Review of Literature
Cervical cancer is an important health problem, being the second most common cancer among women worldwide and first in many developing countries (Parkin, 1992). Disease occurrence varies widely by geographic region with some of the highest rates in the world having been reported from Latin American countries, parts of Asia and Eastern Europe (Potishman and Brinton, 1996). Utilization of regular cytologic (i.e., Papanicolaou or PAP) screening has been shown to reduce effectively the incidence of cervical cancer. In the United States, cervical cancer has a well recognized pattern with ethnicity and socioeconomic status, where high rates have been reported for black and Hispanic women and those with lower educational status and lower incomes.

Cervical cancer occurs in a continuum from dysplasia to carcinoma in situ to invasive cancer. Dysplasia is a microscopic lesion and literally means disordered growth or development. In cervical dysplasia, part of epithelium shows abnormal and rest of the epithelial layers show normal maturation. However, if all the layers show abnormal growth, it is called cervical intraepithelial neoplasia (CIN). Dysplasia may be categorized into mild, moderate or marked depending on the number of layers showing neoplastic changes. Dysplasia and CIN represent a series of changes, which if untreated over a period of many years, may lead to invasive cancer of the cervix.

The transformation of endocervical columnar epithelium to squamous epithelium is a normal physiologic and dynamic process occurring throughout women's reproductive years. This process is influenced by changes in local hormonal milieu and pH. Ectocervix is covered by stratified squamous epithelium which is multilayered. The maturation of the cells progresses from basal layers towards the surface. In cervical neoplasms, the maturation of cells does not occur in an ordered manner.
ETIOLOGY

Cervical cancer exhibits many of the epidemiological features of sexually transmitted diseases. The etiology of cervical cancer is still the subject of debate. It has been found that cervical cancer is more prevalent in women belonging to lower socioeconomic background. Another factor which is strongly related to cervical cancer is sexual behaviour (Franco, 1991; Joffe et al., 1992). Various factors like women's age of marriage, age of commencement of sexual activity, number of sexual partners are thought to be major risk factors (Jones et al., 1958; Boyd et al., 1964).

The wives of the men who have multiple sexual partners are at high risk of developing cervical cancer (Bosch et al., 1995; 1996). Epidemiological studies have also implicated cigarette smoking as a possible risk factor in the development of cervical neoplasia (La Vecchia et al., 1986). Cervical mucus of women smokers contain high levels of cotinine, nicotine, phenols, hydrocarbons and tars which are known chemical carcinogens (Schiffman et al., 1987; Hellberg et al., 1988). The presence of chemical carcinogens is, however, not sufficient to cause cervical cancer since women who smoke but are celibate are not at increased risk (Cox, 1995). The most important effect of cigarette smoking is impairment of local immunity.

An increased incidence of cervical cancer and its precursors has been noted in women using oral contraceptives (Beral et al. 1986, Brinton, 1991). Studies by Zondervan et al. (1996) have suggested that current/recent, long term users of oral contraceptives may have an elevated risk of cervical neoplasia, in particular of invasive carcinoma and carcinoma in situ. A number of studies have indicated a decreased risk of cervical cancer in users of barrier methods of contraception i.e. diaphragm and condom (Boyd et al., 1964; Fasal et al., 1981; Zondervan et al., 1996). These results are further supported by recent findings of Fernandez et al (1995) that oxidation products polyamines, spermine and spermidine and the diaminoputrescine in seminal plasma act as
cofactors in the development of cervical cancer. They have suggested that sexual activity in the absence of barrier method of contraception may lead to generation of mutagenic and immunosuppressive polyamine oxidation products within female genital tract. They have proposed that women with high levels of polyamine oxidase (PAO) and diamine oxidase (DAO) (the enzymes which catalyse the oxidation of polyamines from a male partner's semen), in the cervical mucus may be at increased risk of cancer.

Another risk factor which is thought to be important in the genesis of cervical cancer is multiparity which is considered to be a reflection of sexual activity and age of onset of intercourse (Brinton et al. 1989). The increased susceptibility to cervical cancer with multiparity might occur from the effect of hormones on the cervix or the effect of trauma at the time of delivery upon the cervical epithelium. Some studies have implicated that age at first pregnancy and not the number of pregnancies is important risk factor (Bosch et al., 1992). However, the number of pregnancies has also been shown to be an adverse prognostic factor, as women with six or more child births have a 2-5 fold excess risk of dying from cervical cancer compared to those with three or less births (de Britton et al., 1993).

The influence of nutrient status on risk of cervical neoplasia has received substantial attention. Dietary factors play an important role by acting either as antioxidants or by their effect on local cellular immunity. Two case control studies showed a decreased risk of cervical cancer with high intake of vitamins A, C, E and β-carotene (Verrault et al., 1989, Slattery et al., 1990). Similar observations have also been made by Potischman et al. (1993, 1996) where low vitamin C and carotenoid status were associated fairly consistently with both cervical cancer and its precursors, whereas results with vitamin E status were less consistent.

The association of cervical neoplasia with sexual behaviour motivated the research for sexually transmitted agent(s) which may be responsible for the
neoplasia. A number of infectious agents have been studied in relation to cervical cancer which include; *Treponema pallidum*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, Herpes simplex virus type-2 (HSV-2), Cytomegalovirus and Human papilloma virus (HPV) (Schmauz *et al.*, 1989, Brinton *et al.*, 1992, Lehtinen *et al.*, 1992, Munoz *et al.*, 1995). Exposure to *Chlamydia trachomatis* was assessed serologically and reported as significant and independent risk factor for cervical neoplasia (Schacter *et al.*, 1982, de Sanjose *et al.*, 1994). Other studies also showed an increased risk of cervical neoplasia with chlamydia (Koutsky *et al.*, 1992).

Earlier studies on epidemiology of cervical cancer strongly implicated HSV type 2 (HSV-2) as the causative agent (Rawls *et al.* 1968; Duff and Rapp 1973; Chen *et al.* 1994). The etiological role of HSV-2 was determined mainly from the seroepidemiological studies. In cervical cancer patients, higher titres of antibodies to HSV-2 were found than in controls. However, the role of HSV-2 was not well defined in association with cervical cancer due to lack of better serological assays (Adam *et al.*, 1985; Lehtinen *et al.*, 1992). With the development of modern molecular techniques, it has become possible to detect viral DNA within cervical cancer (Durst *et al.* 1983; Gissmann *et al.*, 1983). HSV-2 DNA has been rarely found in cervical cancer. Many workers suggested that HSV may have synergistic relationship with other viruses like human papilloma virus (HPV) and enhance the development of cancer in some cases (Zur Hausen, 1982, 1986; Kaufman *et al.*, 1988; Hildesheim *et al.*, 1991).

No consistent association was observed for *T. pallidum*, Cytomegalovirus or Epstein bar virus. The human papillomavirus has emerged as etiologically most important agent in cervical neoplasia. A large volume of epidemiological and molecular biological evidence has accumulated which indicates that specific sexually transmitted HPV types are the main cause of most cases of CIN and cervical cancer (Munoz *et al.*, 1992). However HPV plays a part in the development of cervical cancer by interaction with various other factors which
have already been mentioned. The association between HPV and cervical cancer was formally proposed by Zur Hausen et al. (1974). Because HPV could not be propagated in tissue culture, further link between HPV and human disease had to await the advent of molecular hybridization techniques. The first reports of HPV DNA detection in cervical cancer appeared in 1983 and 1984 (Durst et al., 1983; Boshart et al., 1984). The first definitive case-control study associating HPV types 16 and 18 with cervical cancer was published by Reeves et al. (1989). The evidence implicating specific HPV types in etiology of cervical cancer is now strong enough to establish a causative role (Koutsky et al., 1988; 1992).

Papillomaviruses belong to the genus of the Papovaviridae family. These viruses are widely distributed and can infect humans and animals (e.g., bovine, canine, rabbit and deer papillomaviruses have been identified). Although human and animal papillomaviruses share a similar genomic organization, they are highly species specific and do not cross infect other species. Based on DNA homology, they have been divided into distinct genotypes and subtypes. To be classified as new HPV type, a new isolate should have less than 50% homology with the genome of existing HPV types.

Approximately 35 types infect the anogenital mucosa and are generally called genital HPVs, however, these (genital HPVs) may also infect several non-genital sites including upper airways, the conjunctiva and periungual tissues (Moy et al., 1989). Relative risks have been documented for each of the genital HPVs in their association with cervical intraepithelial neoplasia (CIN) and cervical cancer (Lorincz et al., 1992). The low risk types i.e., 6, 11, 42, 43, 44 are commonly associated with condyloma acuminata and are sometimes found in CIN I, but rarely in invasive cervical cancer. Among high risk types, predominantly HPV 16 and 18 have been detected in women with high grade CIN and in persons with invasive cancers of cervix, vulva, penis and anus (Allen and Planner, 1995). A recent study by Huang et al. (1997) in China on women
with cervical cancer has indicated that HPV types 52 and 58 are as prevalent as high risk HPVs 16 and 18. However, these two types i.e., 52 and 58 are relatively uncommon in cervical cancers in America, Europe, Africa and Southeast Asia (Bosch et al., 1995).

**Genomic organization of HPV**

All known papillomaviruses have similar structural and genomic organization. These DNA tumour viruses are nonenveloped; capsid is composed of 72 capsomers, has icosahedral symmetry and measures 45-55 nm in diameter. They have double stranded circular DNA containing 7800-7900 base pairs. The HPVs initiate infection and replicate as episomes only in squamous epithelial cells. Because viral transcription and replication occur as cells become more differentiated most viral DNA replication, structural protein synthesis and viral particle assembly processes occur in superficial keratinizing epithelial layers. The tight linkage of viral replication to terminal cellular differentiation of squamous epithelium makes *in vitro* propagation of HPV difficult. However recently, organotypic cultures that achieve differentiation of cultured cells have provided new tools for studying viral replication, transcription and production of virions (Dollard et al., 1992, Meyers et al., 1992).

HPV genome has been divided into eight open reading frames (ORFs) and a regulatory region called the upstream regulatory region (URR), also known as long control region (LCR) or non coding region (NCR). The six HPV ORFs code for early proteins (E1, E2, E4, E5, E6, E7) that regulate viral transcription, replication and transformation. Two ORFs code for late proteins (L1 and L2) that make up the capsid found in mature particle. The URR is approximately 400 base pair DNA segment. The URR harbours numerous specific DNA binding sites for both viral and cellular proteins involved in viral transcription and replication.
Fig.(a) A linear genomic map of HPV16. The genome is schematically depicted with early region (indicated by genes represented with an E) and late-region (those preceded by L) open reading frames. The approximate size of genome is shown in kilobases, and location of the open reading frames is diagrammed in relation to this scale. Also indicated is the P97 promoter located in the upstream regulatory region.
(Source: Vernon et al., 1996)
The URR also contains the major viral promoter site (the site of transcription initiation) known as P97. It is a major transcriptional promoter and is located upstream of E6 gene. Transcription of HPV E6 and E7 oncogenes is regulated negatively and positively through the binding of cellular and/or viral proteins to the URR and these are referred to as transcription regulatory proteins. It has been suggested that URR may play a critical role in determining the host range of specific types of HPV (Turek et al., 1994).

Early region is located downstream of URR and contains six ORFs. The gene products encoded by E1 and E2 ORFs are critical for viral replication (Chiang et al., 1992). HPV E1 is 80-KD nuclear phosphoprotein with DNA binding, ATPase and GTPase activity (Bream et al., 1993). The E2 is a 42-KD protein and plays a crucial role in viral replication and transcription. It acts as a transcriptional regulator by binding as a dimer to specific DNA sequence in the viral URR (Dostatni et al., 1988; McBride et al., 1989). There are four E2 binding sites in the URR and binding of E2 dimer to two of these results in transcriptional activation of P97 promoter, whereas binding to all four sites leads to repression of transcription (Tan et al., 1992; Ong et al., 1994). E4 protein appears to be important for viral maturation and replication and is expressed in later stages of viral infection when complete virions are being assembled. It plays a primary role in productive viral infection. It is expressed in large quantities in the differentiated cells of papillomas (Breitburd et al., 1987). In human keratinocytes, this protein induces the collapse of cytoplasmic cytokeratin network which may cause characteristic perinuclear clearing or halos (koilocytosis) which is observed histologically and cytologically in HPV-infected cells (Doorbar et al., 1991).

The functional role of E5 protein is not very well known. It is a small hydrophobic protein of approximately 10KD and found predominantly in intracellular membrane compartments such as Golgi apparatus. The major biologic activity of E5 is mitogenic stimulation of cells through interaction with
signal transduction pathways. The E5 interacts with cell membrane receptors, such as EGF and PDGF and may stimulate cell proliferation in HPV infected cells (Conrad et al., 1994). The HPV16 E5 gene has been shown to cooperate with E7 gene in stimulating keratinocyte proliferation and increasing viral gene expression (Bouvard et al., 1994).

E6 and E7 oncoproteins are critical for viral replication as well as host cell immortalization and transformation (Bedell et al., 1987; Phelps et al., 1988; Storey et al., 1988). The E6 protein is composed of 150 amino acids and has a molecular weight of approximately 16KD. The role of HPV E6 protein has been extensively studied and it shows some variation depending on the type of tissue involved. Efficient immortalization of human keratinocytes requires both E6 and E7 viral oncoproteins, whereas mammary epithelium is immortalized by transfection with E6 alone. It has been found that E6 protein forms a complex with tumour suppressor protein p53 leading to its degradation (Werness et al., 1990). This is thought to be an important mechanism in the genesis of cervical cancer.

E7 is a small nuclear phosphoprotein of 99 amino acids with a molecular weight of 10 KD. Although high levels of E7 alone can immortalize human keratinocytes, the addition of E6 increases the efficiency of immortalization. The E7 oncoprotein can bind to tumour suppressor protein retinoblastoma (Rb) and this binding is an important step in immortalization (Dyson et al., 1989; Heck et al., 1992).

L1 and L2 are the structural proteins of human papillomavirus and are known as major and minor capsid proteins, respectively. Transcription products of L1 and L2 ORFs are only rarely observed with neoplastic transformation. This is in accordance with the fact that mature virions have not been demonstrated in invasive cervical cancers.

Electron microscopy has revealed that each of the 72 capsomers of HPV capsid is a pentamer of L1, however, the location of L2 in the capsid is not
known. L1 ORF is highly conserved among different papillomavirus species.

Antibodies made against bovine papillomavirus L1 capsid proteins can cross-react with human papillomavirus and have been used to identify HPV capsid proteins in human tissues. HPV L1 capsid proteins have been produced by several investigators in vitro using vaccinia virus or baculovirus systems which can self-assemble and produce empty viral-like particles.

HPV type-specific antibodies have been found in HPV-infected women (Galloway et al., 1989; Kirnbauer et al., 1994). Further studies to assess the usefulness of these antibodies as clinical markers are still going on (Park et al., 1995). Because HPV is not easily propagated in tissue culture, the L1 and L2 virus-like particles can prove useful for studying papillomavirus cell entry and antibody neutralization. Transcription of L1 and L2 occurs when complete virions are being assembled. Transcription appears to be regulated by cell-derived transcription regulators that are produced by more differentiated squamous epithelial cells in the upper layers of infected epithelium. This explains why virion production and the resultant HPV cytopathic effects are more pronounced in histologically low grade lesions as these retain relatively good differentiation of the squamous epithelial cells.

**HPV testing and its principles**

HPVs are not readily detectable by direct procedures commonly used to diagnose most viral infections. There are no suitable serological tests and HPVs cannot be isolated from clinical specimens by cell culture methods.

In cytomorphic or histological evaluation, cervical smears and biopsies are examined for the presence of koilocytic changes. However, the HPV-type cannot be predicted on the basis of histopathological findings alone (Kadish et al., 1986).

Electron microscopy has been used to locate virions in koilocytes and dyskeratocytes (Hills and Laverty, 1979; Ferenczy et al., 1981; Kadish et al.,
1986; Syrjanen et al., 1986). However, it is not possible to distinguish different HPV types by this method.

Immunohistochemical studies have been carried out to demonstrate HPV associated antigen in the tissues. In this method, antibodies specific for the capsid antigen are used (Jochmus-Kudielka et al., 1989). The most commonly used method to detect HPV-capsid antigen is, the peroxidase antiperoxidase localization of HPV using rabbit antibodies to disrupted papilloma virion (Kurman et al., 1981). Although this method is quite specific, it has little value for typing of HPVs and is not very sensitive (Kadish et al., 1986). Besides, it has been found that HPV-16 produces very little capsid antigen and escapes immunohistochemical detection (Beckman et al., 1988).

Hybridization tests

Despite evolutionary differences in nucleotide sequence of different HPV types, the patterns of genome organization have remained similar and localized areas of homology exist between all HPV types. Three basic DNA tests i.e., Southern blot, the dot blot and the in situ hybridization tests have been the backbone of both clinical and research studies through the 1980s. However, in 1990s, semi-automated adaptations of PCR and solution hybridization reactions have transformed the area of laboratory medicine, giving better results in less time (Ho et al., 1994).

Jourdan et al. (1995) have reported the detection and typing of human papillomaviruses by in situ hybridization with biotinylated oligonucleotide mixtures. Both radiolabelled and biotinylated oligonucleotide probes have been successfully used to detect HPV-DNA in tissue samples by dot and Southern blotting (RaKoczy et al., 1989; Falkner-Jones et al., 1993; Capalash and Sobti, 1996).
Polymerase chain reaction

PCR is a powerful method by which minute amounts of specific target DNA sequences can be amplified several million folds (Mullis and Faloona, 1987). Vossler et al. (1995) have shown that HPV can be detected by PCR in a majority of individuals showing evidence for HPV infection. There are two types of primers i.e., type specific primers which detect a single HPV type and consensus primers which can detect many different HPVs in a single reaction. Two sets of consensus primers popularly used are - MY09/MY11 and GPO primers (Manos et al., 1989; Vanden Brule et al., 1990). Arends et al. (1993) used a type-specific, sensitive PCR-based assay for HPV types 6b, 11, 16, 18 and 33 on 47 cervical carcinomas, 60 cases of cervical intraepithelial neoplasia (CIN) and 24 samples of histologically normal cervix and revealed the combined incidence of common high-risk genital HPVs (types 16 and 18) to be higher in carcinomas (79%) and CIN2/3 (60%), low in CIN I (25%) and nonexistent in normal cervices. Analysis of data, revealed that HPV 18 was more associated with advanced disease.

Using PCR Remmink et al. (1995) demonstrated that the continuous presence of high-risk HPV types in women with cytomorphologically abnormal smears was a strong marker for the progressive CIN disease. A study designed to determine the prognostic significance of HPV DNA in cervical cancer using PCR for HPV types 6,11,16,18,31,33,52 and 58 in 148 patients indicated that HPV-18 positivity is a risk factor for tumour recurrence in surgically treated cancers. (Rose et al., 1996). Woodman et al. (1996) studied the presence of HPV 16 and HPV 18 DNA sequences by PCR in archival histological material removed from a cohort of untreated women with cervical epithelial abnormalities. This study indicated that the detection of HPV 16 or 18 DNA sequences in initial biopsy specimens was associated with a significantly increased risk of subsequent disease progression.
Hybrid capture:

This method can detect up to 16 different HPV types in a single reaction. Experiments conducted in various laboratories have shown that it is comparable to the Southern blot with a precision of 95% or greater (Lorincz 1992a).

The sensitivity and accuracy of hybrid capture was evaluated by taking cervical samples from 520 women (Sun et al., 1995). These samples were assayed for HPV-DNA both by hybrid capture and PCR. HPV DNA was detected in 106 (42%) of 254 samples from women with no evidence of cervical intraepithelial neoplasia and 211 (79%) of 266 with cervical intraepithelial lesions or cervical cancer. Good correlation was found between PCR and hybrid capture. However, no correlation was found between the relative amount of HPV DNA detected and grade of cervical lesion. Thus hybrid capture has been found to be a sensitive and accurate method for identification of HPV types of high and intermediate oncogenic potential (Schiffman et al., 1995).

Strip test: A rapid and non-radioactive molecular hybridization test called 'strip test' was developed for the detection and typing of HPV DNA in fresh and paraffin-embedded clinical specimens (Venuti et al., 1995). This is a reverse blot hybridization in which different recombinant plasmids on immobilized on nylon membrane and cellular DNA is amplified and biotin labelled by PCR. This method has a sensitivity to detect 50 copies of HPV-16 per sample. Using this test HPV-11, 16, 18, 31 and 35 were typed in a number of samples from patients (Venuti et al., 1995). This method appears to be suitable for handling of clinical samples and for screening of type specific infections by HPV.

GENOMIC INSTABILITY IN CERVICAL CANCER

The genetic changes in neoplasia has been a subject of debate for past century. It was David Von Hanseman who made a systematic study of cell division in malignant tumours in 1890 and reported that aberrant mitoses are
very common in cancer cells. He suggested that this phenomenon can be used as criterion for diagnosing malignant state (Von Hansemann, 1890). Later studies suggested that cancer may result from the disturbance of normal chromosomal balance which is essential for normal cell function.

Only a limited number of cytogenetic studies have been carried out in invasive cervical cancer, because it is difficult to obtain good-quality banded chromosomes in solid tumours. Structural and numerical alterations of several different chromosomes have been reported in cervical cancer (Atkin and Baker, 1979; Sreekantaiah et al., 1988; Atkin and Baker, 1990). However the chromosomal picture has been extremely variable and complex and complete karyotyping has been accomplished in only a few cases. Triploidy and tetraploidy are common events, but there is no single cytogenetic abnormality which has been consistently associated with this tumour. Chromosome 1 has been found to be involved in a non-random fashion. Both structural rearrangements as well as numerical changes have been detected in the chromosomes (Atkin and Baker, 1979; 1982; 1984). The commonly found aberrations are isochromosomes, deletions, duplication and associated translocations of both long and short arms. Besides, double minutes (DMs) chromatin bodies have also been observed in cases of invasive cancer and few of carcinoma in situ (Atkin and Baker, 1980; Barbich et al., 1985; Sreekantaiah and Bhargava, 1992). The presence of DMs in cytogenetic preparations of primary tumours may indicate a role in the maintenance and progression of malignant state, because DMs have been suggested to represent amplified genes. Similarly chromosomal abnormalities have also been seen in other chromosomes i.e., 3, 4, 5, 6, 11, 13, 17, 18 and 21 (Atkin and Baker, 1979). Sreekantaiah et al. (1988) detected chromosomal alterations in 95% of the tumours. Loss of chromosome 1 was observed in 54% of the cases. The regions 1p11-p13 and 1q21-q32 were preferentially overexpressed in these cases. Translocations between chromosome 1 and other chromosomes were frequently
detected with the most common partner chromosome being chromosome 3.

**Chromosomal alterations and physical status of HPV.** Cervical cancer is strongly associated with HPV, which may be found in episomal or integrated form (Schneider-Maunoury et al., 1987). Viral integration may result in genomic reorganization. HPVs are clastogenic as are other DNA and RNA viruses. Viral gene products can also contribute to the generation of chromosomal rearrangements. However, in benign lesions, viral DNA is maintained in a free, extrachromosomal, circular form termed as episome. In cervical cancers as well as cell lines derived from cervical cancers and human keratinocytes transformed in vitro with HPV, the viral DNA is integrated into the host genome (Cullen et al., 1991). Integration occurs more frequently in HPV 18 associated tumours, than those will HPV-16. It has been suggested that viral DNA can induce chromosomal abnormalities in both cell (Matlashewski et al., 1988) and raft cultures (McCance et al., 1988).

It has been proposed that integration is an activation mechanism for progression from pre-invasive advanced lesions (CIN III) to cervical cancers (Schwarz et al., 1985). Integration usually occurs within E1 and E2 open reading frames and results in loss of expression of these genes (Schwarz et al., 1985; Choo et al., 1987). E2 protein is an important transcriptional regulator and loss of its function may result in increased expression of E6 and E7 oncogenes of HPV.

Site of integration of HPVs in the host cell’s chromosomal DNA is highly variable and no preferential chromosomal integration has been identified. However, in some HPV- containing tumour cell lines and immortalized primary human keratinocytes, integrated viral DNA has been mapped to or within fragile sites (Smith et al., 1992) which are chromosomal regions susceptible to foreign DNA integration or other mutagens. HPV integration has also been detected near cellular oncogenes such as c-myc (Couturier et al., 1991). Interruption and rearrangement of these regions could result in genomic instability or
activation of cellular oncogenes and might subsequently promote tumour progression. Although integration of HPV DNA is an important step in this process, it is not essential because some tumour cells contain episomal form of HPV DNA. Both forms have also been detected in some cases.

The cervical carcinoma cell lines that have been characterized cytogenetically have single or multiple copies of integrated HPV (Popescu et al., 1990). The cytogenetic analysis of HPV-immortalized cell lines revealed all of them to be aneuploid. A small metacentric marker chromosome i(5p) was observed in FEA and FEH18L cell lines (Atkin and Baker, 1982; Smith et al., 1989). This was regarded as a major marker in cervical tumours followed by structural changes in chromosomes 1, 2, 3, 6, 9, 11 and 17. HPV negative carcinoma cell lines are very rare. The study on such lines has contributed to the recognition of importance of chromosomes and gene alterations to cervical cancer. Zimonjic et al. (1995) conducted cytogenetic studies on two HPV-negative cervical carcinoma cell lines, C-33A and HT-3 using G-banding and fluorescence in situ hybridization (FISH). Several whole chromosomes and satellites were used as probes. Translocations of chromosomal were common to both cell lines. Besides, partial or complete loss of short arm of chromosome as well as loss of chromosome 13 was also observed. The C-33A line had a relatively simple chromosomal constitution. In contrast, HT-3 cell line had complex rearrangements and deletions of chromosomes 1p, 3p, 9p, 10p/q, 11p/q and 17p. All these regions contain known tumour suppressor genes. The deletions observed in HPV-negative cervical carcinoma cell lines could be important in delineating the regions of tumour suppressor genes.

Based on the study of somatic cell hybrids, it has been postulated that cellular genes are capable of suppressing HPV gene expression (Zur Hausen, 1986). Although not directly identified, it has been suggested that an element responsible for this suppression is located on chromosome 11 (Bosch et al., 1990; Jesudasan et al., 1995). Numerical abnormalities of chromosome 11 are
demonstrable in cervical carcinoma-derived cell lines by interphase cytogenetics (Herrington et al., 1995). Break points in cervical cancer tend to cluster at 11q23 (Sreekantaiah et al., 1991), a site at which a high frequency of allele loss has been observed using microdissection and minisatellite PCR techniques (Bethwaite et al., 1995).

Karyotypic abnormalities of chromosomes 11, 17 and X were evaluated directly within intact cervical tissues (Southern et al., 1997). Chromosome 'X' was used as a control, because it is infrequently abnormal in cytogenetic studies. This study suggested that a relative reduction in number of chromosomes 11 and 17 is important in invasive cervical neoplasia and these findings are consistent with the putative presence of relevant tumour-suppressor genes on these chromosomes. However, there was no correlation between the distribution of viral sequences and chromosomal pattern, suggesting that HPV infection precedes the karyotypic changes.

Another parameter which is considered as an index of genomic instability is sister chromatid exchanges (SCEs). An increase in the frequency of SCEs was reported by various workers on their studies on peripheral blood lymphocytes of cervical cancer patients as compared to controls (Mitra et al., 1982; Lukovic, 1992; Dhillon, 1995; Capalash et al., 1996). However, no correlation was found between the SCE rate and the severity of the lesions. It has been suggested that genomic instability in cervical cancer patients may be due to integration of HPV or to release of clastogenic products by HPV (Paz-y-Mino et al., 1992; Capalash et al., 1996).

ROLE OF ONCOGENES AND ANTI-ONCOGENES IN CERVICAL NEOPLASIA

The current information reveals that a variety of cellular factors besides HPV, including cellular tumour suppressor genes and oncogenes are required for the transformation process. At the clinical level, these genes and their products may improve the understanding of the etiology of disease and provide
more precise diagnostic, prognostic and therapeutic characterisation of individual tumours.

**Amplification and overexpression of c-erbB-2.** It is a member of the receptor tyrosine kinase family and is closely related to the epidermal growth factor (EGF) receptor (Schechter *et al.*, 1984). In most of the tumour conditions, c-erbB-2 is either mutated or overexpressed (Suda *et al.*, 1990). Amplification of the c-erbB-2 gene leading to overexpression of the protein has been observed in a high percentage of human breast and ovarian tumour cells (Kraus *et al.*, 1987). It is likely that elevated levels of the c-erbB-2 protein contribute to malignant process (Slamon *et al.*, 1989). There are only a few reports regarding the c-erbB-2 oncogene amplification or oncoprotein expression in cervical carcinoma (Mitra *et al.*, 1994; Kihana *et al.*, 1994; Oka *et al.*, 1994).

Amplification of different oncogenes was evaluated in 50 untreated squamous cell carcinoma of the uterine cervix (Mitra *et al.*, 1994). c-erbB-2 ranged from 5 to 68 copies. In addition, 2 tumours with c-erbB-2 amplification showed additional restriction fragments, suggesting possible mutation or rearrangement of the gene.

Prognostic significance of the overexpression of c-erbB-2 in adeno-carcinoma of uterine cervix was evaluated by Kihana *et al.* (1994). To ascertain the role of c-erbB-2 oncogene in adeno-carcinoma of uterine cervix, formalin fixed, paraffin-embedded tissue sections from 44 cases of cervical adenocarcinoma were immunohistochemically examined for expression of c-erbB-2 protein and for c-erbB-2 gene amplification by slot blot-hybridization analysis. The expression of c-erb B-2 protein was detected in 34 (77%) cases, while strong expression on cell membrane was detected in 11 (25%) cases. Expression of the c-erb B-2 protein was also associated with poor prognosis of patients with cervical adenocarcinoma by comparison of survival curves. A similar report was made by Oka *et al.* (1994) in squamous cell carcinoma of the cervix. A polyclonal antihuman c-erb B-2 oncoprotein antibody was used to
recognize c-erb B-2 oncoprotein in routinely formaldehyde-fixed, paraffin-embedded specimens.

**Role of c-myc in cervical cancer**: C-myc oncogene is present in all eukaryotic cells and plays an important role in the regulation of cellular proliferation and differentiation (Scheiness *et al.*, 1979; Kelly *et al.*, 1986). This is expressed at high levels in rapidly dividing non malignant cells such as regenerating liver (Goyette *et al.*, 1984) and abnormalities in its expression are known to be associated with many malignant tumours including ovarian and cervical carcinomas (Ocadiz, 1987; Yasue, 1987; Pinion, 1991). The c-myc oncogene has been found to be amplified in a wide variety of human cancer cell lines. Ocadiz *et al.* (1987) were first to report alteration in c-myc gene in solid tumours of uterine cervix. These alterations included amplification and rearrangement with or without amplification.

According to Couturier *et al.* (1991) DNA sequences of specific HPV types are integrated in the genome of cell in most invasive genital carcinomas. Their results revealed that HPV genomes are preferentially integrated near myc genes in invasive genital cancers and it was revealed that integration plays a part in tumour progression via activation of cellular oncogenes.

In carcinoma of uterine cervix Northern blot and slot-blot hybridization has shown a clear correlation between elevated c-myc gene expression and risk of relapse and of early distant metastasis (Couturier *et al.*, 1991; Ocadiz *et al.*, 1987; Riou *et al.*, 1987). Immunocytochemical studies using paraffin embedded specimens and monoclonal antibodies for protein encoded by the c-myc gene also demonstrated a direct relation between c-myc protein levels and duration of disease-free state and overall survival (Couturier *et al.* 1991). The overexpression of c-myc gene in uterine cervical cancer was also reported by Tanimoto *et al.* (1992). The patients of cervical cancer with c-myc level more than 10 times as high as the normal uterine cervical tissue, relapsed and died within 2 years after the first treatment. It was suggested that overexpression of
c-myc gene occurred more frequently in advanced than in early uterine cervical carcinoma and it might be useful in prognosis. Similarly Riou et al. (1987) analyzed the H-ras and c-myc protooncogenes in a large series (154 cases) of cervical cancers at various clinical stages. They reported that alterations of H-ras (deletion, mutation) and c-myc (amplification) are frequently observed in cervical cancer and are associated with tumour progression. They also suggested that if alteration in c-myc expression is identified in early cervical cancers, it provides a means of identifying the patients at high risk of early recurrence.

Iwasaka et al. (1993) using monoclonal antibodies to oncogene products in paraffin embedded specimens revealed that only some of cervical invasive carcinomas expressed c-myc protein, ras p21 or EGFR. Northern blot hybridization analysis revealed overexpression of c-myc mRNA in 30% of cervical invasive carcinomas although amplification of c-myc oncogene was detected in only one such case.

Gotoh et al. (1991) established a human tumour cell line, NCC-c-cx-1 (cx-1) from a uterine cervical carcinoma and xenografted it in nude mice. This cell line harboured approximately 50-100 copies of HPV DNA per haploid genome and contained about 16 fold amplified c-myc gene with rearrangement. These genomic alterations found in cervical cancerous cells were also present in both primary and xenografted tumours. This cervical cancer cell line may be a useful model for studying cervical carcinogenesis and particularly with respect to interaction between HPV and c-myc oncogene.

Role of PRADI/BCL-1 (Oncogene) : Over the past few years, cell-cycle control proteins have been discovered. These proteins, known as cyclins, regulate the cell cycle during normal state and perturbations in their expression may lead to various neoplastic processes. These include mitotic cyclins A and B which are associated with the G2/M phase transition and C,D and E types active in G1 phase. They mediate their action by activation of cyclin-dependent protein
kinases which phosphorylate the key regulatory substrates (Kidd et al., 1991). Cyclin D1 is a cell-cycle control gene which was shown to be identical to oncogene known as BCL-1 and PRADI and is implicated in centrocytic lymphomas and parathyroid adenomas, respectively (Berenson et al., 1989; 1990; Motokura et al. 1991; Rosenberg et al., 1991; Raffeld et al., 1991; Medeiros et al., 1996). PRADI is located on chromosome 11q. 13. In both these neoplasms, the PRADI is activated by translocation resulting in marked overexpression of the gene.

PRADI was found to form a complex with the product of retinoblastoma gene which is a tumour suppressor gene. This event leads to inactivation of retinoblastoma gene. Retinoblastoma gene prevents the entry into the S phase during cell cycle, and this growth suppressive function is presumed to result from hyperphosphorylation of retinoblastoma protein. These observations suggested that cell cycle control is mediated atleast in part by the interaction between an oncogene (PRAD1) and an antioncogene (Rb). In carcinoma of the cervix, HPV was implicated as an initiating event and it has been found that E7 oncoprotein of HPV forms a complex with Rb leading to its degradation. There is some overlap between the molecular processes mediated by HPV and PRAD1 oncogene. Kurzrock et al., (1995) analyzed 13 cell lines derived from squamous cell carcinomas of cervix and vulva for abnormalities in the PRAD1 gene or its expression. Seven out of thirteen cell lines showed abnormalities of PRAD1. These included amplification and rearrangement of DNA and overexpression of mRNA. This study suggested that PRAD1 deregulation could be a significant molecular event in the evolution of tumours of cervix and vulva.

**Role of p53 (tumour suppressor gene) in cervical cancer:** Besides the positive regulators, negative regulators (tumour suppressor or antioncogenes) also have an important role to play in the transformed state of cells. Todate, about a dozen tumour suppressor genes have been identified, and their number is expected to increase. p53 is the most frequently mutated tumour
suppressor gene which is mutated in 50% of all tumours (Nigro et al., 1989; Iggo et al., 1990; Harris et al., 1990; Crook et al., 1992).

It is located in 10-20 kb of DNA on the short arm of human chromosome 17 position 17p 13.1. It is composed of eleven exons, the first of which is non-coding and has 213 base pairs. It is located 8-10 kb away from the exons 2-11. The p53 gene has been conserved during evolutionary scale. In cross species comparison, the p53 protein showed five highly (90%) conserved regions among the amino acid residues 13-19 (I), 117-142 (II), 171-181 (III), 234-258 (IV) and 270-280 (V). Sixty eight per cent of the p53 missense mutations reside in the conserved regions II, III, IV, V, while 86% of them were between codons 120 and 290. Both the distribution of mutations and the conservation of amino acid sequences in this region of the protein suggested that an important functional domain resides in this region of the protein. The product of p53 gene is a 393 amino acid nuclear phosphoprotein (about 53 KD in molecular weight). The amino acid residues of the p53 protein have now been divided into three distinct functional domains. On the amino-terminal, 75 amino acids are quite acidic. The carboxy terminal domain composed of amino acid sequences 290-383 contains a set of nuclear localization signals and a region that promotes the protein to form tetramers or other oligomeric forms in solution (Stegner, 1992). Thus, p53 is a transcription factor that enhances the rate of transcription of gene that has p53 responsive element. This has been shown both in vivo and in vitro (Finlay et al., 1989; Eliyahu et al., 1984).

The p53 gene was originally regarded as a dominant oncogene because its overexpression resulted in immortalization of rodent cells and the p53 gene could transform rat embryo fibroblasts in concert with an activated ras gene (Jenkins et al., 1984; Parada et al., 1984). However later on, it was found that many of these p53 clones were mutated forms of the gene and properties of these were different from those of wild type - p53 gene (Lane and Benchimol, 1990; Lane, 1992). The wild type allele was found to suppress the
transformation of rat embryo fibroblasts in cell culture and introduction of the wild type p53 c-DNA into a transformed cell in culture stops cell growth at G1 phase of the cell cycle (Ham et al., 1991; Hartwell, 1992).

In cell proliferation, wild type p53 regulates the transition from G1 to S-phase of the cell cycle (Diller et al., 1990; Hupp et al., 1992; Livingstone et al., 1992; Yin et al., 1992). In addition, it determines the cell death through apoptosis (Yonish-Rouach et al., 1991). The gene p53 appeared to function normally as a G1-S check point control for DNA damage (Kastan et al., 1991; 1992). This gene thus, maintains the integrity of the host genome by acting directly upon DNA as well as via regulation of other gene products. The role of p53 as cell cycle check point permits repair of DNA and prevents DNA damage. In some cell types, high levels of p53 protein act as a switch to turn on a pathway to apoptosis in cell responding to DNA alteration. These regulatory functions may be mediated by the interaction of p53 protein with specific DNA sequences (Kern et al., 1991) which may allow regulation of other genes at transcriptional level (John et al., 1992) or perhaps by initiating DNA replication. In mutated p53 proteins, all these functions are altered.

Cancer cells with p53 mutations enter S-phase and duplicate damaged DNA. They segregate their chromosomes abnormally and these processes result in a good deal of cell death. These cells also fail to enter into the pathway for apoptosis even with extensively altered DNA. The result is the progressive appearance of more abnormal cells and clones of cells that evolve into more aggressive cancers (Soussi et al., 1994).

In cervical cancer, the E6 and E7 genes of high-risk HPVs (type 16 and 18) are regularly expressed in HPV -positive tumours and cervical carcinoma cell lines (Smotkin et al., 1987). The E6 protein binds and forms complexes with p53 protein (Werness et al., 1990). The binding stimulates the degradation of p53 involving the ubiquitin dependent protease system (Scheffner et al., 1991).
Human papillomavirus early protein E6, from high-risk viruses, results in the degradation of p53. This degradation appears to occur via the ubiquitin pathway. E6 and E6-associated protein (E6-AP) are found in a complex with p53. It has been suggested that E6-AP may be similar to one of the proteins in the ubiquitin pathway and thus become targeted for degradation.
(Source: Vernon et al., 1996).
Many studies based on human cervical carcinoma cell lines indicate that inactivation of both p53 and Rb is important in tumourigenesis of the cervix uteri (Crook et al., 1991; Scheffner et al., 1991). It has been proposed that wild-type p53 cell regulating functions are annulled in human cervical cancer either by mutations in human papilloma virus negative cases or as a consequence of their complexing with HPV E6 (Crook et al., 1992). In a study on 28 patients with cervical carcinoma, they found that only 3 patients were HPV negative and had a mutation in p53 in the conserved domains. However, in another study on 36 cases of primary cervical carcinoma, Fujita et al. (1992) detected 7 samples with no HPV infection and no mutation in p53, while two samples had both HPV infection as well as mutation in p53. Similar observations have also been made by other workers (Borresen et al., 1991; 1992; Busbe-Earle et al 1992; Holland et al., 1993). In a study on 92 patients with primary cervical carcinoma, mutations in p53 were detected in only 2 patients and one of these was HPV positive (Helland et al., 1993). However, 4 samples negative for HPV 16 and 18 did not contain p53 mutation, but had an elevated p53 protein expression. Overexpression of p53 protein was detected in 55% (51/92) of the tumours (Helland et al., 1993). The increased level of p53 results from an enhanced stability of p53 protein. In response to DNA damage, the half life of p53 protein increases from 20 -30 minutes in normal cells to several hours (Maltzman, and Czyzyk., 1984). It has been suggested that altered expression of p53 protein may be involved in the progression of the cervical carcinoma (Holm et al., 1993). Cooper et al. (1993) based on a study on 39 cervical cancer cases proposed that p53 protein expression may represent either the wild type promoter form or mutant p53 protein, both of which share the same conformation. This may explain the co-localization of p53 and HPV in some tumours. However they also reported the absence of p53 protein in 50 percent of HPV-negative squamous cell carcinomas suggesting that not all HPV-negative tumours accumulate p53 protein. Similarly mutant p53 was also
detected in some HPV-positive cases by other workers (Park 1994). This indicated that the inverse hypothesis proposed initially is not consistent. Miwa et al. (1995) suggested that in HPV negative tumours, p53 may be inactivated by some unidentified or identified effector molecules other than E6. They also suggested that p53 may be irrelevant to tumourigenesis of HPV-negative cervical cancers. In a study on recurrent/metastatic tumours of cervix, Milde-Langosh (1995) indicated that HPV persistence was an important event for the evolution and maintenance of cervical cancer, while p53 mutation rate was not significant. In a study, Kurvinen et al., (1996) have indicated that HPV lesions with a high percentage of cells that express p53 are more likely to regress than those with low or absent p53. They suggested that assessment of p53 expression patterns may be useful to predict the clinical course of cervical HPV lesions.

Hamada et al. (1996) have suggested the potential use of p53 in the gene therapy for cervical cancer. The wild type p53 was introduced into cervical cancer cell lines via a recombinant adenoviral vector, Ad5CMV-p53 and its effect was analyzed on cell and tumour growth. It was found that Ad5CMV-p53 infected cells undergo apoptosis and cell growth is greatly suppressed. These results indicated that transfection of p53 gene in a Ad5 CMV-p53 can be a potential novel approach to the therapy of cervical cancer.

It has been hypothesized that p53 abnormality leads to increased genomic alterations in primary tumour cells. To examine this hypothesis, Ku et al. (1997) examined 51 primary tumors of cervix and 10 microsatellite markers. These markers were mapped on the short-arms of chromosomes 3 and 5, covering the region 3p13-25 and 5p15.1-15.3. It was found that proportion of abnormal p53 was significantly higher in cases exhibiting loss of heterozygosity (LOH) on 5p. This study supported the hypothesis of presence of p53-dependent pathway to cervical carcinogenesis.
Role of p16 in cervical cancer: Eukaryotic cell division is regulated by a series of protein kinase complexes consisting of cyclin-dependent kinase (cdk) catalytic units and cyclin control units (Liu et al., 1995; Kamb 1994; Weinberg, 1995). Besides that several cyclin-dependent kinase inhibitors have been identified as control system that prevent the progression of cell cycle. Examples of these inhibitors are p21 (Noda et al., 1994), p27 (polyak et al., 1994; Toyoshiba and Hunter, 1994), p15 (Hannon and Beach, 1994) and p18 (Guan et al., 1994). The deregulation of cell cycle was proposed as an important mechanism for the malignant progression of normal cells (Pardee, 1989). For several kinds of cancers, the contribution of mutations in the sequences of p16 (MTS1) tumour suppressor gene to the process of carcinogenesis was proposed. The p16 (MTS1) is a cyclin-dependent kinase (cdk) inhibitor that decelerates the cell cycle by inactivating the cdk that phosphorylate the retinoblastoma tumour suppressor gene (pRb). As a result, pRb binds to and depletes the level of the E2F transcription factor required for the expression of the S-phase oncogenes that activate cell cycle. Therefore, the loss of functional p16 disrupts the pattern of cellular gene expression and initiates carcinogenesis through the deregulation of cell cycle. A number of studies have shown that point mutations and homozygous deletions of p16 for some types of cancers and cancer cell lines are frequent (Guan et al., 1994; Kamb 1995). However alterations of p16 have been reported to be rare events in some cancers and tumour cell lines (Beltinger et al., 1995; Liu et al., 1995; Quesnal et al., 1995), including cervical carcinomas and cell lines (Kelley et al., 1995; Hirama et al., 1996). Therefore the significance of p16 in the evolution of cervical cancer remains uncertain. In cervical cancers, the pRb is inactivated by the HPV E7 oncoproteins or mutatious. The hypothesis of earlier reports that the disruption of p16/cdk -cyclin/Rb cascade is essential for malignant cervical transformation/carcinogenesis. Nakao et al (1996, 1997) detected no point mutations or homozygous deletions of p16 in the in vitro systems or in clinical
specimens. It has been suggested that inactivation of the p16/cdk-cyclin/Rb cascade does not occur during malignant transformation but occurs during the immortalization by HPV in HPV-harbouring premalignant lesions. According to these authors p16 has no role in the specific malignant transformation step from immortal premalignant lesions during the carcinogenesis of HPV-initiated cervical cancer.

Although much research has been carried out to elucidate the carcinogenesis of cervical cancer still we need to further evaluate:

- the association of HPV with cervical cancer patients.
- the possibility of using non invasive and easy to perform ELISA for detection of over expression of p53 from the serum of the patients.
- and to look for any correlation between the presence of HPV and the status of p53 towards the multifactorial carcinogenesis of the cervix.