CHAPTER - I

INTRODUCTION

The growing cancer burden calls for greater investment in health facilities specific to cancer treatment, early diagnosis and for preventive strategies. The steadily increasing trend of cancer incidence gains attention on the key step called "carcinogenesis" for an extensive research. Although research on cancer has been going on for a long time, no solution seems to be in sight because cancer is not a "single disease of a single causative factor". It exists in more than 100 forms and has many causative agents from genetic factors to infectious agents (Morris et al., 1995). No single genetic alteration is sufficient for the induction of cancers, in vivo. A tumour is an abnormal mass of tissue, the growth of which is virtually autonomous and exceeds that of normal tissues. In contrast to non-neoplastic proliferations, the growth of tumours persists after cessation of the stimuli that initiated the change. Malignant tumours are often called cancers and those which arise from epithelial cells are "carcinomas".

It has been realized for many years that cancer can be considered as a "genetic disease" and the genetic injury may be acquired in somatic cells by environmental agents or inherited in the germ line. Cancers develop as clonal progeny of a single genetically damaged progenitor cell and is said to be a "monoclonal growth". Four classes of genes are the targets of genetic damage and they are growth-promoting proto-
oncogenes, growth-inhibiting tumour-suppressor genes, genes that regulate apoptosis and genes that regulate DNA repair. It is only in recent years that the involvement of specific proto-oncogenes in the development of cancer has been demonstrated at the molecular level (Koshida et al., 1997; Lakshmi et al., 1997; Radhakrishna Pillai et al., 1999). The proteins encoded by these proto-oncogenes are known to be the components of cell cycle and cell signalling pathways (Diebold et al., 1996; Sidransky and Hollstein, 1996; Yang and Korsmeyer, 1996).

Carcinogenesis is a multistep process. Malignancy is attributed to invasiveness, excessive growth, escape from the immune system and metastasis and occurs in a stepwise fashion-a process called tumour progression. At the genetic level, progression results from successive mutations or persistent exposure to causative agents (Fearon and Vogelstein, 1990; Ho et al., 1998a,b,c; Ruche et al., 1998). Multiple controls exerted by all the three above-said categories of genes, such as oncogenes, tumour suppressor genes and apoptosis regulating genes, must be lost for the emergence of cancer cells (Aejaz Syeed et al., 2001).

Squamous Cell Carcinoma (SCC) of the Uterine Cervix

Squamous cell carcinoma (SCC) of the uterine cervix is the most frequent among all the genital tract cancers and is one of the most common malignancies affecting women worldwide especially in the developing countries (Shanta et al., 2000). This cancer primarily arises from continuously progressing premalignant lesions called dysplasia (Hines et al., 1995). SCC of the cervix is more amenable for screening
than any other cancer. This is principally because of the existence of a prolonged detectable pre-clinical phase (DPCP), lasting many years in the majority of cases (Miller, 1999).

Cancer of the cervix is of two major types. One is adenocarcinoma which arises from the mucous membrane of the cervical canal and is less common (30%). The other is SCC which arises from the squamous epithelium covering the vaginal portion of the cervix. Most cervical cancers are squamous in type (70%); (Buckley, 1994; Smith et al., 2000) and Epidemiological studies reveal multiple etiological factors including HPV infection which is being implicated as a promoter of cervical carcinoma. All studies on cervical cancer, however, had one common factor viz, sexual activity, the most specific risk factor for cervical carcinogenesis (Shanta et al., 2000).

**Epidemiology**

Globally, cancer is considered as a third major cause of death (12%) after infectious diseases and diseases of circulatory system (WHO, 1997). In India, cancer is a major health problem and about 7 lakh new cases are reported every year and probably 3-3.5 lakhs of people die every year because of cancer (Patel, 1999). Cervical cancer is predominantly a disease of developing countries because of the different distribution of environmental and cultural factors associated with risk of cervical cancer, facilities for screening, early detection and management. About 3,50,000 new cases are identified in developing countries each year and fewer than 100,000 in developed countries (Parkin et al., 1993). The
cervical cancer burden in India alone is estimated as 100,000 in 2001 AD (Shanta et al., 2000).

**International Status of Cervical Cancer**

Cancer of the uterine cervix is the seventh most common cancer in the world when both the sexes are taken into account (Krishnan Nair, 1999). It is also important to note that cervical cancer is the second most common cancer among women worldwide with an estimated 5,24,000 cases in 1995 (WHO, 1997). Globally, the annual estimated number of new cases is 3,71,200 which accounts for 10% of all cancers diagnosed in women. Among which, 80% of them are in developing countries whereas 20% of them are in developed countries (Misra et al., 1997). The highest incidence is seen in sub-Saharan Africa and Central America. India, Pakistan and most of the African countries have an incidence between 20 and 30 per 100,000. Most of the developed countries, China and the Middle East have a low incidence, less than 10 per 100,000. The trends in cervical cancer mortality in the world show a declining trend in all the 36 selected countries. In developed countries the decline in mortality has been substantial and is predominantly due to an organized screening program (Krishnan Nair, 1999). Cervical cancer is responsible for 190,000 deaths annually and about 78% of which is in developing countries, where it is the leading cause of cancer mortality in women (Pisani et al., 1999).
Cervical Cancer Status in India

In India, cervical cancer ranks first among women although it is the second most common malignancy among women in the world, next to breast cancer (National Cancer Registry Programme, 1992). Within the country, the highest incidence of cervical cancer is found in Chennai (AAR:37.7) (Shanta et al., 2000). The crude incidence rate (CIR) of cervical cancer during 1996-1997 was estimated as 22.9 / 100,000. However, cervical cancer ranks second among female cancers in certain areas such as Mumbai, Delhi and Trivandrum during the years 1988 to 1992. The age adjusted incidence of cervical cancer rates in India is the lowest in Trivandrum and highest in Chennai (Fig.1). The CIR of cervical cancer varied between 11.5 / 100,000 women (Trivandrum) to 27.7/100,000 women (Chennai) (cancer incidence in five continents 1988-1992 : IARC, 1995) and is shown in Fig.1. It is the most common cancer among women in India, accounting for about 26% of female cancers and resulting in about 100,000 women developing the disease annually (Ramalingaswamy, 1999).

Cervical Cancer Status in Chennai

Chennai is one of the areas with the highest incidence of cervical cancer in the world, as well as in India, especially in the age group of 35-64 years. A very recent report has shown a CIR of 84.2 / 100,000 in Chennai from Registered cases (National Cancer Registery Programme, 2000). However a slow, but steady decline in the incidence of cervical cancer has been observed in Chennai since 1986 (Cancer incidence, 1995).
Fig. 1 Incidence Rate of Cervical Cancer in India (1988-92)

It is also noted that the survival from cervical cancer in Chennai is the highest reported in India (Nandakumar et al., 1995; Sankaranarayanan et al., 1995; Jayant et al., 1996).

**Clinicopathology of Uterine Cervix**

In cervix the carcinoma arises from the squamous epithelium covering the vaginal portion of the cervix. But adenocarcinoma arises from the mucous membrane of the cervical canal. The preponderance of squamous cancers is in part explained by the tendency of the endocervical epithelium to undergo squamous metaplasia and this tendency increases with age. Squamous cell carcinoma of the cervix assumes one of the 3 clinical types. One form is proliferating cauliflower-like growth (or) exophytic growth which projects into the vagina. These are vascular and produce profuse vaginal bleeding and because of infection and necrosis, lead to an offensive vaginal discharge. The second form is an excavating or endophytic form. It does not cause such profuse haemorrhage, the main symptom being blood stained discharge. The third form is a raised flat induration. (Howkins and Bourne, 1994). The clinical pictures of different stages of SCC of the uterine cervix and normal cervix are given in Fig 2.

**Histopathologic History of Cervical Cancer**

Cancer of the uterine cervix arises from precursor lesions. The precancerous lesions are microscopic and rise within the "transformation zone" which is the squamo-columnar junction. The
a. The gross anatomy of the normal uterus of a postmenopausal woman with the cervix indicated by arrow.
b. Progression of cervical dysplasia to carcinoma.
c. Fungating, stage I squamous cell carcinoma limited to the cervix.
d. Advanced squamous cell carcinoma (Stage IV) infiltrating into vagina.
reserve cells lying beneath the columnar epithelium at the squamo-columnar junction form the metaplastic cells in the transformation zone. Metaplastic cells are usually transformed into mature epithelium, but in some instances, in response to the relative acidity of the vagina, the columnar epithelium is replaced by squamous epithelium as a result of reserve cell proliferation in a process called squamous metaplasia which is atypical and may progress to dysplasia and cervical cancer. The schematic representation of natural history of cervical cancer development and progression is given in Fig.3. It is thought to occur from genetic reprogramming of stem cells known to exist in most epithelia or the persistant exposure of metaplasia to etiological factors. Atypical metaplasia is reversible when the underlying stimulus abates. Cancer cells are undifferentiated in most of the malignant growths and in the mature forms there is a tendency to develop epithelial pearls (Howkins and Bourne, 1994).

**Dysplasia**

Dysplasia represents a change causing an alternation and disorderly arrangement of the undifferentiated basal cells of the stratified squamous epithelium. It is a microscopic non-neoplastic growth characterized by pleomorphism, hyperchromatism and loss of normal orientation. Precancerous lesions are classified according to the degree of epithelial maturation and the distribution of cytologic atypia as cervical intraepithelial neoplasias (CIN), which is divided into three grades as CIN I, II & III.
Fig 3. The Schematic representation of the natural history of cervical dysplasia in the transformation zone of cervix
Invasive Cervical Carcinoma

Invasive cancer is macroscopic, neoplastic growth, manifests itself in three gross morphologic patterns: exophytic or fungating, ulcerating and infiltrative. Histologically, 65% of tumours are large-cell, non-keratinizing and moderately well-differentiated, whereas 25% are large and keratinizing and the rest are composed of small undifferentiated squamous cells. This stage is the clinically observable stage. The Bethesda system of classification is given in Table 1 and the clinical staging based on International Federation of Gynecology and Obstetrics (FIGO) is given in Table 2.

Etiology

Cervical cancer is a multifactorial disease. Epidemiological studies have clearly indicated that both HPV infection and cervical cancer are strongly influenced by measures of sexual activity, in the woman and her partner (IARC Working Group, 1995). The following risk factors are considered as causative agents for cervical cancer at different degrees. The recent increase in incidence has been ascribed to a number of factors including changing sexual partners (Anttila et al., 1999).

Risk factors

1. Socio-demographic factors
2. Sexual determinants
   a) Early age at first intercourse
<table>
<thead>
<tr>
<th>Group</th>
<th>Sub group</th>
<th>Epithelial changes</th>
<th>Characteristic features</th>
<th>Cellular changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopic stages</td>
<td></td>
<td>Undifferentiated cells confined to the lower one third of the epithelium</td>
<td>Nuclear abnormalities seen in cells of deeper layers are, pleomorphism, increase in nuclear:cytoplasmic ratio and large irregular nucleus</td>
<td>Cells have ill-defined boundaries with large nuclei and scanty cytoplasm</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>Mild dysplasia (CIN I &amp; Low grade SIL)</td>
<td>Undifferentiated cells occupy more than one third of the total thickness</td>
<td>Undifferentiated cells occupy more than two third of the total thickness of the epithelium</td>
<td>Cells have ill-defined boundaries with large nuclei and scanty cytoplasm</td>
</tr>
<tr>
<td></td>
<td>Moderate dysplasia (CIN II &amp; high grade SIL)</td>
<td>Undifferentiated cells occupy more than two third of the total thickness of the epithelium</td>
<td>Dysplastic changes occupy the entire thickness of the epithelium. No surface stratification and basement membrane is intact with no infiltration</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Severe dysplasia (high grade SIL &amp; CIN III)</td>
<td></td>
<td></td>
<td>All the cellular changes of dysplasia are seen and are even more vigorous. Polynuclei is prominent.</td>
</tr>
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<td></td>
<td>Carcinoma <em>In situ</em> (preinvasive stage)</td>
<td></td>
<td></td>
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<tr>
<td>Group</td>
<td>Subdivision</td>
<td>Characteristic features</td>
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<tr>
<td><strong>Macroscopic stage</strong>&lt;br&gt;(Clinically observable)</td>
<td></td>
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<tr>
<td>Invasive cancer&lt;br&gt;(Malignant Neoplasm)</td>
<td><strong>Local Invasion</strong>&lt;br&gt;<strong>Forms of tumor growth</strong>&lt;br&gt;1. Exophytic or fungating&lt;br&gt;(Cauliflower growth)&lt;br&gt;2. Ulcerating growth&lt;br&gt;3. Infiltrating growth</td>
<td>Local infiltration occurs and destroys the normal tissue surrounding them. But do not penetrate the capsule on the surrounding normal tissues</td>
<td></td>
<td></td>
</tr>
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<td></td>
<td><strong>Metastasis</strong></td>
<td>It is a distinguishing feature of malignant tumors. It involves invasion of the lymphatics, blood vessels and body cavities, followed by transport and growth of secondary tumor, which is discontinuous with primary tumor.</td>
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<td></td>
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Table 2: The clinical staging based on FIGO classification

<table>
<thead>
<tr>
<th>Stage</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Carcinoma <em>In situ</em></td>
</tr>
<tr>
<td>I</td>
<td>Carcinoma confined to the cervix</td>
</tr>
<tr>
<td>IA</td>
<td>Preclinical carcinoma diagnosed only by microscopy</td>
</tr>
<tr>
<td>IB</td>
<td>Histological invasive carcinoma greater than 5 mm in depth</td>
</tr>
<tr>
<td>II</td>
<td>Carcinoma extends beyond the cervix but not into the pelvic wall; into the vagina but not the lower third.</td>
</tr>
<tr>
<td>III</td>
<td>Carcinoma extends to the pelvic wall or lower third of the vagina.</td>
</tr>
<tr>
<td>IV</td>
<td>Carcinoma has extended beyond the pelvis</td>
</tr>
</tbody>
</table>
b) High frequency of intercourse

c) Multiparity

3. Human Papilloma Virus Infection (HPV)

4. Infectious agents other than HPV
   a) Herpes simplex virus (HSV-2)
   b) Bacterial vaginosis (BV)
   c) Gonococcal

5. Immunosuppression

6. Tobacco use

7. Exposure to oral contraceptives

8. Poor educational status

9. Poor hygiene of the female as well as her sexual partner

10. Nutritional deficiencies

**Symptoms of cervical cancer**

When invasive disease is established, symptoms include vaginal bleeding or blood-stained discharge, pelvic pain and eventually rectal and urinary complications. Four main symptoms are haemorrhage, discharge, cachexia and pain. The irregular haemorrhage and blood-stained vaginal discharge are the most important early symptoms. Vaginal bleeding and watery vaginal discharge in women of post-menopausal age should also be suspected. Cachexia is well marked in advanced growth. The woman is anaemic and shows symptoms and signs of incipient uraemia, with loss of appetite, headaches and sickness. Weight loss is obvious and sometimes extreme. Pain in the knee and along the inner side of the
thigh are the late symptoms. If the bladder is infiltrated with growth, urinary symptoms and pelvic pain develop. Symptoms that arise in late cases are painful and frequent micturition, incontinence of urine, painful defaecation and obstruction (Howkins and Bourne, 1994).

**Importance of this study**

The cause of cancer is not only a consequence of uncontrolled cell growth but may also be due to loss of growth suppressing activities. One of the most common and fatal malignancies in women worldwide is cervical cancer. Substantial evidence has been gathered linking cervical cancer with certain genotypes of HPV.

This study would assess the factors associated with premalignant stages of cervical cancer such as cervical intraepithelial lesions (CIN I, II and III), grouped as dysplasias and invasive cervical cancer, with special attention to HPV infection. The association of HPV infection with the occurrence of cervical neoplasia, in particular high-risk HPV types 16 and 18 is now clearly established world-wide but the prevalence of HPV subtypes may vary with geographic variation (Bosch et al., 1995). The role of HPV subtypes in the progression of cervical cancer is poorly understood. Therefore, an attempt was made to study the prevalence of HPV subtypes, in Chennai, India, and its significant role during the progression of cervical cancer from dysplastic stage to invasive cancer.

Apoptosis and cell proliferation are two factors which are critical in tissue homeostasis (Reed, 1994). The disruption in the homeostatic
control accounts for the basic molecular mechanism involved in carcinogenesis (Bravo et al., 1987). This implies that the HPV may disrupt this homeostatic balance in order to establish its effect which may lead to cervical cancer. An understanding of the mechanism of the interaction between HPV and host factors is still incomplete (Vassallo et al., 2000). Studies are therefore needed on apoptosis regulatory factors and cell proliferation factors in relation with HPV infection in order to get a better understanding of the mechanism. Therefore, this study was aimed at understanding the mechanism of HPV-associated cervical carcinogenesis and the involvement of other oncogenic factors in the development of cervical cancer along with HPV infection.