Chapter 1: Introduction and Review of Literature

1.1 INTRODUCTION

1.2 REVIEW OF LITERATURE
1.2.1 Incidence of cervical cancer
1.2.2 Natural History of Cervical Cancer
1.2.3 Etiology of cervical cancer
   1.2.3.1 Human papillomavirus (HPV) Infection
      1.2.3.1.1 HPV Genome
      1.2.3.1.2 Viral life cycle
      1.2.3.1.3 Molecular Pathophysiology of HPV infection-
                     Role of E6 and E7
      1.2.3.1.4 HPV types
         Methods for HPV detection and genotyping
         Incidence and prevalence of different HPV types in cervical cancer
      1.2.3.1.5 Viral load
      1.2.3.1.6 Viral integration
         Methods of detecting HR-HPV integration
         Significance of viral integration
         ‘Hotspots’ of viral integration - whether a random event
   1.2.3.2 Genetic Alterations
      Next generation sequencing - a new face of cancer research
   1.2.4 Molecular biomarkers in cervical cancer – from diagnosis to prognosis
Chapter 2: Determination of HPV status in cervical cancer biopsies

2.1 INTRODUCTION

2.2 MATERIALS AND METHODS

2.2.1 Clinical Sample accrual

2.2.2 Processing of tumour samples

2.2.3 Genomic DNA isolation

2.2.4 Agarose gel electrophoresis

2.2.5 Genotyping of HPV

2.2.5.1 Genotyping of HPV by high throughput Luminex assay

2.2.5.2 Genotyping of HPV by using MY09/11 and SPF1/2 primers

2.2.6 Statistical analysis

2.3 RESULTS

2.3.1 Genotyping of HPV by high throughput Luminex assay

2.3.2 Genotyping of HPV by using MY09/11 and SPF1/2 primers

2.3.3 Correlation with clinical outcome

2.4 DISCUSSION

2.5 CONCLUSION

Chapter 3: Identification of integration site of the virus by Amplification of Papillomavirus Oncogene Transcripts (APOT) assay

3.1 INTRODUCTION

3.2 MATERIALS AND METHODS

3.2.1 RNA extraction from cervical cancer biopsies

3.2.2 Formaldehyde agarose gel electrophoresis of RNA
Table of Contents

3.2.3 DNase treatment of RNA 81
3.2.4 Amplification of Papillomavirus Oncogene Transcripts (APOT) 82
3.2.5 Validation of the recurrent integration sites 87
3.2.6 Estimation of viral load by qRT-PCR 87
3.2.7 Association with clinical data 89

3.3 RESULTS
3.3.1 Determination of physical state of the virus by APOT assay 89
3.3.2 Association of physical state of the virus with clinical outcome 90
3.3.3 Identification of viral integration sites in the genome 91
3.3.4 Validation of the recurrent integration sites 92
3.3.5 Association of site of viral integration with clinical outcome 93
3.3.6 Features associated with HPV integration 94
3.3.7 Determination of viral load by quantitative qRT-PCR 96
3.3.8 Physical state of the virus, viral load and clinical outcome 98

3.4 DISCUSSION 99
3.5 CONCLUSION 104

Chapter 4: Identification of genomic alterations in cervical cancer biopsies by exome sequencing 105-129

4.1 INTRODUCTION 106
4.2 MATERIALS AND METHODS
4.2.1 Isolation of genomic DNA by DNA Mini Kit 108
4.2.2 Exome capture and sequencing 108
4.2.3 Analysis for identification of single nucleotide variations and indels 109
4.2.4 Validation of the data by Sanger sequencing 109
4.2.5 Validation of the data by Customized SNP Array 110
4.2.6 Analysis for detection of Copy Number Variation (CNV) and Loss of Heterozygosity (LOH) 111
4.2.7 Validation of copy number variation by qRT-PCR 111
4.3 RESULTS

Dataset-I

4.3.1 Exome capture, sequencing and analysis

4.3.2 Screening for identical variations from the OMIM, HGMD and COSMIC databases

4.3.3 Validation of the data by Sanger sequencing

4.3.3 Validation of the data by Customized SNP Array

4.3.4 CNV and LOH identification

4.3.5 Validation of copy number variation by qRT-PCR

Dataset-II

4.3.4 Exome capture, sequencing and analysis

4.3.5 Validation of the data by Sanger sequencing

4.4 DISCUSSION

4.5 CONCLUSION

Chapter- 5: General discussion and study perspective

Chapter- 6: Summary and conclusion

Bibliography

Appendix I

Appendix II

Reprints of published articles