Chapter 1

Introduction

1.1 Background:

JC polyomavirus or John Cunnigham Virus (JCPyV) is a member of the *Polyomaviridae* family and is responsible for a rare fatal brain infection known as Progressive Multifocal Leucoencephalopathy (PML). PML occurs due to immunosuppression resulting from Human Immunodeficiency Virus (HIV) infection, hematological malignancies, organ transplant and recipients of immunosuppressive and immunomodulatory therapy for treatment of inflammatory and autoimmune disease such as multiple sclerosis (MS), rheumatoid arthritis, systemic lupus erythromatous (SLE) and Crohns Disease (Ferenczy *et al.,* 2012). PML involves productive infection in both oligodendrocytes and astrocytes. Histologically, PML is characterized by subcortical demyelinated plaque, enlarged oligodendrocytes containing intranuclear inclusion bodies and bizarre astrocytes. Symptoms include cognitive deterioration, coordination abnormalities, limb paresis and seizure activity.

In 1958, histopathological studies of two patients with progressive dementia, motor dysfunction and vision loss, rapidly followed by death displayed severe demyelination, abnormal oligodendroglial nuclei and giant astrocytes. Both the patients had an underlying lymphoproliferative disorder (Astrom *et al*., 1958). In 1965, papova-like virions in nuclei of injured oligodendrocytes were displayed by electron microscopy (Zu Rhein and Chou, 1965). The isolation of the virus was done in 1971 from brain tissue of a 38-year-old man suffering from Hodgkin's disease and was named JC, after his initials (Padgett *et al*., 1971). Prior to the onset of the HIV-1 pandemic, PML was considered a very rare viral disease (White and Khalili, 2011). PML has also been observed in patients receiving monoclonal antibody such as natalizumab (Langer-Gould *et al*., 2005). Currently there is no effective treatment for PML. The best available therapy is reversal of the immune-deficient state.

Like other polyomaviruses, JCPyV was found to have oncogenic potential in experimental animal models. Viral DNA and proteins have been detected in a wide range of tumours such as gliomas, ependymomas and medulloblastomas, colorectal carcinoma

but their role in human malignancies is still not clear (Burnett-Hartman *et al*., 2008; Delbue *et al*., 2017).

1.2 JCPyV Genomic Organization:

JCPyV is a non-enveloped virus having a diameter of 42 nm, with a circular double-stranded DNA genome of about 5000 base pairs (Frisque *et al.*, 1984). The viral genome comprises early and late coding regions separated by a non-coding control region (NCCR). The early region encodes a large tumour antigen (T-Ag) and small tumour (t-Ag) antigen and various splice variants via alternative splicing of one major pre-mRNA transcripts known as T'135, T'136 and T'165. These genes have important roles in viral DNA replication and late region gene transcription. The late region encodes three capsid proteins VP1, VP2 and VP3 and a small multi-functional protein (Agnoprotein). The viral capsid DNA is composed 360 copies of major capsid protein VP1 molecules assembled into of 72 pentamers and each one of them is associated with one unit of the minor VP2 and VP3 proteins (Jiang *et al*., 2009). The early proximal side of the NCCR is highly conserved and contains the origin of viral DNA replication. The late proximal side of the NCCR contains the repetitive enhancer elements and undergoes rearrangements that account for most of the differences between different strains of the same virus.

Two types of molecular variations are observed in JCPyV: deletion, duplication in the regulatory region of the genome and nucleotide substitutions in the coding region that may lead to amino acids sequence changes (Yogo and Sugimoto, 2001).

1.3 NCCR architecture:

The NCCR architecture has been found to be mainly of two types: archetype and prototype. The naturally occurring variant of the NCCR found in CY strain is termed as 'archetype'. The archetypal strain has rarely been associated with PML and is mainly found in urine and kidney cells. The prototype NCCR is detected in brain, CSF and blood of PML patients and is derived from archetype NCCR during reactivation via rearrangement, deletion and insertion of sequences. The prototype strain is different from

archetype strain because of the presence of a 98-bp tandem repeat resulting in the duplication of TATA-Box and increase in number of transcription factor binding sites.

Replication and transcriptional regulatory proteins of the host cells bind on to the NCCR and determine the course of both virus life cycle and tissue specific expression patterns of the viral proteins. Several transcription factors have been implicated in the regulation of JCPyV gene expression which include NF-κB (Ranganathan and Khalili, 1993), NFAT4 (Wollebo *et al*., 2012), upstream Target or up-TAR (Chowdhury *et al*., 1993), Tst-1 (Wegner *et al*., 1993), Sp-1 (Henson *et al*., 1992), Spi-B (Marshall *et al*., 2010), GBP-i (Raj and Khalili, 1994), Y-box binding protein 1 (YB-1) and Purα (Chen and Khalili, 1995), Nuclear factor 1 or NF-1 (Amemiya *et al*. 1989), CREB/ATF-1 (Lonze and Guinty, 2002), Activator Protein 1 (AP-1) family members (Sadowska *et al*., 2003), p53 (Ariza *et al*., 1994), Early growth response-1 protein or Egr-1 (Romagnoli *et al*., 2008), Bcl-2-associated athano gene-1 or BAG-1 (Devireddy *et al*., 2000) and CAAT/enhancer binding protein beta or C/EBPβ (Romagnoli *et al*., 2009). In most of the cases, binding of host transcription factors activates viral gene expression.

1.4 JCPyV Genotypes:

Nucleotide changes in the 610 bp intergenic (IG) region of JCPyV genome have allowed identification of 12 genotypes EU, Af1, Af2, Af3, B1-a, B1-b, B1-c, B1-d, B2, CY, MY and SC (Sugimoto *et al*., 1997; Guo *et al*., 1998) in different regions of the world. Some genotypes were further subdivided. Eu-a, Eu-b, B1-C are mainly found in Europe and Mediterranean areas, Af2 is spread throughout Africa and West and South Asia, Af1 and Af3 are mainly localized in West and Central Africa. B1-a, B1-b, B1-d, B2, CY, MY and SC are distributed throughout East Asia. In another study, sequencing of full-length JCPyV sequences identified 8 JCPyV types that are numbered 1 through 8, each with multiple subtypes (Cubitt *et al*., 2001). Type 5 was determined to be a minor member of Type 3 (Agostini *et al*., 1997).

JCPyV have been used to study migration of human population. It was hypothesized that Type 6 found in Africa is the original JCPyV type (Pavesi, 2003).

Types 1 and 4 which are closely interrelated to each other have been found mostly in Europe and in some indigenous populations living in Japan, Siberia and Canada. Types 3 and 6 are found in the population of Africa, Type 8 in regions of Papua New Guinea and the Pacific Islands and Types 2, 5 and 7 in Asian population. Type 2D is the Indian subtype (Yanagihara *et al*., 2002; Cui *et al*., 2004).

1.5 JCPyV infection:

Initial infection with JCPyV is found to occur through oral or respiratory routes. Viral protein or DNA have been detected in about 70% to 90% of the human population (Agostini *et al*., 1997; Shackelton *et al*., 2006) throughout the world. The virus can remain latent in organs like kidney and B-lymphocytes until its reactivation (Knowles, 2006).

The JCPyV genome has been detected in various cell types and tissues such as tonsillar stromal cells, B-lymphoid cells, kidney epithelial cells, upper and lower parts of the gastrointestinal tract, urine, brain autopsy and CNS tissues, serum/peripheral blood leukocytes and bone marrow aspirates (Laghi *et al*., 1999; Gu *et al*., 2003; Delbue *et al*., 2008; Tan *et al*., 2009; Husseiny *et al*., 2010). In the immunocompetent individuals, it is rarely found outside the urinary tract (Koralnik *et al*., 1999). However, during severe immunosuppression the virus may establish a lytic infection in the oligodendrocytes which lead to the occurrence of PML in such patients (Koralnik, 2004).

Polymerase Chain Reaction (PCR) is mainly employed these days to identify virus from different types of specimen. Real-time PCR method has also emerged as a new tool for both detection and quantification of the viral DNA (Watzinger *et al*., 2004). It has the ability to identify even small copy numbers of viral DNA (Pal *et al*., 2006).

1.6 Significance of the study:

Reactivation of JCPyV infection may occur in healthy individuals but occurs more frequently under conditions of immunosuppression (Chesters *et al.,* 1983; Gardner *et al.,* 1984). Therefore, the risk of viral reactivation should be considered in individuals

with underlying chronic viral infection during immunosuppressive therapy. Reactivation of JCPyV is also a common event during pregnancy, especially during third trimesters of pregnancy, which may be attributed to changes in immune system (Coleman *et al.,* 1980; Markowitz *et al.,* 1991; Markowitz *et al.,* 1993; Greenlee, 1997). JCPyV have also been detected in urine of immunocompetent older-patients which may be associated to a decline in host-immunity with old age (Kitamura *et al.,* 1990; Chang *et al.,* 2002). Nutritional deficiency might also result in an immunodeficient condition which in turn enhances the susceptibility towards infection and disease (Harbige, 1996).

Study of JCPyV genotypes and variations in it may help in providing an idea about its virulence and its association with disease severity. The NCCRs of different JCPyV isolates differ due to rearrangements in the late proximal region of the NCCR. Analyzing NCCR variants will help in determining the rearrangements that may have a role in pathogenesis. Distribution of JCPyV subtypes has been studied in different regions of the world. Genotyping of JCPyV will help gain an overall picture of the distribution pattern of JCPyV subtypes circulating in this part of India.

The focus of this study was to explore the incidence of JCPyV in both immunocompromised and non-immunocompromised individuals of the sub-Himalayan part of West Bengal. As per available reports, this was the first study to explore the circulating JCPyV NCCR variants in urine, blood and CSF of different population of this region. Genomic variation in NCCR, VP1 and T-Ag regions of the viral isolates were also studied. Quantification of viral load was also done in individuals infected with the virus.

1.7 Objectives of the Study:

- To detect and identify prevalent JCV types from urine and blood of immunocompromised and nonimmunocompromised general population of sub-Himalayan West Bengal.
- To analyse the sequences of Non coding control region (NCCR) and/or Viral Protein(s) of endemic viral isolates from both immunocompromised and nonimmunocompromised population of this region to understand disease potential.
- To quantify the viral DNA load in the urine/blood samples from both immunocompromised and nonimmunocompromised population of sub-Himalayan West Bengal.