Discussion
A number of studies on the epidemiology of cervical cancer have indicated that this disease occurs in sexually active women (Bosch et al., 1996). The present study is also in agreement with this finding. All the patients in the present study were married. Some religious or ethnic groups that particularly promote celibacy until marriage and monogamous relationship have lowest risk for the development of cervical cancer (Cox, 1995). Two sexual behavioural characteristics that have traditionally varied between high and low risk populations are the age at first intercourse (Rotkin, 1967) and number of sexual partners. The risk is highest for the women whose first intercourse is closest to the menarche (Peters et al., 1986). A study by Edebiri et al. (1990) has indicated that the risk of development of cervical cancer gradually decreases to a plateau with first intercourse between the ages of 20 and 23. In the present study, majority of the patients (44%) got married between the age of 15-17 and 7% before the age of 15 and only 6% above the age of twenty. The immature metaplasia of the active transformation zone of young women may be most susceptible to potentially transforming sexually transmitted agents (Coppelson and Reid, 1968). Due to social constraints, the information regarding the number of sexual partners could not be obtained in the present study.

Majority of the patients (98%) in the present study were multiparous. An increased risk of both CIN and invasive cancer is seen with increased parity (Brinton et al., 1989). Such increased susceptibility might occur due to hormones or the effect of trauma at the time of delivery upon the cervical epithelium. However, some studies have suggested that age at first pregnancy and not the number of pregnancies, is an important risk factor (Bosch et al., 1992). In the present study, as many as 58% of patients had their 1st pregnancy between the age 14-20 years.

An increased incidence of cervical cancer and its precursors has been noticed in women using oral contraceptives (Beral et al., 1988). It has been
stated that oral contraceptives may directly affect the cervical metaplastic epithelium. However, the possible physiological effects of oral contraceptives on cervical epithelium that might influence cervical cancer risk are yet to be proved. In the present study, 60% of the patients were not using any method of contraception and none had consumed oral contraceptives, so the exact role of the later could not be ascertained.

Epidemiological evidence has implicated cigarette smoking as a possible contributing factor in the development of cervical neoplasia (La Vecchia et al., 1986). High levels of cotinine, nicotine, phenols, hydrocarbons and tars have been detected in cervical mucus of women smokers (Schiffman et al., 1987, Hellberg et al., 1988). Also the cellular immunity to sexually transmitted agents like HPV is lowered in smokers due to decrease in the density of Langerhan's and antigen presenting cells (Cox et al., 1995). However, in the present study, none of the subjects was a smoker.

The age of the patients in the present study ranged from 30-70 years. Although the number of patients was less (16%) in the age group of 30-39, still this indicates the need of screening even in the young sexually active women. The maximum number of patients were, however, in the age group of 40-60 years.

The present study indicates that incidence of squamous cell carcinoma was higher (96%) than the adenocarcinoma (4%) in this region. Even in other parts of world, the incidence of adenocarcinoma is quite low representing only 5-20% of all cervical cancer cases (Brand et al., 1988). However, in Finland a relative frequency of 26% has been reported for adenocarcinoma (Nieminen et al., 1995). In the present study, no significant association of the cervical cancer was seen with the preexisting diseases like diabetes, tuberculosis or hypertension. This is in agreement with the finding by Brinton et al (1987). Most of the patients (66%) presented themselves at stage III of cancer, indicating, thereby, lack of early screening.
As many as 70% of cases belonged to rural areas with poor socioeconomic, educational and nutritional status. Despite being a major health problem, no population based cytologic screening programmes for control of cervical cancer have been undertaken due to lack of resources (Luthra and Rengachari, 1986). For developing countries with limited resources, the World Health Organization recommended an approach to downstage screening by visual inspection of cervix by paramedical workers, because earlier stages are more amenable to treatment. Some studies have indicated that visual inspection may be useful in areas where there is lack of cytologic screening (Jayant et al., 1995). Gajalakshmi et al. (1996) have suggested that in India even village health nurses could be trained for visual examination of the cervix, that could downstage the cancer to earlier stages.

CYTOGENETIC ANALYSIS

In the present study, a significant increase in the frequency of SCEs was found in the cultured lymphocytes of patients over those of controls. In tumour conditions, the analysis of frequency of SCEs can reveal genomic instability and some cell cycle abnormalities (Sandberg et al., 1990). In some preneoplastic states and overt neoplasia, significant deviations from normal SCE levels have been reported (Lukovic and Milasin 1992; Dhillon et al., 1995; Dhillon et al. 1996). Elevated levels of SCEs in bone marrow cells and peripheral blood lymphocytes of patients with breast cancer and lung cancer have also been reported (Dhillon et al., 1995; Banerjee et al., 1982). However, there is a lack of consistency in these findings, because in cases of malignant lymphoma (Crossen et al., 1981), chronic myelogenous leukemia (Cheng et al., 1979) no change in SCE levels was found as compared to controls (Adhvaryu et al., 1985).

In recent years, the goal of cancer research is to look for specific tumour markers in cancer and in this context, SCEs phenomenon is being widely...
considered (Yokota et al., 1989). The precise mechanism of SCE formation is unknown and it is still difficult to evaluate entirely the significance of variations in SCE levels in different malignancies, yet the analysis of SCE patterns is used as the reflection of genomic instability.

In the current study the frequency of SCEs was not found to increase with the severity of disease. A similar observation was reported by Lukovic and Milasin (1992) where it was suggested that once the malignant process is established, SCE levels do not increase further with the progression of disease. The frequency of SCEs may vary in relation to a large number of factors such as age, sex, genetic make up of the individual or environmental factors like smoking, alcohol intake, radiation or chemical exposure (Hedner et al., 1982; Livingston et al., 1983). The patients included in the present study were not exposed to radiotherapy and none of them was smoker or alcoholic or user of oral contraceptives. No significant correlation of SCE frequency was found in patients as well as in controls with age. Similar observations have been made by other workers (Livingston et al., 1983; Yokota et al., 1989). The number of pregnancies and that of abortions were not correlated with SCE frequency in both cancer and control groups.

In cervical cancer, the association of human papillomavirus is very well known (Zur Hausen 1989). A correlation between the chromosomal fragility and the presence of human papilloma virus in cervical cancer has been reported (Couturier et al., 1991). In the present study also, a high incidence of HPV infection (70%) has been seen and it might have been responsible for elevation in the level of SCEs. Besides that, there are report that metabolic stress on the system due to the tumour growth (Banerjee et al., 1982) or the clastogenic products released by tumour cells (Werkmeister et al., 1980; Park and Grimm, 1982) may be responsible for increase in the level of SCEs.
INCIDENCE OF HPV

The current study revealed a high incidence of HPV infection in patients with cervical cancer (70%) as well as those with benign lesions and cervical abnormalities (63.6%) by dot blot hybridization. HPV 16 was the dominant type (70%) in invasive cases followed by HPV 18 (30%) and HPV 11 (15%). However, in cases with benign lesions and cervical abnormalities, HPV 16 was positive in 45.4% and HPV 11 in 36.7% of the cases, while none of the samples was positive for HPV 18. Although HPV 11 was detected in invasive cases, its incidence was lower (15%) than that with cervical abnormalities (36.7%). Similar trend was also projected in an earlier study on Indian women, where HPV 11 was detected in invasive cases (Das et al., 1991). A number of studies have suggested that HPV 6 and 11 commonly occur in benign lesions and HPV types 16, 18, 31 and 33 in the malignant lesions (Durust et al., 1983; Gissman et al., 1983; Shimada et al., 1990; Azocar et al., 1990). A recent study on Chinese women indicated that HPV 52 and 58 were as prevalent as the high-risk types 16 and 18 in the invasive cervical cancer (Huang et al., 1997). However, these are relatively uncommon in cervical cancer in the United States, Europe, Africa and South East Asia (Bosch et al., 1995).

It has been observed that the incidence of different HPV types also varies with the histopathology of the disease. Squamous cell carcinoma are generally associated with HPV 16, adenocarcinoma and adenosquamous carcinoma with HPV 18 (Lorincz et al., 1992). In the present study there was only one case of adenocarcinoma and that was positive for HPV 16 and 11. However, in squamous cell carcinomas, the incidence of HPV 18 was quite low (30%) as compared to HPV 16 (70%). This is in agreement with the finding of other workers, where HPV 16 was detected in 80-90% of squamous cell carcinoma, but HPV 18 was found in only 1-15% of these tumours (Galloway et al., 1989; Koutsky et al., 1988; 1992; Lorincz et al., 1992b, Hording et al.,...
The reason for the association of HPV 16 with squamous cell carcinoma and HPV 18 with adenocarcinoma is not known.

In the present study, infection with multiple HPV types (11, 16 and 18) was detected in low frequency in invasive cervical cancer patients and those with minor cervical abnormalities. A high frequency of mixed infections of high risk HPVs -16, 18, 31 in women with minor cervical lesions has been reported (Cuzick et al., 1995; Londesborough et al., 1996). Somoylova et al., (1995) detected double infection with HPV 16 and 18 in 2 cases of squamous cell carcinoma by PCR, but in both of these Southern analysis gave a positive signal for HPV 18 only. This was due to great difference in the copy number of HPV 18 and HPV 16 in the total population of tumour cells. Mixed infections with two HPV types were identified in 8% of squamous cell carcinoma and 5% of adenosquamous carcinomas by Iwasawa et al. (1996) in Finland. But the exact significance of double infection is not clear. More studies are required to understand the epidemiological significance of persistence of more than one type of HPVs with in the same lesion. The present study may be an underestimation of cases with multiple infection due to the low copy number of one HPV type than the other which might be missed by dot blot hybridization.

It is evident from the literature that there is a lot of variation in the reports regarding the incidence of HPVs. The detection of HPV varies with geographical differences, focal heterogeneity of viral DNA replication within the lesions or more likely because of the difference in the sensitivity of the method of detection (Arends et al., 1990). The incidence of HPV - 16 has been reported to be 34.8% in cervical cancer biopsies in Kenya and Brazil and 61% in Germany (Durst et al., 1983), while it has been 45% in England (Scholl et al., 1985). Dot blot analysis of the cases of precancerous lesions has revealed incidence to the 38 and 58%, respectively (Shirasawa et al., 1986). Filter in situ hybridization (FISH) studies, supplemented with in situ hybridization (ISH) results have shown it to be 33% (Ostrow et al., 1987), while it has been 66% by
Southern hybridization in another study on invasive cancer of cervix (Meanwell et al., 1987).

To eliminate the possibility of false negative and to confirm the results of dot blot hybridization in the present study, some of the samples were subjected to PCR using L1 consensus primers which promote the amplification of an approximately 450 bp product from at least 25 distinct genital HPVs (Manos et al., 1989; Ting et al., 1990). PCR amplification resulted in an increase (77%) in the overall incidence of HPV in invasive cervical cancer cases as compared to dot blot hybridization (70%). However, in cases with benign lesions and other cervical abnormalities, the incidence of HPV remained same by both the methods.

In India, using PCR, an incidence of HPV was found to be as high as 98% in cervical cancer (Das et al., 1992). Chen et al. (1994) reported the amplification of HPV sequences in 81% (58/72) of patients with squamous cell carcinoma or CIN III using LI consensus primer pairs. However, as in the present study, the incidence of HPV was quite low in patients with cervicitis where only 3 patients out of 14 were positive for HPV and only one with HPV 16.

In benign lesions and other cervical abnormalities (erosion, cervicitis) of the cervix, a high incidence of HPV 16 (45.4%) was detected. The presence of HPV DNA of high risk types in low-grade lesions of the cervix has also been documented by other workers (Woodman et al., 1996; Londesborough et al., 1996). A greater persistence of oncogenic HPV types has also been reported in women with normal cytology (Hildsheim et al., 1994; Ho G.Y. F et al., 1994; Remmink et al., 1995). It is suggested that presence and persistence of high risk HPVs is a prerequisite for the development of high-grade lesions and cancer (Melchers et al., 1991). Londesborough et al. (1996) suggested that women with persistent HPV infection particularly with type 16 require careful monitoring even in the absence of a visible high-grade lesion. All these studies
suggest that there is a strong need for a marker that will define the natural history of cervical intraepithelial neoplasia (CIN) and detect true precursor lesions or malignant disease. In this context Remmink et al (1995) have suggested that the continuous presence of high-risk HPV types in women with consistently abnormal pap smears can act as a marker for progressive CIN disease, however, women infected with low-risk HPVs do not show progression of the disease.

The progression and regression of preclinical lesions (dysplasia, carcinoma in situ) has been linked with the age of the patients at which the lesions appear (Ley et al., 1991; Vanoortmarssen et al., 1991). This is further supported by the finding that HPV infection is quite common in sexually active women in the age groups of 20-25 years after which its prevalence decreases (Evander et al., 1995). A majority of women normally clear viral DNA within 2 years (Hildsheim et al., 1994). Regression is maximum (84%) when the age is < 3 years as compared to 40% regression above this age (Vanoortmarssen et al, 1991). The mean age of the women with cervical abnormalities in the present study was on the higher side (>3 years), thus, suggesting the need of prolonged follow up of patients with low grade lesions and infected with high risk HPVs.

Approximately 30% of cervical carcinoma cases subjected to dot blot hybridization and 23% subjected to PCR in the present study were not associated with demonstrable HPV DNA. It is possible that specific HPV DNA plays a part in the early stages of tumour progression and that viral DNA sequences are lost in later stages. The loss of viral DNA in lymph node metastases from cervical cancer which contained nonintegrated HPV 16, has been reported (Fuchs et al., 1989). Some cervical carcinomas may be associated with HPVs only distantly related to known genital HPV types and remain undetected. In a recent study Huang et al (1997) have detected HPV types 52 and 58 at same frequency as most frequently encountered HPV types
Some cervical carcinomas might also develop in the absence of HPV infections since HPV DNA has not been detected in these and also in some cell lines derived from these tumours. Mutations of p53 tumour suppressor gene might be involved in the causation of these HPV negative cervical carcinomas (Crook et al., 1991), representing a biologically distinct subset of tumours with a poor prognosis (Galloway et al., 1989).

STATUS OF p53 IN PATIENTS WITH CERVICAL CANCER

As in most of the cancers, the carcinogenesis of cervix is multifactorial involving the association of human papilloma virus in a majority of cases (Zur Hausen, 1991). Evidence suggests that viral infection by HPVs is necessary, but not sufficient to induce invasive cancer. Since only a percentage of infected individuals develop cancer after a long latency period, cervical epithelium must undergo additional genetic changes caused by other factors for malignant transformation to occur in vivo (Zur Hausen, 1989). DNA tumor viruses appear to exert some of their proliferative and oncogenic effects on the host cell by the interaction of viral encoded oncoproteins with critical proteins in the cell cycle regulation (Helland et al., 1993). It is in this context that alterations in protooncogenes and tumour suppressor genes (p53 and RB) have been widely studied in cervical cancer. The p53 gene has an important role in the growth and maintenance of the genomic integrity of the cells. In situations of p53 protein dysfunctioning, genetically damaged cells may survive and contribute to the malignancy process. During the last few years, many studies have revealed DNA changes in or near the p53 gene in many cancers (Hollstein et al., 1991; Oliner et al., 1992).

The expression of p53 using immunohistochemical method has been widely studied in cervical as well as other cancers (Crook et al., 1992; Holm et al., 1993; Cooper et al., 1993, Helland et al., 1993). Alterations in p53 protein expression are important in the pathology of cervical carcinomas and may act
as a useful tumour marker as in a variety of other cancers (Thor et al., 1992, Votejesek et al., 1993). However, while using immunohistochemical methods for studying p53 expression, results depend on the type of fixative and conditions of fixation. To overcome this problem, two-site ELISA assay has been used as an alternative approach to determine the levels of p53 protein in cytosol extracts (Thor et al., 1992).

In the present study, p53 expression was analyzed from the serum of cervical cancer patients along with the healthy individuals as controls. The detection of p53 protein in serum, which otherwise is a nuclear phosphoprotein suggested that it might have been released from the cells during the tumour necrosis. This is also supported by the detection of antibodies to p53 in cancer patients (Crawford et al., 1992; Kurvinen et al., 1996) which can only be formed, if p53 protein comes in contact with B-cells or is expressed on the cell surface (Schlichtholtz et al., 1992; Soussi et al., 1994).

An overexpression of p53 was observed in 61.5% of cases. Maximum level of p53 was found in the lone case of carcinoma in situ, while it was not detected in a case with adenocarcinoma. Kurvinen et al. (1996) have reported high levels of p53 expression in high percentage (83%) of low grade squamous intraepithelial lesions as compared to high grade lesions. In another study by Holm et al. (1993), p53 expression was detected in 62% of invasive cervical squamous cell carcinoma, 11% of invasive adenocarcinoma and 7% of carcinoma in situ, but the low grade lesions were negative thus, suggesting the involvement of p53 in progression of cervical cancer. The present study has not revealed any correlation between the stage of disease and the p53 expression. A study with more cases at early stage of cancer, would be more conclusive.

The wild type p53 has a short half life and hence under normal conditions is undetectable in tissues (Gronstajaski et al., 1984; Finlay, 1988). The accumulation of p53 could be due to its stabilization which is often the result of
point mutations leading to changes in the conformation of protein (Soussi et al., 1994). Monoclonal antibody PAb 1801 used in the present study can in fact detect both the mutant and the wild type p53, thus, the overexpression seen in the cancer patients could be due to the accumulation of mutant p53 or to the overexpression of wild protein needing further analysis. The detection of overexpression also varies with the type of antibody used. Helland et al. (1993) detected increased level of p53 protein in 51 of 92 primary tumours using polyclonal antibody (CMI) and in 17 of 92 samples with monoclonal antibody (PAb 1801). The discrepancy between the two antibodies could be due to overexpression of normal p53 or mutated p53 with a configuration detected by the polyclonal, but not the monoclonal antibody. However, some studies have indicated that p53 protein overexpression is not always associated with the presence of mutations (Helland, 1993). Overexpression in the absence of mutations could be caused by binding to another cell protein (Moll et al., 1992; Momand et al., 1992; Leach et al., 1993; Reifenberger et al., 1993).

The status of p53 in relation to HPV infection in the patients revealed 42.8% (3/7) cases positive for HPV, but negative for p53. This could be due to the binding and degradation of p53 by oncoprotein E6 of high risk HPV types i.e., 16 and 18 (Crook et al., 1994). The loss of wild type p53 can, thus, provide a growth advantage to the cells expressing E6 (Scheffner et al., 1990). However, in cancer patients with high level of wild type p53, the non-functioning could be due to the mutations in other genes controlling cell proliferation upstream or downstream of p53 (Scheffner et al., 1990; Demers et al., 1994). The present study showed 28.2% (2/7) of cases to be positive for both HPV and p53. Cooper et al. (1993) have also reported the co-localization of HPV and overexpression of p53 using immunohistochemical method with antibody PAb 240 which could detect both the wild type promoter form of p53 and the mutant p53 as both of them share the same conformation. In addition, mutation in p53 gene may render p53 protein non-degradable by E6 of high risk.
HPV types and could provide a further growth advantage in HPV associated carcinoma even in the presence of active viral E6 expression (Scheffner et al., 1990).

The high level of p53 observed in carcinoma in situ in our study could be due to infection by low grade HPVs as has also been pointed out by Kurvinen et al. (1996), where 86% of the lesions infected with HPV 6/11/42 revealed high levels of p53 expression. E6 oncoprotein of low risk HPVs can modulate the activity of p53, but does not lead to its degradation (Crook et al., 1994). The absence of HPV as well as p53 has also been reported by Cooper et al. (1993) suggesting that not all the HPV negative tumours accumulate p53 protein. Moreover, the samples negative for HPV DNA by dot blot may harbour HPV DNA in low amounts or may have other HPV types not picked up by the probes used in the present study. Thus, the pattern of p53 expression and HPV status in cervical cancer patients observed in the present study suggests that alteration in p53 expression and the HPV infection may be related or independent events in the carcinogenesis of cervix. However the assessment of p53 levels may be useful to predict in clinical course of cervical HPV lesions. But a considerably larger number of cases and an increased observation time would be required to confirm the prognostic value of p53 overexpression in cases with cervical cancer.

In the light of above discussion it becomes evident that cervical cancer results from a complex interplay of a number of common behavioural risk factors as well as molecular and cytogenetic events.