

TABLE OF CONTENTS

Content	Page No.
Preface	I
Abstract	III
Acknowledgement	VII
List of Tables	XV
List of Figures	XIX
List of abbreviations	XXIII
Chapter 1: Introduction	
1.1: Background	3
1.2: JCPyV Genomic Organization	4
1.3: NCCR architecture	4
1.4: JCPyV Genotypes	5
1.5: JCPyV infection	6
1.6: Significance of the study	6
1.7: Objectives of the Study	8
Chapter 2: Review of Literature	
2.1: Virus	11
2.2: DNA Virus	12
2.3: Polyomavirus	13
2.3.1: Structure and Genome	15
2.4: Human Polyomavirus	16
2.4.1: Infection in Host Cell	20
2.4.2: Epidemiology	23

2.5: JC Polyomavirus	25
2.5.1: Genome and Structure	26
2.5.2: Types of JC virus based on NCCR architecture	28
2.5.3: JC Virus Genotypes	31
2.5.4: Progressive Multifocal Leucoencephalopathy or PML	32
2.5.4.1: Diagnosis	35
2.5.4.2: Treatment	35
2.5.4.3: Prognosis	36
2.6: JCPyV and its association with Cancer	36
2.7: Detection of JCPyV	39
Chapter 3: Materials and Methods	
3.1: Study Population	45
3.2: Sample Collection	46
3.3: Designing of Oligonucleotides	47
3.4: Viral DNA isolation from urine samples by boiling method	
3.4.1: Materials	49
3.4.2: Methods	49
3.5: Viral DNA isolation from blood samples by Phenol-Chloroform extraction method	
3.5.1: Materials	50
3.5.2: Methods	53
3.6: Viral DNA isolation from urine, blood and CSF samples using high pure viral nucleic acid kit (Roche, Switzerland)	
3.6.1: Materials	54
3.6.2: Methods	54

3.7: Viral DNA isolation from urine, blood and CSF samples using QIAmp DNA mini kit (Qiagen, Germany)	
3.7.1: Materials	55
3.7.2: Methods	55
3.8: Amplification of NCCR	56
3.9: Amplification of T-antigen	57
3.10: Amplification of VP1	58
3.11: Gel Electrophoresis	
3.11.1: Materials	59
3.11.2: Methods	59
3.12: Purification of PCR products	
3.12.1: Materials	60
3.12.2: Methods	60
3.13: Sequencing of positively amplified NCCR, VP1 and T-Antigen and submission of sequences in NCBI	61
3.14: Quantification of Viral Load by Real Time PCR	61
3.15: Statistical analysis by SPSS	62
3.16: Multiple sequence alignment using CLUSTAL X	63
3.17: Pairwise alignment using Genomatix	63
3.18: Transcription factor binding site prediction in NCCR by Genomatix	64
3.19: Phylogenetic analysis based on VP1 region of JC virus	64
3.20: Phylogenetic analysis based on T-antigen region of JC virus	65
Chapter 4: Results and Discussion	
4.1: Amplification of NCCR, VP1 and T-Ag	69
4.2: Prevalence of JC virus	72

4.3: Sequences submission in GenBank with their accession numbers	78
4.4: Pairwise and Multiple sequence alignment of NCCR sequence	89
4.5 Transcription factor binding analyses of NCCR sequences	96
4.6: Pairwise and Multiple sequence alignment of VP1	109
4.7: Pairwise and Multiple sequence alignment of T-Ag sequences	115
4.8: Phylogenetic analyses based on VP1 and T-Antigen	120
4.9: Viral load estimation by real time PCR	128
Chapter 5: Comprehensive Discussion	
5.1: JCPyV prevalence in the sub Himalayan part of West Bengal	135
5.2: NCCR Architecture among the endemic JCPyV strains	137
5.3: JC virus genotypes based on viral protein VP1 and T-Ag Sequences	140
Chapter 6: Summary and Conclusion	145
Bibliography	149
Index	193
Appendices	
Appendix A: List of Publications	197
Appendix B: Seminar and Conferences	198
Appendix C: Institutional Ethical Committee	199
Appendix D: Consent Form	201