# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>List of Tables</td>
<td>i-x</td>
</tr>
<tr>
<td>List of Figures</td>
<td>xi-xvi</td>
</tr>
<tr>
<td>Abbreviations</td>
<td>xvii-xxii</td>
</tr>
<tr>
<td><strong>CHAPTER 1 – INTRODUCTION</strong></td>
<td>1-7</td>
</tr>
<tr>
<td>1.1 Aims and Objectives</td>
<td>7</td>
</tr>
<tr>
<td><strong>CHAPTER 2 - REVIEW OF LITERATURE</strong></td>
<td>8-93</td>
</tr>
<tr>
<td>2.1 Epidemiology and Etiology</td>
<td>9</td>
</tr>
<tr>
<td>2.1.1 The global picture of cancer</td>
<td>9</td>
</tr>
<tr>
<td>2.1.2 Worldwide incidence of cervical cancer</td>
<td>10</td>
</tr>
<tr>
<td>2.1.3. Cervical cancer : Indian perspective</td>
<td>13</td>
</tr>
<tr>
<td>2.2 Cervical cancer</td>
<td>16</td>
</tr>
<tr>
<td>2.2.1 Histology of cervical cancer</td>
<td>17</td>
</tr>
<tr>
<td>2.2.2 Causes and Risk Factors</td>
<td>17</td>
</tr>
<tr>
<td>2.2.2.1 HPV Infection</td>
<td>17</td>
</tr>
<tr>
<td>2.2.2.1.1 Morphology</td>
<td>18</td>
</tr>
<tr>
<td>2.2.2.1.2 Life cycle of HPV</td>
<td>20</td>
</tr>
<tr>
<td>2.2.2.1.3 Molecular pathophysiology in cervical cancer</td>
<td>22</td>
</tr>
<tr>
<td>(I) HPV E7 and retinoblastoma protein</td>
<td>22</td>
</tr>
<tr>
<td>(II) HPV E6 and p53</td>
<td>23</td>
</tr>
<tr>
<td>2.2.2.2 Co-factors of cervical cancer</td>
<td>24</td>
</tr>
<tr>
<td>2.2.2.2.1 Smoking</td>
<td>25</td>
</tr>
<tr>
<td>2.2.2.2.2 Sexually transmitted diseases</td>
<td>26</td>
</tr>
<tr>
<td>(a) Chlamydia</td>
<td>26</td>
</tr>
<tr>
<td>(b) Human immunodeficiency virus (HIV)</td>
<td>26</td>
</tr>
<tr>
<td>2.2.2.3 Oral contraceptives</td>
<td>27</td>
</tr>
<tr>
<td>2.2.2.4 Low socioeconomic status</td>
<td>28</td>
</tr>
<tr>
<td>2.2.2.5 Age at sexual intercourse</td>
<td>28</td>
</tr>
<tr>
<td>2.2.2.6 Sexual history of the woman’s male partners</td>
<td>29</td>
</tr>
<tr>
<td>2.2.2.7 Multiple pregnancies</td>
<td>29</td>
</tr>
<tr>
<td>2.2.2.8 Deficient diet</td>
<td>29</td>
</tr>
<tr>
<td>2.2.2.9 Family history of cervical cancer</td>
<td>30</td>
</tr>
<tr>
<td>2.2.2.10 Age</td>
<td>30</td>
</tr>
<tr>
<td>2.2.3 Stages of Cervical Cancer</td>
<td>30</td>
</tr>
</tbody>
</table>
2.3 The Molecular Pathology of Cancer
   2.3.1 Oncogenes
   2.3.2 Tumor Suppressor Genes
2.4. Apoptosis
   2.4.1 Pathways
   2.4.2 Apoptotic Dysfunction as a Cause of Cancer
2.5. DNA Repair Regulation
2.6. Epigenetics
   2.6.1 Possible mechanisms of epigenetic inheritance
      2.6.1.1 DNA methylation- the black box of epigenetics.
      2.6.1.2 DNA Methyltransferases
      2.6.1.3 Methyl-CpG Binding Domain Proteins (MBDs)
      2.6.1.4 Global genomic hypomethylation
   2.6.1.3 Histone Modifications
      2.6.1.3.1 The Chromatin Architecture
      2.6.1.3.2 Histone Methylation
      2.6.1.3.3 Histone Acetylation
      2.6.1.3.4 Histone Deacetylation
      2.6.1.3.5 Histone Phosphorylation
      2.6.1.3.6 Histone Ubiquitination
      2.6.1.3.7 Sumoylation
   2.6.2 Chromatin remodeling
   2.6.3 Interplay between DNA methylation and Histone modifications
2.7 Cell Cycle Regulation
   2.7.1 Cyclin Dependent Kinase Inhibitors
2.8 Multistep Molecular Carcinogenesis
2.9 Structure and function of candidate Tumor suppressor genes
   2.9.1 ARF (Alternative Reading Frame) tumor suppressor.
   2.9.2 The INK4 Family
   2.9.3 The CIP/KIP CDKI Family
   2.9.4 The p53-family
      2.9.4.1 p53 and Cancer
      2.9.4.2 p73 gene
   2.9.5 RARβ2 gene
   2.9.6 FHIT gene
      2.9.6.1 FHIT mutations, aberrant transcripts and hypermethylation
   2.9.7 Rb and cancer
      2.9.7.1 RB tumor suppressor gene
      2.9.7.2 The Rb family
3.15 Complementary DNA synthesis 112
3.16 Determination of relative level of gene expression 113
  3.16.1 Semi-quantitative RT-PCR (Reverse Transcription PCR) 113
  3.16.2 Real-time PCR (RT-PCR) 113
3.17 Statistical analysis 116
3.18 Cell lines 117
  3.18.1 Cell Culture 117
    3.18.1.1 Preparation of RPMI-1640 117
    3.18.1.2 Trypsinization of adherent cells 117
  3.18.2 Screening of dietary compounds and plant extracts for their potential to cause reversal of promoter hypermethylation using HeLa and SiHa cervical cancer cell lines 118
    3.18.2.1 Preparation of plant extracts 118
    3.18.2.2 Preparation of stock solutions of the test compounds and plant extracts 118
    3.18.2.3 Cell viability assay 118
    3.18.2.4 Morphological changes 119
    3.18.2.5 DNA extraction from Adherent Cells 119
    3.18.2.6 DNA fragmentation assay 120
    3.18.2.7 Reversal of epigenetic changes 121
    3.18.2.8 Reactivation of methylated tumor suppressor genes 121

CHAPTER 4 – RESULTS 122-228
4.1 Epidemiological characteristics 122
4.2 HPV infection and HPV-16 typing 123
4.3 Hypermethylation of Tumor Suppressor Genes in Patients 125
  4.3.1 Status of promoter hypermethylation of p14ARF and INK family 125
    4.3.1.1 p14ARF 125
      4.3.1.1.1 Bisulfite sequencing of p14ARF 129
      4.3.1.1.2 Effect of promoter hypermethylation of p14ARF on gene expression 129
      4.3.1.2 Semi-Quantitative RT-PCR 129
      4.3.1.2.1 Semi-Quantitative RT-PCR 129
      4.3.1.2.2 Quantitative RT-PCR 129
    4.3.1.2 p15INK4b 130
      4.3.1.2.1 Bisulfite sequencing of p15INK4b 133
      4.3.1.2.2 Effect of promoter methylation of p15INK4b on gene expression 134
      4.3.1.2.2.1 Semi-Quantitative RT-PCR 134
      4.3.1.2.2.2 Quantitative RT-PCR 134
    4.3.1.3 p16^INK4a 135
      4.3.1.3.1 Bisulfite sequencing of p16^INK4a 138
      4.3.1.3.2 Study on the effect of promoter methylation of p16^INK4a on gene expression 138
      4.3.1.3.2.1 Semi-Quantitative RT-PCR 138
      4.3.1.3.2.2 Quantitative RT-PCR 138
4.3.1.4 Cumulative effect of hypermethylation of p14ARF and INK family

4.3.2 Study of the methylation status of CIP/KIP family

4.3.2.1 p21CIP1
4.3.2.1.1 Bisulfite sequencing of p21CIP1
4.3.2.1.2 Study on effect of promoter methylation of p21CIP1 on gene expression
4.3.2.1.2.1 Semi-Quantitative RT-PCR
4.3.2.2 p27KIP1
4.3.2.2.1 Bisulfite sequencing of p27KIP1
4.3.2.2.2 Study on effect of promoter methylation of p27KIP1 on gene expression
4.3.2.2.2.1 Semi-Quantitative RT-PCR
4.3.2.3 p57KIP2
4.3.2.3.1 Bisulfite sequencing of p57KIP2
4.3.2.3.2 Study on effect of promoter methylation of p57KIP2 on gene expression
4.3.2.3.2.1 Semi-Quantitative RT-PCR
4.3.2.3.2.2 Quantitative RT-PCR
4.3.2.4 Cumulative effect of hypermethylation of genes of CIP/KIP family

4.3.3 p53 and p73 genes

4.3.3.1 p53
4.3.3.2 p73
4.3.3.2.1 Bisulfite sequencing of p73
4.3.3.2.2 Study on effect of promoter methylation of p73 on gene expression
4.3.3.2.2.1 Semi-Quantitative RT-PCR
4.3.3.2.2.2 Quantitative RT-PCR
4.3.3.3 Cumulative effect of hypermethylation of p53 and p73 gene

4.3.4 RARβ2
4.3.4.1 Bisulfite sequencing of RARβ2
4.3.4.2 Study on effect of promoter methylation of RARβ2 on gene expression
4.3.4.2.1 Semi-Quantitative RT-PCR
4.3.4.2.2 Quantitative RT-PCR

4.3.5 FHIT
4.3.5.1 Bisulfite sequencing of FHIT
4.3.5.2 Study on effect of promoter methylation of FHIT on gene expression
4.3.5.2.1 Semi-Quantitative RT-PCR
4.3.5.2.2 Quantitative RT-PCR

4.3.6 RB1
4.3.6.1 Bisulfite sequencing of RB1
4.3.6.2 Study on effect of promoter methylation of RB1 on gene expression
4.3.6.2.1 Semi-Quantitative RT-PCR

4.3.7. STAT1
4.3.7.1 Study on effect of promoter methylation of STAT1 on gene expression
4.3.7.1.1 Semi-Quantitative RT-PCR

4.3.8. DAPK
4.3.8.1 Bisulfite sequencing of DAPK
4.3.8.2 Study on effect of promoter methylation of DAPK expression
4.3.8.2.1 Semi-Quantitative RT-PCR
4.3.8.2.2 Quantitative RT-PCR

4.4 Correlation of methylation status of the tumor suppressor genes with the stage of cervical cancer

4.5 Methylation status of tumor suppressor genes in paired biopsy and serum samples of cervical cancer patients
4.5.1 Methylation status of tumor suppressor genes in serum samples from different stages of cervical cancer

4.6 Screening of natural compounds for their potential to cause reversal of promoter hypermethylation and reactivation of RARβ2 in cervical cancer cell lines
4.6.1 MTT Assay
4.6.2 Morphological changes in SiHa and HeLa cell lines after treatment with test compounds and plant extracts
4.6.3 DNA Fragmentation Assay
4.6.4 Reversal of promoter hypermethylation of RARβ2 gene in cervical cancer cell lines using natural compounds
4.6.5 Reactivation of RARβ2 gene caused by reversal of hypermethylation by the dietary factors

CHAPTER 5 – DISCUSSION

5.1 Promoter hypermethylation of tumor suppressor genes in cervical cancer
5.1.1 p14ARF
5.1.2 p15INK4b
5.1.3 p16INK4a
5.1.4 Cumulative effect of hypermethylation of p14ARF and INK family
5.1.5 p21CIP1
5.1.6 p27KIP1
5.1.7 p57KIP2
5.1.8 Cumulative effect of hypermethylation of genes of CIP/KIP family
5.1.9 p53
5.1.10 p73
5.1.11 Cumulative effect of hypermethylation of p53 and p73 genes 248
5.1.12 RARβ2 249
5.1.13 FHIT 251
5.1.14 RB1 253
5.1.15 STAT1 254
5.1.16 DAPK 255
5.1.17 Correlation of the Methylation status of the genes and the stage of cervical cancer 257

5.2 Comparison of methylation status of tumor suppressor genes in paired biopsy and serum samples 259
5.3 Reversal of promoter hypermethylation of RARβ2 gene in cervical cancer cell lines 260

CHAPTER 6 - SUMMARY AND CONCLUSIONS 267-269

CHAPTER 7 – REFERENCES 270-310