Hepatitis C virus (HCV) was first detected in 1989 using molecular biology techniques after testing of serum from experimentally infected animal. It was characterized to be an RNA virus belonging to the Flaviviridae family and genus Hepacivirus. Ever since the discovery of HCV it became clear that this virus was the cause of acute hepatitis after a blood transfusion that was neither related to hepatitis A nor to hepatitis B. The estimated global prevalence of Hepatitis C virus (HCV) infection is around 2%, with 170 million persons chronically infected and 3 to 4 million persons getting infected each year (Shepard, Finelli, and Alter 2005). It is now widely considered as one of the common etiological agents of liver cirrhosis. Most European countries have a prevalence of HCV in the general population of 0.5 to 2% (“Hepatitis C--Global Prevalence (update)” 1999). As many as two to four million persons are chronically infected in the United States, five to ten million in Europe, and around twelve million in India and most do not have the knowledge of their infection. Around 150000 new cases occur annually in the US and in Western Europe, and in Japan the number hovers around 350000. Out of these, around 25% are symptomatic, but 60 to 80% may progress to chronic liver disease, and 20% of these may develop cirrhosis. About 5%-7% of patients may ultimately die of the infection.

The hepatitis C virus genome is comprised of a single stranded positive-sense RNA with a single opening reading frame of 9.6 kb in length encoding for a single polyprotein precursor of approximately 3000 residues flanked by untranslated regions (UTRs) at both ends (Choo et al. 1989). The precursor is cleaved into 10 different proteins: the structural proteins which include Core, E1, E2 and p7, as well as the non-
Introduction

structural proteins NS2, NS3, NS4A, NS4B, NS5A and NS5B. A constituting feature of the HCV genome is its high degree of variability in the genome. Mutation rates vary in the various regions in the viral genome. The E1 and E2 regions are the most variable, whereas the 5'UTR and the 3'UTR have the highest degree of sequence conservation. This high mutation rate is a manifestation of imperfect proof reading ability of the viral RNA-dependent RNA polymerase. Consequently different mutants of the parent strain co-exist as quasi-species in a single infected individual (Martell et al. 1992). HCV genome between strains from different geographical regions vary largely allowing the virus to be classified into six major genotypes (Simmonds et al. 2005). Genotypes of the virus have not been found to be associated with disease presentation or severity of disease but have been identified as one of the predictor of response to antiviral therapy.

Hepatitis C can present as acute or chronic hepatitis with the later being most prominent. Acute hepatitis C cases are mostly asymptomatic. This virus usually does not cause fulminant hepatitis in immune-competent individuals and the he only acute life threatening illness is a variant called fibrosing cholestatic hepatitis which is seen in patients with liver transplant (Taga et al. 1998). In chronic HCV infected patients development of portal hypertension leads to ascites, hepatic encephalopathy, spontaneous bacterial peritonitis, hepatorenal syndrome and variceal haemorrhage. Extra-hepatic manifestations as a result of chronic hepatitis C infection such as cryoglobulinemia, porphyria cutaneatarda, arthralgia, membranoproliferative glomerulonephritis, Raynaud’s syndrome, Sjogren’s syndrome, idiopathic thrombocytopenic purpura and non-Hodgkin’s lymphoma have been reported (Liang et al. 2000). The patients with hepatitis C cirrhosis are at a higher risk of hepatocellular carcinoma. The common modes of spread of hepatitis C infection are blood transfusion,
unsafe therapeutic injections, health care related procedures and injection drug use. In
developed countries the predominant route of hepatitis C infection is drug use, whereas
in India, blood transfusions and unsafe therapeutic injections are the predominant
modes of transmission of hepatitis C. A lot of studies have been carried out to increase
the understanding of HCV proteins and its interaction with the host immune factors,
both innate and adaptive. HCV possesses relentless adroitness in disrupting the co­
ordinated activity of the innate immune cells and thus result in deficient adaptive
immune response and abrogates HCV elimination.

The complement system functions as an immune surveillance system that rapidly
responds to infection. The complement system consists of 30 soluble and membrane
bound proteins, and is activated by 3 distinct pathways. There are three established
mechanisms of complement activation; these are known as the classical, alternative and
lectin pathways. Activation of these pathways are dependent on different molecules for
their initiation. The complement system is a major component of innate immunity and
consists of both soluble factors and cell surface receptors that interact to sense and
respond to invading pathogens. The complement system links the innate and adaptive
immune responses by a variety of mechanisms including enhancing modulating T cell
function, regulating antibody effector mechanisms and humoral immunity. All of the
complement cascades culminate in the central cleavage of C3 and the generation of its
active fragments C3a and C3b. Opsonization of foreign surfaces by covalently attached
C3b fulfils three major functions: cell clearance by phagocytosis; amplification of
complement activation by the formation of a surface-bound C3 convertase; and
assembly of the C5 convertases. Cleavage of C5 induces the formation of a
multiprotein pore complex (the membrane-attack complex (MAC)), which leads to cell
lysis. Both the covalent attachment of C3b and the stabilization of the C3 convertase by the complement regulator properdin are greatly encouraged by hydroxyl-rich surface of pathogens. A series of complement receptors arbitrate the recognition of opsonized cells by leukocytes, which results in phagocytosis and the stimulation of the adaptive immune system. Finally, the anaphylatoxins C3a and C5a are released during complement activation and trigger a range of chemotactic and pro-inflammatory responses. In this way, the complement cascade also supports and promotes the function of downstream mechanisms of the immune response. Although this carefully regulated protein complexes, receptors and cascade of enzymes ensures the rapid recognition and elimination of foreign molecules, it also offers many sites of hindrance that can disrupt this balanced network of protein interactions. Studies have found that pathogens utilize these sites in complement system to leverage their pathogenicity. Many pathogens have been able to successfully persist in host cells by evading the complement system by incorporating techniques like closely mimicking host regulators on their surfaces; acquiring regulators by which they bind stably to circulating RCA and protect themselves from complement mediated attack. Some viruses have found to shed or internalize antigen complexes from their surface to avoid complement activation and phagocytosis. Some viruses expresses glycoproteins that are recognized by complement receptors on cells and get internalized resulting in infectivity. In addition to these roles in normal host immune responses, the complement system also has pathogenic roles in a variety of ischemic, inflammatory, and autoimmune diseases. Studies have found that HCV also developed strategies for compromising complement system. Hepatits C virus infection has been associated with reduced levels of certain complement component like C3, C4 which is due to HCV viral protein repressing
transcriptional capabilities of complement components (Dumestre-Perard et al: 2002a; Banerjee et al. 2011a; Budhaditya Mazumdar et al. 2012; Mawatari et al. 2013a). It has also been recently found that HCV also incorporates (Regulator of Complement Activation) RCA at levels that provide resistance to antibody-dependent complement-mediated lysis (ADCML). There have been reports that show that direct addition of RCA blockers into plasma samples from patients chronically infected with HCV render endogenous plasma virions sensitive to complement-mediated destruction (Spear et al. 1995; Huber-Lang et al. 2006; Amet et al. 2012).

The important mediator of humoral immunity, the complement system, has received relatively little attention up to now. There has been few reports where it has been stated that polymorphism in complement system components are associated with diseases like Age Related Macular Degeneration (ARMD), atypical Hemolytic Uremic Syndrome (aHUS), Systemic Lupus Erythematosus (Miyagawa et al. 2008). Very recently there have been findings which indicate that complement system component levels are altered in case of an infection and that levels of complement system component correlate to the degree of severity as well as response to treatment. This alteration in complement system component levels has been attributed to the infectious agent’s adroitness to compromise complement system and also due to some host genetic factors. Therefore it becomes very necessary to correctly quantify the complement system components during an infection and to pursue its impact on treatment and disease severity. The objectives of this study are as follows:

- SNP related deficiencies of complementary system components in HCV infected patients and healthy control will be investigated. [C3, C4 & CFH].
Introduction

• Quantification of complementary system components at mRNA level in HCV infected and control samples.

• Determination of HCV genotypes and viral load among HCV infected patients.

• Correlation of complement gene SNP's, complementary system component levels, HCV viral load and genotype with disease progression in HCV infected patients and comparison with controls.