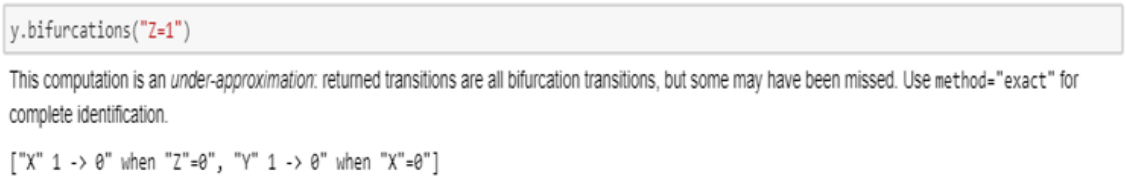

Figure 3.6: Bifurcations implies the diversion of the pathway away from the path leading towards stable state. State of interest X would be impeded when state Z becomes zero while reachability towards state Y would be hampered when state X becomes zero. Together both will impede the reachability towards the diseased state

3.3.8 Cut sets

In case of immense biological regulatory networks, assuming an initial state for every vertex of the automata network, the goal designated as the stable state or disease-causing state is only attainable provided that there subsist a series of steps that brings about a state where target goal commences. After elaborated static evaluation, the Pint enables list of cut sets that comprises of local states within an automaton just as each trajectory extending to the goal is inclusive of a trace embracing any one of those specified local states. Upon model-checking, in case the reachability attribute towards fixpoint gives answer of false, the given set of local states is verified as shown in Figure 3.7. Henceforth, incapacitating all the trajectories having prior condition bisecting with the cut set will abolish all the paths steered towards the goal [146, 160].

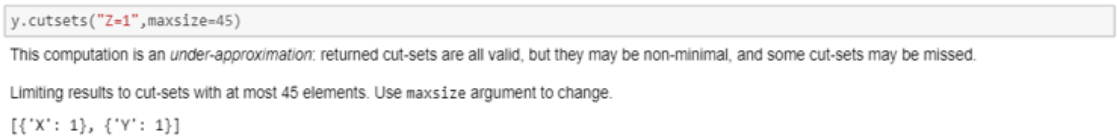

Figure 3.7: In Cut sets Analysis, the given cut sets encompasses the list of all those states that lead to the reachability towards fix point. Hampering all the states X and Y through their knock out or cut down impedes the reachability towards that stable state Z

3.3.9 Model Reduction

The static deliberation provided by Pint distinguishes the definite local states mentioned as cut sets that are unquestionably outreached just before the concerned reachability goal. Based on the stable state (disease state/fixpoint), the cut sets that validated the goal reachability, enables a catalogue of entities whose related transitions are abolished from the model through the function of model reduction. The static evaluation also spots those traces that no longer contribute any role towards reaching the fix point as shown in Figure 3.8. Hence those trajectories also get removed during the disabling of cut sets provided entities and the resulting reduced model gets further tractable with its reduced dynamics [153, 160].

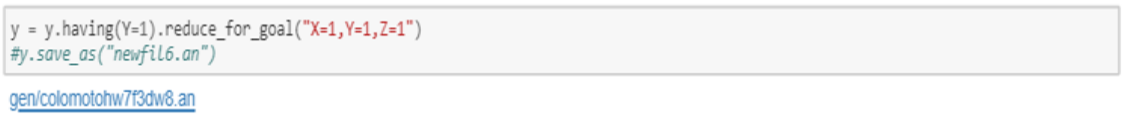

Figure 3.8: Based on the derived cut sets and all those entities that no longer play any role in the reachability towards stable state, the Model Reduction represents the reduced file obtained after removal of unwanted entities and their transitions from the complete BRN pathway of X,Y and Z states

3.3.10 Reachable State Graph

The scalability of the software Pint running under the parasol of Process Hitting framework is determined by its attribute of enabling an extensive reachability state graph-based evaluation as shown in Figure 3.9. Coming from some set of initial states, it

indicates the definite reachability towards all attainable states in the given complex biological automata network of HCV.

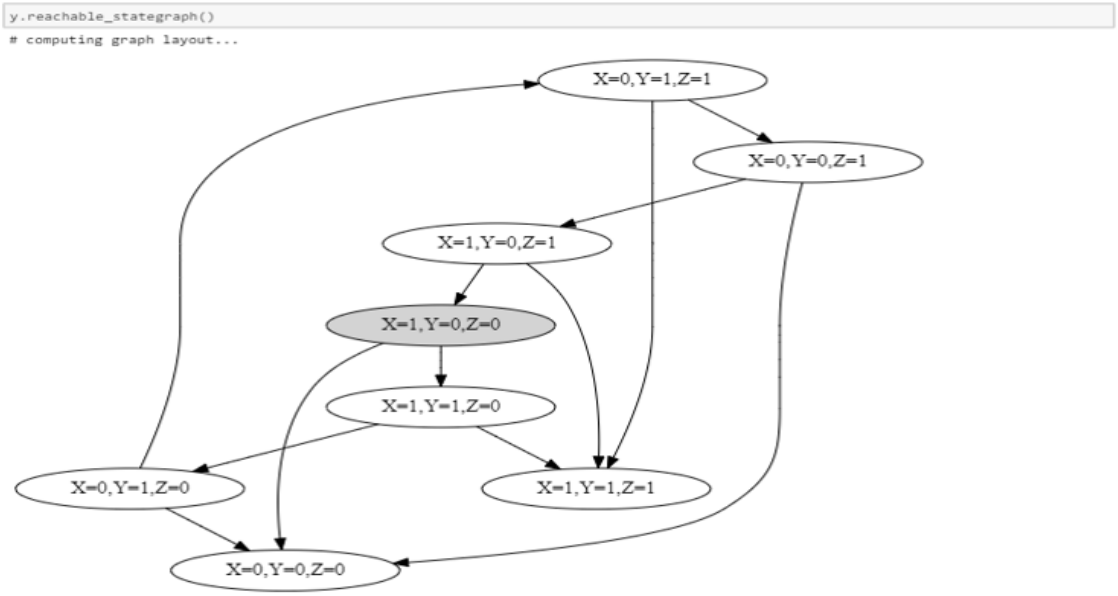

Figure 3.9: State Graph Analysis State graph of the entities X,Y and Z linked BRN. Nodes here represents the states while edges refer to the evolution of states within the BRN. Specified state encompasses the expression levels of X,Y and Z. The stable state with expression levels as (1,1,1) indicates the high expression levels of X,Y and Z leading to the occurrence of disease

Chapter 4

Results and Discussion

4.1 Results

Infectious agents like blood-borne Hepatitis C Virus confers nearly 15% of the human's cancers. It is one of the vital public health issues owing to the lack of its early diagnosis and management. Most of the cases are recognized to suffer from chronic infec-tion and cirrhosis followed by Hepatocellular carcinoma. owing to the usage of injecting drugs practices primarily in our developing country like Pakistan, HCV is endemic and is the cause of infection of 10 million individuals while, other cases may not be reported. A comprehensive understanding of the complex biological system of HCV is an open challenge till now. The present study seeks to model the diverse underlying mechanism and comes up with the unique findings contributing to the disease diagnosis along with its treatment.

4.1.1 Fabrication of Extensive Biological Regulatory Network of HCV pathway

In the initial steps, the complete biological regulatory network of Hepatitis C pathway was developed after thorough literature review and curation of additional missing links in the pathway on the Ginsim software Figure4.1. Unlike other computational techniques, no additional kinetic parameters were required to model the pathway in Pint software.

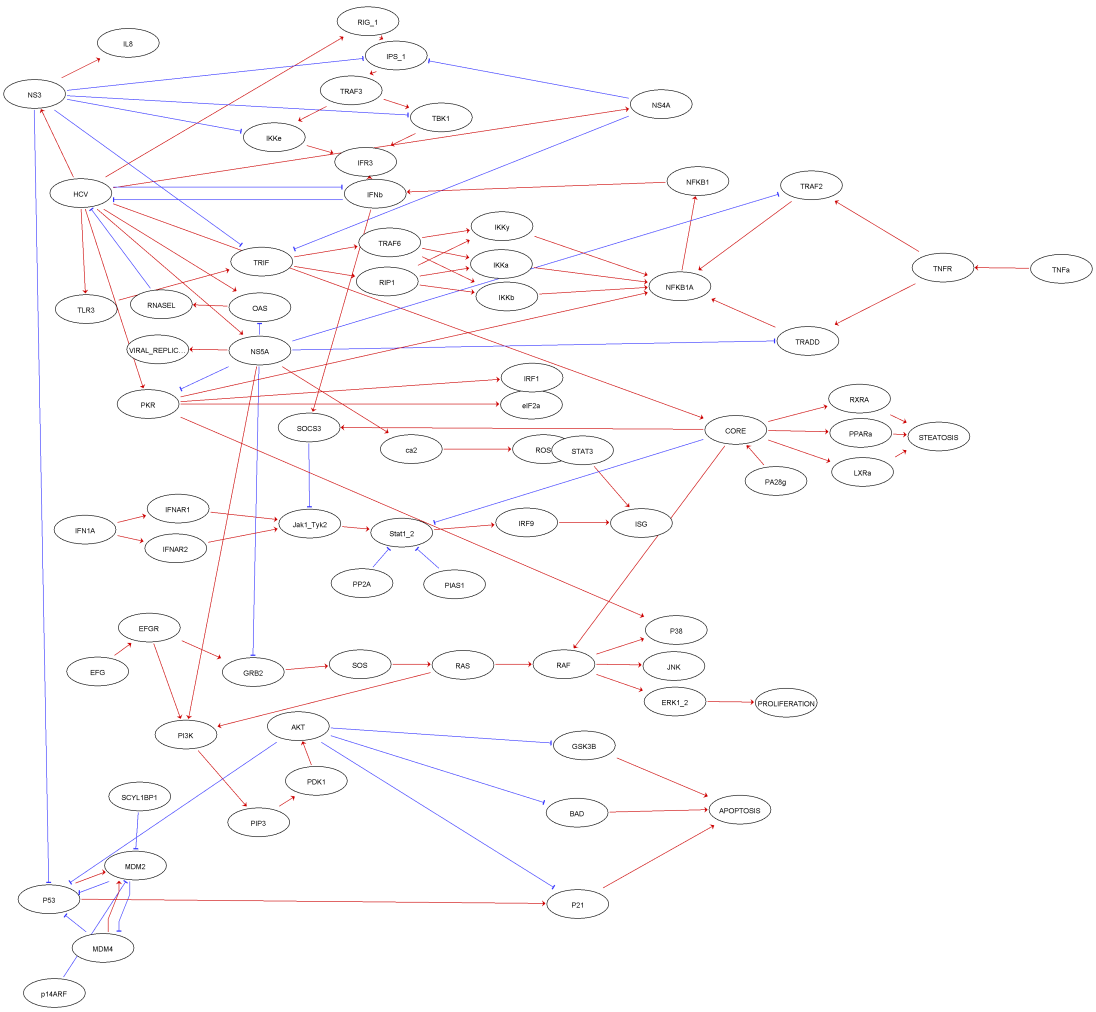

Figure 4.1: Represents the biological automata network of Hepatitis C pathway constructed on the software Ginsim. It encompasses 73 nodes having the prerequisite entities of the pathway while, vertices represent the transitions as causing either inhibition or upregulation of the other state. These interactions are marked down on the basis of the estimation of parameters as specified in the .an file

4.1.2 Estimation of Interaction Related Parameters

Based on the explicit interactions, the entire sets of cartesian products of all positive interactions were elucidated in the Ginsim file, that would then serve as input to the Pint software as shown in Table 4.1,4.2,4.3,4.4,4.5,4.6,4.7,4.8,4.9.

4.1.3 Evaluation of Pint Modeling to Generate Stable States

The Process Hitting framework provided an elaborated list of Biomarkers in the form of its stable state or fix point. As illustrated instead of directly impeding the virus, we targeted its associated functions so that, the remaining biological system retains its homeostatic balance and may not be triggered towards disease onset. In case of diseased state, we obtained two stable states based on the presence and absence of negative feedback of IFN-beta on HCV. Among these, the states with positive expression levels of 1 serve as altered in the diseased condition. The elucidated list of Biomarkers is inclusive of as follows in Figure 4.2, 4.3.

Stable state in case of host immune defense mechanism dominates the HCV virus during infection:	
Upregulated	**AKT, EGF, EGFR, ERK1_2, GRB2, IFN1A, IFNAR1, IFNAR2, IFNb, JNK, LXRa, MDM4, NFKB1, NFKB1A, P38, PA28g, PDK1, PI3K, PIAS1, PIP3, PP2A, PPARa, PROLIFERATION, RAF, RAS, RXRA, SCYL1BP1, SOCS3, SOS, STEATOSIS, TNFR, TNFa, TRADD, TRAF2, CORE, P14ARF**
Downregulated	**APOPTOSIS, BAD, GSK3B, HCV, IRF3, IKKa, IKKb, IKKe, IKKy, IL8, IPS_1, IRF1, IRF9, ISG, Jak1_Tyk2, MDM2, NS3, NS4A, NS5A, OAS, P21, P53, PKR, RIG_1, RIP_1, RNASEL, ROS, STAT3, Stat1_2, TBK1, TLR3, TRAF3, TRAF6, TRIF, VIRAL_REPLICATION, Ca2, eIF2a**

Figure 4.2: Represents the derivation of the list of fix points obtained after extensive static analysis through software Pint working under the framework of Process Hitting. In case of interferon mediated immune response generated against HCV, the stable state indicates the hampering of viral components with the active regulation of host immune components. The following table indicates the list of upregulated and downregulated entities within the stable state.

According to the first stable state, owing to the presence of HCV infectious pathogen,

Stable state in case of Virus dominating the host immune defense system during infection:	
Up regulated	**AKT, EGF, EGFR, ERK1_2, IFN1A, HCV, IFNAR1, IFNAR2, IL8, ISG, JNK, LXRa, MDM4, NS3, NS4A, NS5A, P38, PA28g, PDK1, PI3K, PIAS1, PIP3, PP2A, PPARa, PROLIFERATION, RAF, RIG_1, RXRA, SCYL1BP1, SOCS3, ROS, STEATOSIS, STAT3, TLR3, TNFR, TNFa, VIRAL_REPLICATION, Ca2, CORE, P14ARF**
Down regulated	**APOPTOSIS, BAD, GRB2, GSK3B, IRF3, IKKa, IKKb, IKKe, IKKy, IPS_1, IRF1, IRF9, Jak1_Tyk2, IFNb, MDM2, NFKB1, NFKB1A, OAS, P21, P53, PKR, RAS, RIP_1, SOS, RNASEL, Stat1_2, TBK1, TRADD, TRAF2, TRAF3, TRAF6, TRIF, eIF2a**

Figure 4.3: Represents the derivation of fix point in case the host mediated interferon based immune response gets impeded by the viral components. At various points, the HCV and its non-structural proteins hamper the defense mechanism of the host thus, leading to the development of chronic infection.

the host triggers its initial antiviral response through interferon-beta that is produced because of RIG_1 mediated defense mechanism. Now, either the immune defense mechanism takes hold of the virus and becomes able to combat it or in other case as shown in the second stable state, the virus impedes host defense mechanism and through series of its non-structural proteins, hinders the immune response generated by the host.

4.1.4 Non-Viral Pathway Analysis

On the other hand, the stable states generated in case of diseased condition can be compared to that generated in the normal non-viral pathway. Despite of presence of virus and its components in the body, it is not able to trigger the onset of infection as can be observed in the stable state generated for non-viral condition as shown in Figure 4.4.

Stable state in case of host immune defense system completely takes over the virus leaving no point for onset of infection:	
Upregulated	**APOPTOSIS, EGF, EGFR, ERK1_2, GRB2, IFN1A, IFNAR1, IFNAR2, IFNb, IRF1, JNK, NFKB1, NFKB1A, P21, P53, P38, PIAS1, PKR, PP2A, PROLIFERATION, RAF, RAS, SCYL1BP1, SOCS3, SOS, TNFR, TNFa, TRADD, TRAF2, P14ARF, eIF2a**
Downregulated	**AKT, BAD, GSK3B, HCV, IRF3, IKKa, IKKb, IKKe, IKKy, IL8, IPS_1, , IRF9, ISG, Jak1_Tyk2, LXRa, MDM4, MDM2, NS3, NS4A, NS5A, OAS, PA28g, PDK1,PI3K, PIP3, PPARa, RIG_1, RIP_1, RNASEL, RXRA, ROS, STAT3, STEATOSIS, Stat1_2, TBK1, TLR3, TRAF3, TRAF6, TRIF, VIRAL_REPLICATION, Ca2, CORE**

Figure 4.4: Represents the obtained stable state in case the host immune defense mechanism completely takes over the virus and its resultant infection. The HCV virus and its components are at expression level 0 with the host antiviral components at active expression level of 1. In this case, the virus despite of its presence, could not trigger the onset of disease state.

4.1.5 One-Shot Mutations and Analysis

The software Pint also applied mutations on the disease triggering states such as on AKT, PROLIFERATION, RAF,JNK, STEATOSIS as shown in Figure 4.5, 4.6, 4.8, 4.7.

The list had the altered expression of levels of all those entities that when applied could as it is impede the reachability towards stable state. There was no definite application of Bifurcations function since the pathway is itself triggered by the virus and it gets hampered later on at various points through viral components. Henceforth, no explicit alteration of the paths of states could be identified for entities involved in HCV pathway.

```
y.oneshot_mutations_for_cut("AKT = 1")
```

This computation is an *under-approximation*: returned mutations are all valid, but they may be non-minimal, and some solutions may be missed.

Limiting solutions to mutations of at most 5 automata. Use `maxsize` argument to change.

```
[{'PI3K': 0},
 {'PDK1': 0},
 {'PIP3': 0},
 {'EFG': 0, 'IFNb': 1},
 {'EFGR': 0, 'IFNb': 1},
 {'EFG': 0, 'NS5A': 0},
 {'EFG': 0, 'RNASEL': 1},
 {'EFG': 0, 'HCV': 0},
 {'EFGR': 0, 'NS5A': 0},
 {'EFGR': 0, 'RNASEL': 1},
 {'EFGR': 0, 'HCV': 0}]
```

Figure 4.5: Application of Mutations function on AKT

```
y.oneshot_mutations_for_cut("PROLIFERATION = 1")
```

This computation is an *under-approximation*: returned mutations are all valid, but they may be non-minimal, and some solutions may be missed.

Limiting solutions to mutations of at most 5 automata. Use `maxsize` argument to change.

```
[{'RAF': 0},
 {'ERK1_2': 0},
 {'CORE': 0, 'EFG': 0},
 {'CORE': 0, 'EFGR': 0},
 {'CORE': 0, 'GRB2': 0},
 {'CORE': 0, 'SOS': 0},
 {'CORE': 0, 'RAS': 0},
 {'CORE': 0, 'NS5A': 1},
 {'EFG': 0, 'IFNb': 1, 'PA28g': 0},
 {'EFGR': 0, 'IFNb': 1, 'PA28g': 0},
 {'GRB2': 0, 'IFNb': 1, 'PA28g': 0},
 {'IFNb': 1, 'PA28g': 0, 'SOS': 0},
 {'IFNb': 1, 'PA28g': 0, 'RAS': 0},
 {'IFNb': 1, 'NS5A': 1, 'PA28g': 0},
 {'EFG': 0, 'PA28g': 0, 'RNASEL': 1},
 {'EFG': 0, 'HCV': 0, 'PA28g': 0},
 {'EFGR': 0, 'PA28g': 0, 'RNASEL': 1},
 {'EFGR': 0, 'HCV': 0, 'PA28g': 0},
 {'GRB2': 0, 'PA28g': 0, 'RNASEL': 1},
 {'GRB2': 0, 'HCV': 0, 'PA28g': 0},
 {'PA28g': 0, 'RNASEL': 1, 'SOS': 0},
 {'HCV': 0, 'PA28g': 0, 'SOS': 0},
 {'PA28g': 0, 'RAS': 0, 'RNASEL': 1},
 {'HCV': 0, 'PA28g': 0, 'RAS': 0},
 {'NS5A': 1, 'PA28g': 0, 'RNASEL': 1},
 {'HCV': 0, 'NS5A': 1, 'PA28g': 0}]
```

Figure 4.6: Application of Mutations function on Proliferation

```
y.oneshot_mutations_for_cut("STEATOSIS = 1")
```

This computation is an *under-approximation*: returned mutations are all valid, but they may be non-minimal, and some solutions may be missed.

Limiting solutions to mutations of at most 5 automata. Use `maxsize` argument to change.

```
[{'CORE': 0},
 {'IFNb': 1, 'PA28g': 0},
 {'PA28g': 0, 'RNASEL': 1},
 {'HCV': 0, 'PA28g': 0},
 {'LXRa': 0, 'PPARa': 0, 'RXRA': 0},
 {'LXRa': 1, 'PPARa': 1, 'RXRA': 0}]
```

Figure 4.7: Application of Mutations function on STEATOSIS

```
y.oneshot_mutations_for_cut("RAF = 1,JNK = 1")
```

This computation is an *under-approximation*: returned mutations are all valid, but they may be non-minimal, and some solutions may be missed.

Limiting solutions to mutations of at most 5 automata. Use `maxsize` argument to change.

```
[{'CORE': 0, 'EFG': 0},
 {'CORE': 0, 'EFGR': 0},
 {'CORE': 0, 'GRB2': 0},
 {'CORE': 0, 'SOS': 0},
 {'CORE': 0, 'RAS': 0},
 {'CORE': 0, 'NS5A': 1},
 {'EFG': 0, 'IFNb': 1, 'PA28g': 0},
 {'EFGR': 0, 'IFNb': 1, 'PA28g': 0},
 {'GRB2': 0, 'IFNb': 1, 'PA28g': 0},
 {'IFNb': 1, 'PA28g': 0, 'SOS': 0},
 {'IFNb': 1, 'PA28g': 0, 'RAS': 0},
 {'IFNb': 1, 'NS5A': 1, 'PA28g': 0},
 {'EFG': 0, 'PA28g': 0, 'RNASEL': 1},
 {'EFG': 0, 'HCV': 0, 'PA28g': 0},
 {'EFGR': 0, 'PA28g': 0, 'RNASEL': 1},
 {'EFGR': 0, 'HCV': 0, 'PA28g': 0},
 {'GRB2': 0, 'PA28g': 0, 'RNASEL': 1},
 {'GRB2': 0, 'HCV': 0, 'PA28g': 0},
 {'PA28g': 0, 'RNASEL': 1, 'SOS': 0},
 {'HCV': 0, 'PA28g': 0, 'SOS': 0},
 {'PA28g': 0, 'RAS': 0, 'RNASEL': 1},
 {'HCV': 0, 'PA28g': 0, 'RAS': 0},
 {'NS5A': 1, 'PA28g': 0, 'RNASEL': 1},
 {'HCV': 0, 'NS5A': 1, 'PA28g': 0}]
```

Figure 4.8: Application of Mutations function on RAF,JNK

4.1.6 Understanding and Application of Cut Sets in the HCV Pathway

Next, it enabled us with the catalogue of cut sets that serve as our drug targets and lay the basis of the future perspective of therapeutic strategies. Our proposed drug targets are PI3K, PIP3, PDK1, ERK1/2, PA28-gamma, LXR-alpha, PPAR-alpha and RXR-alpha taken from lists of cut sets as shown below in Figure 4.9, 4.11, 4.12, 4.10.

```
y.cutsets("AKT = 1",maxsize=45)
```

This computation is an *under-approximation*: returned cut-sets are all valid, but they may be non-minimal, and some cut-sets may be missed.

Limiting results to cut-sets with at most 45 elements. Use `maxsize` argument to change.

```
[{'PI3K': 1},
 {'PDK1': 1},
 {'PIP3': 1},
 {'EFG': 1, 'IFNb': 0},
 {'EFGR': 1, 'IFNb': 0},
 {'EFG': 1, 'EFGR': 0},
 {'EFG': 1, 'RAS': 0},
 {'EFG': 1, 'NS5A': 1},
 {'EFG': 1, 'RNASEL': 0},
 {'EFG': 1, 'HCV': 1},
 {'EFGR': [0, 1]},
 {'EFGR': 1, 'RAS': 0},
 {'EFGR': 1, 'NS5A': 1},
 {'EFGR': 1, 'RNASEL': 0},
 {'EFGR': 1, 'HCV': 1}]
```

Figure 4.9: Application of Cutsets function on AKT

```
y.cutsets("STEATOSIS = 1",maxsize=45)
```

This computation is an *under-approximation*: returned cut-sets are all valid, but they may be non-minimal, and some cut-sets may be missed.

Limiting results to cut-sets with at most 45 elements. Use `maxsize` argument to change.

```
[{'CORE': 1},
 {'IFNb': 0, 'PA28g': 1},
 {'RXRA': [0, 1]},
 {'PA28g': [0, 1]},
 {'PA28g': 1, 'RNASEL': 0},
 {'HCV': 1, 'PA28g': 1},
 {'PPARa': [0, 1], 'RXRA': 1},
 {'LXRa': 0, 'PPARa': 0, 'RXRA': 1},
 {'LXRa': 1, 'PPARa': 1, 'RXRA': 1},
 {'LXRa': [0, 1], 'RXRA': 1}]
```

Figure 4.10: Application of Cutsets function on STEATOSIS

```
y.cutsets("PROLIFERATION = 1",maxsize=45)
```

This computation is an *under-approximation*: returned cut-sets are all valid, but they may be non-minimal, and some cut-sets may be missed.

Limiting results to cut-sets with at most 45 elements. Use `maxsize` argument to change.

```
[{'RAF': 1},
 {'ERK1_2': 1},
 {'CORE': [0, 1]},
 {'CORE': 1, 'EFG': 1},
 {'CORE': 1, 'EFGR': 1},
 {'CORE': 1, 'GRB2': 1},
 {'CORE': 1, 'SOS': 1},
 {'CORE': 1, 'RAS': 1},
 {'CORE': 1, 'NS5A': 0},
 {'CORE': 0, 'IFNb': 0, 'PA28g': 1},
 {'EFG': 1, 'IFNb': 0, 'PA28g': 1},
 {'EFGR': 1, 'IFNb': 0, 'PA28g': 1},
 {'GRB2': 1, 'IFNb': 0, 'PA28g': 1},
 {'IFNb': 0, 'PA28g': 1, 'SOS': 1},
 {'IFNb': 0, 'PA28g': 1, 'RAS': 1},
 {'IFNb': 0, 'NS5A': 0, 'PA28g': 1},
 {'CORE': 0, 'PA28g': [0, 1]},
 {'CORE': 0, 'PA28g': 1, 'RNASEL': 0},
 {'CORE': 0, 'HCV': 1, 'PA28g': 1},
 {'EFG': 1, 'PA28g': [0, 1]},
 {'EFG': 1, 'PA28g': 1, 'RNASEL': 0},
 {'EFG': 1, 'HCV': 1, 'PA28g': 1},
 {'EFGR': 1, 'PA28g': [0, 1]},
 {'EFGR': 1, 'PA28g': 1, 'RNASEL': 0},
 {'EFGR': 1, 'HCV': 1, 'PA28g': 1},
 {'GRB2': 1, 'PA28g': [0, 1]},
 {'GRB2': 1, 'PA28g': 1, 'RNASEL': 0},
 {'GRB2': 1, 'HCV': 1, 'PA28g': 1},
 {'PA28g': [0, 1], 'SOS': 1},
 {'PA28g': 1, 'RNASEL': 0, 'SOS': 1},
 {'HCV': 1, 'PA28g': 1, 'SOS': 1},
 {'PA28g': [0, 1], 'RAS': 1},
 {'PA28g': 1, 'RAS': 1, 'RNASEL': 0},
 {'HCV': 1, 'PA28g': 1, 'RAS': 1},
 {'NS5A': 0, 'PA28g': [0, 1]},
 {'NS5A': 0, 'PA28g': 1, 'RNASEL': 0},
 {'HCV': 1, 'NS5A': 0, 'PA28g': 1}]
```

Figure 4.11: Application of Cutsets function on Proliferation

```
y.cutsets("RAF = 1,JNK = 1",maxsize=45)
```

This computation is an *under-approximation*: returned cut-sets are all valid, but they may be non-minimal, and some cut-sets may be missed.

Limiting results to cut-sets with at most 45 elements. Use `maxsize` argument to change.

```
[{'_pint_goal': 1},
 {'CORE': [0, 1]},
 {'CORE': 1, 'EFG': 1},
 {'CORE': 1, 'EFGR': 1},
 {'CORE': 1, 'GRB2': 1},
 {'CORE': 1, 'SOS': 1},
 {'CORE': 1, 'RAS': 1},
 {'CORE': 1, 'NS5A': 0},
 {'CORE': 0, 'IFNb': 0, 'PA28g': 1},
 {'EFG': 1, 'IFNb': 0, 'PA28g': 1},
 {'EFGR': 1, 'IFNb': 0, 'PA28g': 1},
 {'GRB2': 1, 'IFNb': 0, 'PA28g': 1},
 {'IFNb': 0, 'PA28g': 1, 'SOS': 1},
 {'IFNb': 0, 'PA28g': 1, 'RAS': 1},
 {'IFNb': 0, 'NS5A': 0, 'PA28g': 1},
 {'CORE': 0, 'PA28g': [0, 1]},
 {'CORE': 0, 'PA28g': 1, 'RNASEL': 0},
 {'CORE': 0, 'HCV': 1, 'PA28g': 1},
 {'EFG': 1, 'PA28g': [0, 1]},
 {'EFG': 1, 'PA28g': 1, 'RNASEL': 0},
 {'EFG': 1, 'HCV': 1, 'PA28g': 1},
 {'EFGR': 1, 'PA28g': [0, 1]},
 {'EFGR': 1, 'PA28g': 1, 'RNASEL': 0},
 {'EFGR': 1, 'HCV': 1, 'PA28g': 1},
 {'GRB2': 1, 'PA28g': [0, 1]},
 {'GRB2': 1, 'PA28g': 1, 'RNASEL': 0},
 {'GRB2': 1, 'HCV': 1, 'PA28g': 1},
 {'PA28g': [0, 1], 'SOS': 1},
 {'PA28g': 1, 'RNASEL': 0, 'SOS': 1},
 {'HCV': 1, 'PA28g': 1, 'SOS': 1},
 {'PA28g': [0, 1], 'RAS': 1},
 {'PA28g': 1, 'RAS': 1, 'RNASEL': 0},
 {'HCV': 1, 'PA28g': 1, 'RAS': 1},
 {'NS5A': 0, 'PA28g': [0, 1]},
 {'NS5A': 0, 'PA28g': 1, 'RNASEL': 0},
 {'HCV': 1, 'NS5A': 0, 'PA28g': 1}]
```

Figure 4.12: Application of Cutsets function on RAF,JNK

4.1.7 Evaluation of Reduction of the HCV Model for further Implementations

The application of cut set on AKT enabled us to regain the homeostasis by reviving the apoptosis through lacerations applied on PI3K, PIP3 and PDK1. Based on these above cut sets, the 73 entities-based colossal model of HCV was reduced to 48 entities encompassing the stable state that no longer can proceed to the infection onset by maintaining the dynamics within the homeostatic cycles as enlightened in the reduced graph.

4.2 Discussion

Owing to the growing incautious attitudes towards needles and syringes-based drug abuse, deadly infections like Hepatitis C Virus are spreading at a pace. But, the findings of the above research could be implemented in the wet lab experimentations to further validate the outcomes. The suppression of the entities mentioned d as s drug targets or knock out experiments could reveal the definite impede the triggering of HCV infection. Despite of the presence of viral components in the biological system, it was validated that all those states that were knock down from the automata network were the real culprits towards disturbing the natural homeostatic functioning of the body and obstructing he host body's immune function to combat that. Further wet lab analysis is expected to validate the sanctity of these findings.

4.3 Conclusion

HCV is one the growing public health issue that prevails at an enhanced pace in the developing countries inclusive of Pakistan, owing to the low standards of international practices of injection-based drug usage. the model implemented in the present research displays the complete pathogenic pathway of HCV and the resulting immune response generated against it. Now the stage sets between the viral pathogen and host immune defense mechanism and who so ever dominates the other determines the course of response. The results obtained in the form of stable state indicates that in case, the host immune response gets able to combat the virus and its resulting infection, the body

develops strategy to cope up with that infection. On the other hand, in case virus takes over the immune system, it resultantly gets able to impede it at different cellular levels. To compare the normal cellular responses with those of during the viral infection onset, the two stable states in each case demonstrate how normal working attributes of the cell gets altered according to virus promoting conditions.

The HCV Automata Network File in Pint
"Stat1_2" 0 -> 1" when "CORE"=0 and
"Jak1_Tyk2"=1 and "PP2A"=0 and "PIAS1"=0"
"EFG" 0 -> 1"
"TRAF6" 0 -> 1" when "TRIF"=1"
"GRB2" 0 -> 1" when "NS5A"=0 and "EFGR"=1"
"TBK1" 1 -> 0" when "TRAF3"=0"
"IPS_1" 0 -> 1" when "NS3"=0 and "RIG_1"=1
and "NS4A"=0"
"IKKy" 0 -> 1" when "RIP1"=1 and "TRAF6"=0"
"GSK3B" 1 -> 0" when "AKT"=1"
"PP2A" 0 -> 1"
"MDM2" 1 -> 0" when "P53"=1 and "MDM4"=1
and "p14ARF"=1"
"IRF1" 1 -> 0" when "PKR"=0"
"IRF9" 0 -> 1" when "Stat1_2"=1"
"TRAF3" 0 -> 1" when "IPS_1"=1"
"TRAF2" 1 -> 0" when "TNFR"=0"
"APOPTOSIS" 1 -> 0" when "GSK3B"=0 and
"BAD"=0 and "P21"=0"
"RAS" 0 -> 1" when "SOS"=1"
"IFNAR1" 1 -> 0" when "IFN1A"=0"
"Jak1_Tyk2" 1 -> 0" when "IFNAR1"=1 and
"SOCS3"=1"
"IPS_1" 1 -> 0" when "NS3"=1 and "RIG_1"=1"
"VIRAL_REPLICATION" 0 -> 1" when "NS5A"=1"
"NFKB1A" 0 -> 1" when "IKKa"=1 and "PKR"=0
and "TRADD"=1 and "IKKy"=0 and "TRAF2"=0
and "IKKb"=0"
"NFKB1A" 0 -> 1" when "IKKa"=1 and "PKR"=1
and "IKKy"=1 and "IKKb"=1"

Table 4.1: The HCV Automata Network File in Pint Parameters 1

The HCV Automata Network File in Pint
"TRIF" 1 -> 0" when "TLR3"=1 and "NS3"=1"
"P53" 0 -> 1" when "NS3"=0 and
"AKT"=0 and "MDM4"=0 and "MDM2"=0"
"MDM2" 1 -> 0" when "P53"=0"
"TLR3" 1 -> 0" when "HCV"=0"
"STAT3" 0 -> 1" when "ROS"=1"
"IKKb" 0 -> 1" when "TRAF6"=1"
"RAF" 1 -> 0" when "CORE"=0 and "RAS"=0"
"Jak1_Tyk2" 1 -> 0" when "IFNAR1"=0 and
"SOCS3"=1 and "IFNAR2"=1"
"IFN1A" 0 -> 1"
"IL8" 1 -> 0" when "NS3"=0"
"Jak1_Tyk2" 1 -> 0" when "IFNAR1"=0 and
"IFNAR2"=0"
"NFKB1A" 1 -> 0" when "IKKa"=0 and "PKR"=0
and "IKKy"=0 and "TRAF2"=0 and "IKKb"=0"
"P53" 1 -> 0" when "NS3"=0 and "MDM2"=1
and "AKT"=0"
"IFNAR2" 0 -> 1" when "IFN1A"=1"
"IFNAR1" 0 -> 1" when "IFN1A"=1"
"NS3" 1 -> 0" when "HCV"=0"
"JNK" 0 -> 1" when "RAF"=1"
"P38" 0 -> 1" when "PKR"=0 and "RAF"=1"
"PA28g" 0 -> 1âĂİ
"IFR3" 0 -> 1" when "TBK1"=1"
"PIAS1" 0 -> 1âĂİ
"MDM4" 1 -> 0" when "MDM2"=1"
"NFKB1A" 1 -> 0" when "IKKa"=0 and "PKR"=0
and "TRADD"=0 and "IKKy"=0 and
"TRAF2"=1 and "IKKb"=0"

Table 4.2: The HCV Automata Network File in Pint Parameters 2

The HCV Automata Network File in Pint
"IPS_1" 1 -> 0" when "RIG_1"=0"
"STEATOSIS" 0 -> 1" when "PPARa"=0 and
"RXRA"=0 and "LXRa"=1"
"TBK1" 1 -> 0" when "TRAF3"=1 and "NS3"=1"
"RIP1" 1 -> 0" when "TRIF"=0"
"IKKy" 0 -> 1" when "TRAF6"=1"
"GRB2" 1 -> 0" when "NS5A"=1 and "EFGR"=1"
"PPARa" 1 -> 0" when "CORE"=0"
"P53" 1 -> 0" when "NS3"=0 and "AKT"=0 and
"MDM4"=1 and "MDM2"=0"
"eIF2a" 1 -> 0" when "PKR"=0"
"RXRA" 0 -> 1" when "CORE"=1"
"RIP1" 0 -> 1" when "TRIF"=1"
"RXRA" 1 -> 0" when "CORE"=0"
"NFKB1A" 1 -> 0" when "IKKa"=0 and "PKR"=0
and "TRADD"=0 and "IKKy"=0 and
"TRAF2"=0 and "IKKb"=1"
"IFR3" 1 -> 0" when "TBK1"=0 and "IKKe"=0"
"RNASEL" 0 -> 1" when "OAS"=1"
"PIP3" 1 -> 0" when "PI3K"=0"
"ERK1_2" 1 -> 0" when "RAF"=0"
"EFGR" 0 -> 1" when "EFG"=1"
"NFKB1A" 1 -> 0" when "IKKa"=1 and "PKR"=0
and "TRADD"=1 and "IKKy"=1 and "TRAF2"=1
and "IKKb"=0"
"NFKB1A" 0 -> 1" when "IKKa"=0 and "IKKy"=1
and "IKKb"=1"
"RAS" 1 -> 0" when "SOS"=0"
"NS4A" 1 -> 0" when "HCV"=0"
"RNASEL" 1 -> 0" when "OAS"=0"

Table 4.3: The HCV Automata Network File in Pint Parameters 3

The HCV Automata Network File in Pint
"PKR" 0 -> 1" when "HCV"=1 and "NS5A"=0"
"P53" 1 -> 0" when "NS3"=1 and "AKT"=0"
"HCV" 1 -> 0" when "IFNb"=0 and "RNASEL"=1"
"TNFR" 0 -> 1" when "TNFa"=1"
"IKKe" 0 -> 1" when "TRAF3"=1 and "NS3"=0"
"LXRa" 1 -> 0" when "CORE"=0"
"TRAF2" 1 -> 0" when "TNFR"=1 and "NS5A"=1"
"IKKa" 1 -> 0" when "RIP1"=0 and "TRAF6"=0"
"IKKa" 0 -> 1" when "RIP1"=1 and "TRAF6"=0"
"STEATOSIS" 0 -> 1" when "RXRA"=1"
"STEATOSIS" 1 -> 0" when "PPARa"=1 and
"RXRA"=0 and "LXRa"=1"
"VIRAL_REPLICATION" 1 -> 0" when "NS5A"=0"
"NFKB1A" 0 -> 1" when "IKKa"=0 and "PKR"=1
and "IKKy"=1 and "TRAF2"=0 and "IKKb"=0"
"P38" 0 -> 1" when "PKR"=1"
"PPARa" 0 -> 1" when "CORE"=1"
"JNK" 1 -> 0" when "RAF"=0"
"RAF" 0 -> 1" when "CORE"=1"
"HCV" 0 -> 1" when "IFNb"=0 and "RNASEL"=0"
"Stat1_2" 1 -> 0" when "CORE"=1"
"TNFR" 1 -> 0" when "TNFa"=0"
"RAF" 0 -> 1" when "CORE"=0 and "RAS"=1"
"IKKa" 0 -> 1" when "TRAF6"=1"
"APOPTOSIS" 0 -> 1" when "GSK3B"=1"
"RIG_1" 1 -> 0" when "HCV"=0"
"SOCS3" 1 -> 0" when "CORE"=0 and "IFNb"=0"
"TRAF2"=1 and "IKKb"=1"
"AKT" 1 -> 0" when "PDK1"=0"
"GRB2" 1 -> 0" when "EFGR"=0"

Table 4.4: The HCV Automata Network File in Pint Parameters 4

The HCV Automata Network File in Pint
"IFNb" 1 -> 0" when "IFR3"=0 and "HCV"=1
and "NFKB1"=1"
"NS4A" 0 -> 1" when "HCV"=1"
"OAS" 0 -> 1" when "HCV"=1 and "NS5A"=0"
"PDK1" 1 -> 0" when "PIP3"=0"
"TRADD" 1 -> 0" when "TNFR"=0"
"SOCS3" 0 -> 1" when "CORE"=1 and "IFNb"=0"
"NFKB1A" 0 -> 1" when "IKKa"=0 and "PKR"=0
and "TRADD"=1 and "IKKy"=0 and "TRAF2"=1
and "IKKb"=0"
"IRF1" 0 -> 1" when "PKR"=1"
"NFKB1A" 1 -> 0" when "IKKa"=1 and
"PKR"=1 and "IKKy"=1 and
"IKKb"=0 and "TRADD"=1"
"IFNAR2" 1 -> 0" when "IFN1A"=0"
"CORE" 0 -> 1" when "PA28g"=0 and "HCV"=1"
"PKR" 1 -> 0" when "NS5A"=1"
"CORE" 0 -> 1" when "PA28g"=1"
"ISG" 1 -> 0" when "STAT3"=0 and "IRF9"=0"
"ROS" 1 -> 0" when "ca2"=0"
"SOCS3" 0 -> 1" when "IFNb"=1"
"PI3K" 0 -> 1" when "EFGR"=1"
"ca2" 0 -> 1" when "NS5A"=1"
"PI3K" 1 -> 0" when "RAS"=0 and "NS5A"=0
and "EFGR"=0"
"Stat1_2" 1 -> 0" when "CORE"=0 and
"Jak1_Tyk2"=1 and
"PP2A"=0 and "PIAS1"=1"
"Stat1_2" 1 -> 0" when "CORE"=0 and
"Jak1_Tyk2"=1 and "PP2A"=1"

Table 4.5: The HCV Automata Network File in Pint Parameters 5

The HCV Automata Network File in Pint
"STAT3" 1 -> 0" when "ROS"=0"
"TNFa" 0 -> 1âĂİ
"OAS" 1 -> 0" when "HCV"=0 and "NS5A"=0"
"PI3K" 0 -> 1" when "RAS"=1 and "EFGR"=0"
"NFKB1A" 0 -> 1" when "IKKa"=1 and
"PKR"=0 and "IKKy"=1 and "TRAF2"=0"
"TRAF3" 1 -> 0" when "IPS_1"=0"
"GSK3B" 0 -> 1" when "AKT"=0"
"NFKB1A" 0 -> 1" when "IKKa"=1 and
"PKR"=0 and "IKKy"=0 and
"TRAF2"=1 and "IKKb"=0"
"NFKB1A" 1 -> 0" when "IKKa"=0 and
"PKR"=1 and "TRADD"=1 and "IKKy"=1 and
"TRAF2"=1 and "IKKb"=0"
"ISG" 0 -> 1" when "STAT3"=1 and "IRF9"=0"
"Stat1_2" 1 -> 0" when "CORE"=0 and
"Jak1_Tyk2"=0"
"PROLIFERATION" 1 -> 0" when "ERK1_2"=0"
"NFKB1A" 0 -> 1" when "IKKa"=0 and "PKR"=1
and "IKKy"=0"
"BAD" 0 -> 1" when "AKT"=0"
"TRIF" 1 -> 0" when "TLR3"=0"
"ca2" 1 -> 0" when "NS5A"=0"
"ISG" 0 -> 1" when "IRF9"=1"
"NFKB1A" 0 -> 1" when "IKKa"=1 and "PKR"=1
and "IKKy"=0 and "TRAF2"=0"
"P53" 1 -> 0" when "AKT"=1"
"NFKB1A" 0 -> 1" when "IKKa"=1 and "PKR"=1
and "IKKy"=1 and "IKKb"=0 and "TRADD"=0"
"ERK1_2" 0 -> 1" when "RAF"=1"

Table 4.6: The HCV Automata Network File in Pint Parameters 6

The HCV Automata Network File in Pint
"APOPTOSIS" 0 -> 1" when "GSK3B"=0 and
"BAD"=0 and "P21"=1"
"Jak1_Tyk2" 0 -> 1" when "IFNAR1"=1 and
"SOCS3"=0"
"ROS" 0 -> 1" when "ca2"=1"
"TBK1" 0 -> 1" when "TRAF3"=1 and "NS3"=0"
"IFNb" 1 -> 0" when "IFR3"=1 and "HCV"=1"
"HCV" 1 -> 0" when "IFNb"=1"
"NFKB1" 0 -> 1" when "NFKB1A"=1"
"P38" 1 -> 0" when "PKR"=0 and "RAF"=0"
"IKKe" 1 -> 0" when "TRAF3"=1 and "NS3"=1"
"IFNb" 0 -> 1" when "IFR3"=1 and "HCV"=0"
"TRAF2" 0 -> 1" when "TNFR"=1 and "NS5A"=0"
"AKT" 0 -> 1" when "PDK1"=1"
"IFNb" 0 -> 1" when "IFR3"=0 and "HCV"=0
and "NFKB1"=1"
"PI3K" 0 -> 1" when "RAS"=0 and "NS5A"=1
and "EFGR"=0"
"CORE" 1 -> 0" when "PA28g"=0 and "HCV"=0"
"RIG_1" 0 -> 1" when "HCV"=1"
"IKKy" 1 -> 0" when "RIP1"=0 and "TRAF6"=0"
"TRIF" 0 -> 1" when "TLR3"=1 and "NS4A"=0
and "NS3"=0"
"MDM2" 0 -> 1" when "P53"=1 and "MDM4"=1
and "p14ARF"=0 and "SCYL1BP1"=0"
"IKKb" 1 -> 0" when "RIP1"=0 and "TRAF6"=0"
"NFKB1A" 0 -> 1" when "IKKa"=0 and "PKR"=0
and "TRADD"=1 and
"IKKy"=0 and "TRAF2"=0 and "IKKb"=1"
"IKKy"=0 and "TRAF2"=1"

Table 4.7: The HCV Automata Network File in Pint Parameters 7

The HCV Automata Network File in Pint
"IKKe" 1 -> 0" when "TRAF3"=0"
"PIP3" 0 -> 1" when "PI3K"=1"
"OAS" 1 -> 0" when "NS5A"=1"
"PROLIFERATION" 0 -> 1" when "ERK1_2"=1"
"BAD" 1 -> 0" when "AKT"=1"
"IL8" 0 -> 1" when "NS3"=1"
"NS3" 0 -> 1" when "HCV"=1"
"NFKB1A" 0 -> 1" when "IKKa"=1 and "PKR"=0
and "TRADD"=0 and "IKKy"=1 and "TRAF2"=1
and "IKKb"=0"
"P21" 1 -> 0" when "AKT"=1"
"NFKB1" 1 -> 0" when "NFKB1A"=0"
"IPS_1" 1 -> 0" when "NS3"=0 and "RIG_1"=1
and "NS4A"=1"
"PKR" 1 -> 0" when "HCV"=0 and "NS5A"=0"
"IKKb" 0 -> 1" when "RIP1"=1 and "TRAF6"=0"
"NS5A" 1 -> 0" when "HCV"=0"
"IRF9" 1 -> 0" when "Stat1_2"=0"
"NFKB1A" 1 -> 0" when "IKKa"=1 and "PKR"=0
and "IKKy"=1 and
"IFNb" 1 -> 0" when "IFR3"=0 and "NFKB1"=0"
"TRADD" 0 -> 1" when "TNFR"=1 and "NS5A"=0"
"SOS" 0 -> 1" when "GRB2"=1"
"NFKB1A" 1 -> 0" when "IKKa"=0 and
"PKR"=0 and "TRADD"=0 and "IKKy"=1 and
"TRAF2"=0 and "IKKb"=0"
"STEATOSIS" 0 -> 1" when "PPARa"=1 and
"RXRA"=0 and "LXRa"=0"
"MDM2" 1 -> 0" when "P53"=1 and "MDM4"=1 and
"p14ARF"=0 and "SCYL1BP1"=1"

Table 4.8: The HCV Automata Network File in Pint Parameters 8

The HCV Automata Network File in Pint
"MDM4" 0 -> 1" when "MDM2"=0"
"Jak1_Tyk2" 0 -> 1" when "IFNAR1"=0 and
"SOCS3"=0 and "IFNAR2"=1"
"TRIF" 1 -> 0" when "TLR3"=1 and "NS4A"=1 and
"NS3"=0"
"NFKB1A" 0 -> 1" when "IKKa"=1 and "PKR"=0 and
"IKKy"=0 and "IKKb"=1"
"p14ARF" 0 -> 1âĂİ
"STEATOSIS" 1 -> 0" when "PPARa"=0 and
"RXRA"=0 and "LXRa"=0"
"NFKB1A" 0 -> 1" when "IKKa"=0 and "PKR"=0 and
"IKKy"=0 and
"TRAF2"=1 and "IKKb"=1"
"NFKB1A" 0 -> 1" when "IKKa"=0 and "PKR"=0 and
"TRADD"=1 and "IKKy"=1 and "TRAF2"=0 and
"IKKb"=0"
"TRADD" 1 -> 0" when "TNFR"=1 and "NS5A"=1"
"IFR3" 0 -> 1" when "TBK1"=0 and "IKKe"=1"
"TRAF6" 1 -> 0" when "TRIF"=0"
"SOS" 1 -> 0" when "GRB2"=0"
"NS5A" 0 -> 1" when "HCV"=1"
"EFGR" 1 -> 0" when "EFG"=0"
"P21" 0 -> 1" when "AKT"=0"
"NFKB1A" 1 -> 0" when "IKKa"=1 and "PKR"=1
"SCYL1BP1" 0 -> 1âĂİ
"APOPTOSIS" 0 -> 1" when "GSK3B"=0 and
"BAD"=1"
"NFKB1A" 1 -> 0" when "IKKa"=1 and "PKR"=1

Table 4.9: The HCV Automata Network File in Pint Parameters 9

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