CHAPTER 5

HPV AND RELATED CYTOLOGY
CHAPTER 5: HPV VIRUS AND RELATED CYTOLOGY

5.1: INTRODUCTION

According to WHO projections, in 2005 there were over 500,000 new cases of cervical cancer, of which over 90% were in developing countries. It is estimated that over 1 million women worldwide currently have cervical cancer, most of whom have not been diagnosed, or have no access to treatment that could cure them or prolong their life. In 2005, almost 260,000 women died of the disease, nearly 95% of them in developing countries, making cervical cancer one of the gravest threats to women’s lives. In many developing countries, access to health services is limited and screening for cervical cancer either is non-existent or reaches few of the women who need it. In these areas, cervical cancer is the most common cancer in women and the leading cause of cancer death among women. The primary underlying cause of cervical cancer is infection with one or more high risk types of the human papillomavirus (HPV), a common virus that is sexually transmitted.

Fig. 1 (a) Winner of Nobel Prize 2008 for discovery of HPV as a Key factor for cervical carcinoma (German virologist, Harald zur Hausen)
Most new HPV infections resolve spontaneously; if it persists, infection may lead to the development of pre-cancer, which left untreated, can lead to cancer (WHO, 1995).

Human papillomaviruses (HPV) are members of the papillomaviridae family of DNA viruses. Because HPV cannot be cultured easily in the laboratory, HPV infection is most commonly diagnosed by detecting HPV DNA. Differences in sequences of DNA are used to determine different HPV types. More than 120 HPV types have been indentified, over 30 of which infect the genital area. Although the majorities of infections causes no symptoms and are self-limited, genital HPV is of public health concern because persistent infection with certain types can cause cervical cancer in women.

Genital HPV infections are categorized according to their association with cervical cancer. Infections with low-risk types, primarily 6 and 11, can cause benign or low-grade cervical cell changes and genital warts, but are not associated with cervical cancer. Infection with high-risk types, primarily types 16, 18, 31 and 45 can cause low-grade cervical cell abnormalities, high grade cervical abnormalities that are precursors to cancer, and genital cancers. Most genital infections with either high risk or low risk HPV types go away on their own, without clinical consequences. Currently, one HPV DNA test is FDA-approved for use in women for cervical cancer screening; no HPV test is available for men.
The sequel of genital HPV infection with greatest public health importance is cervical cancer. Cervical cancer is relatively uncommon in the United States because widespread cervical Papanicolaou (Pap) testing can detect precancerous lesions before they develop into cancer. However, in many developing countries where cervical cancer screening activities are limited, cervical cancer is the most common cancer in women. Based on multiple lines of evidence, both the International Agency for Research on Cancer and National Institute of Health (NIH, 1996) have concluded that high-risk genital HPV infections act as carcinogens in the development of cervical cancer. While infection with high-risk types appears to be “necessary” for the development of cervical cancer, it is not “sufficient” because cancer
doesn't develop in the vast majority of women with HPV infection (WHO, 1995). Other co-factors appear to be necessary for the development of cervical cancer. HPV infection is also associated with anogenital cancers at other sites including the vulva, vagina, penis and anus. Each of these is substantially less common than cervical cancer, with the exception of anal cancer in homosexual men (Koblin et al., 1996). The association of genital types of HPV with non-genital cancer is less well established, but studies support a possible role in a subset of head and neck (Herrero et al., 2003) and esophageal cancers (Syrjanen, 2002).

Data on prevalence and incidence of HPV infection are limited because there is no routine screening for HPV infection, and it is often unclear whether a newly diagnosed infection is recently acquired or longstanding. Neither HPV infection nor genital warts are routinely reported to state health departments for the following reasons: (a) no standard justification for recommending STD case reporting (e.g., patient care measures such as curative treatment for patients and their sex partners, or monitoring ongoing prevention programs) exists for genital HPV infection or warts, (b) most infections clear spontaneously, and (c) case reporting would create a large burden for providers, health departments and laboratories given the prevalence of infection (Division of STD Prevention. Prevention of Genital HPV infection and Sequelae : Report of an External Consultant' Meeting (1999).

However, because cervical cancer is a rare and late manifestation of HPV infection, cancer surveillance provides limited information on the burden and current trends of HPV infections.

It has been estimated that at least 50% of sexually active men and women acquire genital HPV infection at some point in their lives; a
recent estimate suggests that 80% of women will have acquired genital HPV by age 50 (Koutsky et al., 1988; Myers et al., 2000). An estimated 9.2 million sexually active adolescents and young adults 15 to 24 years of age are currently infected with HPV (Weinstock et al., 2000).

Studies have found that the prevalence of HPV infection is lowest in women who have never had sexual intercourse (Fairley et al., 1992; Winer et al., 2003). Genital HPV infection is especially common among sexually active young women (less than 25 years of age), with prevalence decreasing with older age (Baken et al., 1995; Burk et al., 1996).

By far, the most common infections are with the high-risk types. Infection with multiple types of HPV occurs in approximately 5-30% of infected women (Bauer et al., 1991). HPV infection is most likely to be detected in women who have cervical cancer precursors; in one study, over 85% of women with cervical cancer precursors had detectable HPV DNA (Herrero et al., 2000).

These findings are supported by studies of incident (new) genital HPV infections, which can more accurately determine rates, as well as behavioral risk factors for infection. Studies of HPV incidence have been conducted in a variety of settings with variable follow-up periods. Incidence of HPV infection in college women studied for two to three years was 32-43% (Ho et al., 1998; Sellors et al., 2003). Other studies assessing populations of women using routine gynecological or family planning services found incidence of 11-32% in one year, and 44-55% in three years (Giuliano et al., 2002; Franco et al., 1999; Woodmen et al., 2001). The incidence of high-risk type, such as HPV-16, is higher than the incidence of low-risk types. For example, in one study the incidence
in one year was 32% for high-risk HPV types compared with 18% for low risk HPV types.

The risk factors consistently associated with HPV infection in women are young age (age less than 25 years) and sexual behavior, specifically number of sex partners. Other risk factors identified include early age of first sexual intercourse, and male partner sexual behavior. Less consistently identified risk factors include smoking, oral contraceptive use, nutritional factors, and lack of circumcision of male partners (Wheeler et al., 1993). Many of the identified risk factors are likely markers for unmeasured sexual behavior (Ley et al., 1991, Franco et al, 2001), Davidson et al., 1994). In addition, immune suppression is associated with HPV detection. Studies in women with HIV infection, undergoing dialysis, or after kidney transplant, demonstrate that HPV detection is particularly common with immune suppression (Fairley et a., 1994, Sun et al., 1997).
5.2: NATURAL HISTORY OF GENITAL HPV INFECTION

Most HPV infections are transient and asymptomatic, causing no clinical problems. Studies have shown that 70% of new HPV infections clear within one year, and such as many as 91% clear within two years (Molano et al., 2003; Moscicki et al., 1998). The median duration of new infections is typically eight months. HPV-16 is more likely to persist than other HPV types; however, most HPV-16 infections become undetectable within two years. Factors associated with persistence include older age, high-risk HPV types, infection with multiple HPV types, and immune suppression (Ho et al., 1995; Hildesheim et al., 1994). The gradual development of an effective immune response is thought to be the likely mechanism for HPV DNA clearance.

HPV infection that persists is the most important risk factor for cervical cancer precursors and invasive cervical cancer (Schlecht et al., 2003). A recent study found that the risk for developing cervical cancer precursors was 14 times higher for women who had at least three positive tests for high-risk HPV compared with that for women who had negative tests. However, most women with persistent HPV infection do not develop low-grade cervical cell abnormalities, cervical cancer precursors or cervical cancer (Koutsky et al., 1992).

Skin and mucosal changes caused by genital HPV infection—both genital warts and cervical cell abnormalities—often go away without treatment, probably as a result of the development of an effective immunologic response. Rates of spontaneous clearance and progression to cancer without treatment vary for low-grade and high-grade cervical cell abnormalities. Low-grade cervical cell abnormalities usually clear spontaneously (60% of cases) and rarely progress to cancer (1%), while
should be increased to once every 10 years, and then once every 5 years for women aged 35-55 years. If resources are high and a large proportion of the target groups are being screened, screening should be extended, first to older women (up to age 60) and then to younger women (down to age of 25). If additional resources are available and a high proportion of the target group are being screened every 5 years, the frequency of screening should then be increased to once every 3 years for women ages 25-60 years - World Health Organization, 1992.
5.3: REVIEW OF LITERATURE

Carvalho et al., (2003) in their study detection of human papillomavirus DNA by the hybrid capture assay from Rio de Janeiro, Brazil did pap smears and HPV testing on 1055 women attending their clinic for routine examination. The average age of the participants was 31.5 years. Five hundred and ten (48.3%) of the women presented HPV infection, as detected by Hybrid Capture. Two hundred thirty three out of the 510 (45.7%) infected women were from 21-30 years old; this age interval had the highest rate of HPV infection.

There were significant differences in the mean age of the patient in the different Pap (severity) groups. The Pap 1 group (22.1) was significantly younger than the severe Pap IV (HSIL) group (22.1 and 44.3 years, respectively).

Among the 510 HPV-positive women, 60 (11.8%) had HPV DNA with a low risk for cancer, 269 (52.7%) had high-risk types and 181 (35.5%) had both types. Four hundred and fifty (88.2%) of the women were infected by at least one high risk HPV type.

The HPV DNA prevalence was determined for each cytological diagnosis group (Pap test): the overall prevalence of HPV DNA in the studied group was 58.4% (265/454) ranging from 29.6% (8/27) in Pap I to 84.8% (28/33) in Pap IV. Significant differences were detected in HPV infection of cervix between Pap I (normal smears) and Pap IV (carcinomas).

Low risk types (Group A) were detected in 3.5% of Pap II, in 7.3% of Pap II+ HPV and in 8.7 % of Pap III cases (LSIL); while in Pap IV, no low risk HPV was found alone, indicating a downward trend. Group B types, comprising high risk HPV types were detected in most of the cases,
with increasing prevalence according to the severity of the cytological diagnosis; from 10% (17/169) in Pap II to 85% (28/33) in Pap IV.

On comprising value obtained for Pap I and LSIL (for 10.9 to 185.0), they found significant differences for low risk HPV infections, suggesting an association between viral load and risk of SIL. The same trend was found for high SILs (from 22.1 to 723.1 in HSIL). Multiple infections (Group A/B) gave the highest RLU values, even in PAP I smears, but the differences were still significant (from 64.2/223.4 in PAP I to 345.1/850.7 in HSIL).

They found that HSILs were exclusively associated with high-risk HPVs. No low-risk virus was found alone in the smears. High risk HPV was present in every case of HSIL, showing a 100% correlation between high-risk HPV infection and cancer risk.

Louise et al., (2000) reported their study Human Papillomavirus DNA testing for Cervical Cancer Screening in Low-Resource Setting. Cervical samples from 2944 previously unscreened South African women aged 35-65 years were tested for high-risk types of HPV with the use of the Hybrid Capture I (HCI) assay. Women also had a Pap smear, direct visual inspection of the cervix, and Cervicography. Samples from women with biopsy-confirmed, low-grade squamous intraepithelial lesions (SILs) (n = 95), high-grade SILs (n = 74), or invasive cervical cancer (n = 12) and a random sample of women with no cervical disease (n = 243) were tested for HPV DNA with the use of the more sensitive Hybrid Capture II (HCII) assay. High-risk HPV DNA was detected in 73.3% and 88.4% of 86 women with high-grades SIL or invasive cancer and in 12.2% of 2680 and 18.1% of 243 women without evidence of cervical disease, with the use of the HCI and HCII assays.
respectively. HPV DNA testing with the HCII assay was more sensitive than cytology for detecting high-grade SIL and invasive cancer (McNemar’s test), and testing with the HCI assay was of equivalent sensitivity. Cytology had a better specificity (96.8%) than either the HCI assay (87.8%) or the HCII assay (81.9%). Receiver operating characteristic curves identified test cutoff values that allow HPV DNA testing to identify 57% of women with high-grade SIL or cancer, while classifying less than 5% of women with no cervical disease as HPV DNA positive. They concluded that HPV DNA testing has sensitivity equivalent to, or better than, that of cytology. Since HPV DNA testing programs may be easier to implement than cytologic screening, HPV testing should be considered for primary cervical cancer screening in low-resource setting.

Shin et al., (2003) in their study prevalence of human papillomavirus infection in women in Busan, South Korea examined a randomly selected sample of 863 sexually active women (age range =20-74 years, median 44) and 103 self reported virgins from Busan. The presence of DNA of 34 different HPV types in cervical exfoliated cells was tested among sexually active women by means of a PCR-based assay. The overall prevalence of HPV DNA was 10.4% (95% confidence interval, CI: 8.5-12.7%). The most often found HPV DNA types were HPV 70, HPV 16 and HPV 33; 19.8% (95% CI: 17.2-22.0) of sexually active women had antibodies against one or more HPV types. The most common anti-VLPs were against HPV 18, 31 and 16.

Schiffman et al., (2000) in their study HPV DNA testing in cervical cancer screening result from women in a high-risk province of Costa Rica, 8554 non pregnant, sexually active women without hysterectomies
underwent initial HPV DNA testing using the original Hybrid Capture Tube test; a stratified sub sample of 1119 specimen was retested using the more analytically sensitive second generation assay, the Hybrid Capture II test.

In their results an analytic sensitivity of 1.0 pg/ml using the second generation assay would have permitted detection of 88.4% of 138 high-grade lesions and cancers (all 12 cancers were HPV-positive), with colposcopic referral of 12.3% of women.

Sankarnarayan et al., (1999) did a study titled Comparative efficacy of visual inspection with 4% acetic acid, HPV testing and conventional cytology in cervical cancer prevention : A cluster randomized control screening trial in Osmanabad district, Maharashtra, India. Women aged 30-59 years in 52 cluster of 497 villages in Osmanabad district, India, were randomized to a single round of screening by trained midwives with either VIA (N=34,149), Cytology (N=32,136), HPV testing (N=34,515) or to a control group (N=30,378). All laboratory tests were done locally. Test-positive women have further investigations. (colposcopy /biopsy) and treatment in the base of hospital. Data on participation, test positivity, cervical intraepithelial neoplasia (CIN) detection and treatments rates were analyzed. A randomized controlled trial was done with 52 primary health centres randomized into 3 intervention arms (VIA, cytology, HPV testing) and one control arm and 34,000 eligible women (30-59 years) in each arm. They concluded over 75% of women complied with invitation to screening. Over 80% of screen-positive women complied with diagnostic investigations and treatment. VIA has a higher test positivity rate (14%) than cytology (7%) and HPV testing (10%). VIA detected significantly higher CIN 1 lesions as
compared to cytology and HPV testing. VIA detected significantly lower CIN 2-3 lesions than cytology; cytology and HPV testing had similar detection rates of CIN 2-3 lesions. VIA is a useful alternative, but requires careful monitoring and quality assurance, HPV testing is not associated with improved detection of CIN 2-3 lesions compared to cytology, despite high investments. The ultimate effectiveness of the 3 approaches will become clear with follow up for cancer incidence and mortality.

Ho et al., (1998) in Natural history of cervicovaginal papillomavirus infection in young women, reported the cumulative 36-month incidence of HPV infection was 43 percent (95 percent confidence interval, 36 to 49 percent). An increased risk of HPV infection was significantly associated with the younger age, Hispanic ethnicity, black race, an increased number of vaginal sex partners, high frequencies of vaginal sex and alcohol consumption, anal sex, and certain characteristics of partners (regular partners having an increased number of lifetime partners and not being in school). The median duration of new infections was 8 months (95 percent confidence interval, 7 to 10 months). The persistence of HPV for >6 months was related to older age, types of HPV associated with cervical cancer, and infection with multiple types of HPV but not with smoking. The risk of an abnormal Pap smear increased with persistent HPV infection, particularly with high-risk types (relative risk, 37.2; 95 percent confidence interval, 14.6 to 94.8). They concluded that the incidence of HPV infection in sexually active young college women is high. The short duration of most HPV infections in these women suggests that the associated cervical dysplasia should be managed conservatively.
Oh et al., (2001) analyzed the concurrent cervical smears and biopsy, and correlated them with the HPV infection status. They also evaluated histologically proven cases with ASCUS smears according to HPV infection. HPV DNA was identified in eight (0.7%) if 1144 cytologically normal patients; nine (10.5%) of 86 ASCUS; seven (25.0%) of 28 LSIL; 26 (78.8%) of 33 HSIL; and in all of three squamous cell carcinomas (SCC). HPV positivity was significantly associated with cytohistological diagnosis for HSIL of more. In addition, HPV positive ASCUS cases were found to be associated with histological abnormality rather than HPV-negative. The results indicate that high-risk HPV testing by PCR could be a useful adjunct tool for Pap smear in primary cervical screening. The combination of Pap smear and high-risk HPV testing by PCR might reduce unnecessary colposcopy-guided biopsy of women with cytological diagnosis of ASCUS.

Tena et al., (2005) investigated the usefulness of high risk human papillomavirus detection in women with abnormal pap smears as an adjunct to screening by cervical cytology. A total of 75 women underwent colposcopy, histological study and HR-HPV detection using the hybrid capture II test, in the pap-smear studies, HR-HPV was detected in 31.2% of women with ASCUS and 65.7% women with LG-SIL. Histological examination showed HR-HPV in 46.4% of women without lesions, 69.55 of those with LG-SIL and 75% with HG-SIL. Negative predictive values for HG-SIL in women with cytological diagnosis of ASCUS and LG-SIL were 81.8% and 92.3%, respectively.

Bigras et al., (2005) compared in a study involving 13,842 women and 113 gynaecologists, liquid based cytology and HPV testing for detecting cervical cancer. A total of 1334 women were found to be
positive for one or both tests and were invited for colposcopy with biopsy. A total of 1031 satisfactory biopsies on 1031 women were thereafter collected using a systematic biopsy protocol, which was random in the colposcopically normal appearing cervix or directed in the abnormal one. In all, 502 women with negative tests were also biopsied. A total of 82 histologic high-grade squamous intraepithelial lesions (HSIL) were reported in biopsies, all from the group with one or both tests positive. Sensitivity and specificity to detect histologic HSIL were 59 and 97% for cytology, and 97 and 92% for HPV. In total, 14% of reviewed negative cytological preparations associated with histologic HSIL contained no morphologically abnormal cells despite a positive HPV test. This suggested a theoretical limit for cytology sensitivity. HPV viral load analysis of 1143 HPV-positive samples showed a direct relationship between abnormal Pap test frequency and HPV viral load. Thus not only does the HPV testing have a greater sensitivity than cytology but the probability of the latter being positive can also be defined as a function of the associated HPV viral load.

*Flores et al., (2003)* describe some of the results of Moreles HPV study. The main objective of the Morelos HPV Study was to evaluate the use of human papillomavirus (HPV) DNA testing, as compared to the papanicolaou (Pap) test, for cervical cancer (CC) screening. The Morelos HPV study results indicate that HPV testing has a greater sensitivity to detect cervical intraepithelial neoplasia (CIN) 2/3 and CC than the Pap test. Their results also indicate an over all lower acceptability of the Pap test as compared to the self-collected procedure. The results of the CEA and CBA indicate that screening women between the ages of 20-80 for CC using some types of HPV testing is
always more cost effective than screening for CC using the Pap test. They concluded that self-and clinician-collected HPV testing could be used in CC prevention programs, as an effective complement or substitute for the Pap test.

*Mroueh et al., (2002)* determined the prevalence of HPV virus and more specificity HPV 16 in a group of Lebanese women. The population included 1026 women, 18-76 years, seeking routine gynaecological care at tertiary care center. Demographic & behavioral data were collected HPV DNA was detected in cervical scrapes by PCR using consensus primers. Cervical cytology abnormalities were detected using pap smears. Their mean age of population was 40+ 11.3 years. General HPV DNA was detected in 50 patients (4.9%), HPV 16 was detected in 31 patients (3%). The age-specific prevalence of HPV increased with age and peaked at 60-69 years.

*Brito et al., (2002)* evaluated the prevalence of HPV infection in American Indian women, from a tribe in Brazilian Amazonia. Demographic data, pap smears and cervical samples for HPV DNA detection by PCR were obtained for women aged above 10 years old. In all 79 women were interviewed, 78 and 49 women allowed collection of pap smears and PCR samples respectively. Cytological signs of HPV infection were observed in 11 patients; 6 of these were probed for HPV infection and 1 found to be HPV 16. Overall prevalence of HPV infection detected by PCR was 14.3%. 3 patients with high-risk HPV types; 2 HPV 16 and 1 co-infection of HPV 16 and 58.

*Lukaszuk et al., (2001)* estimated the HPV infection prevalence in healthy, asymptomatic women. Pap smears from 255 women were investigated according to The Bethesda Terminology. HPV DNA was
detected by PCR method. They were able to detect high "oncogenic risk" HPV types: 16, 18, 31, 33, 35, 39, 45, 52b, 58 and "low oncogenic risk"- HPV6 and 11 and found only 15 cases (5.9%) of HPV DNA presence. Most of them were HPV16- in 5 patients (33.3%) and HPV 58 in 3 (20%).

Costa et al. (1998) in their study of 221 patients with positive Pap smear (ages 16-65) to evaluate the association of cervicography and HPV DNA test with the probability of biopsy and final histology diagnosis of CIN2 or worse and identified the combinations of results on cervicography and HPV DNA test associated with the absence of such lesions, and also to estimated the cost of a potential triage protocol for patients with HPV-CINI smear. The probability of biopsy showed a univariate association with the severity of the smear result and the cervicography classification but not the HPV DNA test. In the multivariate analysis, only the cervicography result was a significant predictor of biopsy. The final histology diagnosis shows a univariate association with each of the three tests and a multivariate association with the degree of cytology positivity and the cervicography result. Among patients with HPV-CINI smears, only a negative cervicography (with any HPV DNA test result) was always associated with the absence of severe histologic lesions. This pattern accounted only for 7% of such patients. The additional costs of a potential triage protocol based on cervicography were estimated to exceed the saving resulting from the reduced colposcopy rate.
5.4: MATERIALS AND METHODS

The present study was carried out in the Department of Obstetrics & Gynaecology, Silchar Medical College & Hospital, Silchar, Assam. The study was carried from 2005 to 2009 (48 months). Out of 4,198 gynaecological admissions in this hospital during the said period, 100 cases were diagnosed as carcinoma cervix (invasive) and were included in the present study. The cases were selected on the following basis:

1. Women between 25 to 80 years of age.
2. Parity from nullipara to multipara irrespective of age.
3. Cases were selected from unhealthy cervix (selected screening).
4. Women under hormonal contraceptive.

Also a total of 500 women had cervical smear for detection of cervical cancer were also included in the study. The cases were selected on the following basis.

(a) Women between 25 to 50 years of age, irrespective of parity.
(b) Parity up to 4 or more irrespective of age.
(c) Women having cervical erosion, cervicitis, hypertrophied cervix, cervical polyp and prolapse with ulceration were considered in the group with unhealthy cervixes.
(d) Women using contraception.

A detailed clinical history including age, age at marriage & first child birth, number of children, socio-economic status, number of partner, religion and clinical complaints were recorded in a proforma and cervical smears were collected for cytological examination. Patients showing varying degrees of dysplasia e.g. moderate, severe & malignancy on cytology were subjected to histopathological examination on their subsequent visit. Repeat Pap smear was advocated in mild dysplasia.
# PROFORMA

- Case No. : 
- Date of Examination : 
- MRD No. : 
- Hosp. No. : 

**Clinical History:**
- Patients Name : 
- Age : (Yrs) 
- Religion : 
- Ethnic group : 
- Occupation : 
- Address : 

**History related to husband:**
- Husband’s Name : 
- Age : (Yrs) 
- Education : 
- No. of wife/wives : All living/divorces/died (If died cause of death)

- No. of sexual partner/ partners other than wife : 
- Health of the partner : 

Socio-economic status:

- Total monthly income:
- Husband:
- Wife:
- Others:

  (Low income group/middle income group/high income group)

- Total family members:
- Education of husband & wife:
- Living condition: Good/Fair/Poor

Marital status:

(i) Unmarried/ Married/ Remarried/ Divorced/ Widow.
(ii) Age at marriage
(iii) Wife – 1st/ 2nd/ 3rd
(iv) Duration of married life (in years)

Coital practice:

- Age at first coitus:
- Pre-marital/Marital:
- Frequency (per week):
- No. of partner, if other than husband:
5.1: Obstetrical History:

<table>
<thead>
<tr>
<th>No. of pregnancy</th>
<th>Duration of pregnancy</th>
<th>Mode of delivery</th>
<th>Complication of labour</th>
<th>Puerperium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(i) Infertility:  
(a) Primary  
(b) Secondary  

(ii) Family planning method used:  
(a) IUCD & its duration  
(b) Oral pills and its duration  
(c) Other conventional contraceptives  
(d) Sterilization: -Minilaparotomy/Laparoscopic sterilization  
(e) None

Menstrual History:  
(a) Age of menarche:  
(b) Cycle: Regular/Irregular  
(c) Flow: Scanty/Average/Heavy (based on number of pads changed per day)  
(d) Associated pain: Present/Absent  
(e) Vaginal discharge:
(f) Change in menstrual flow :

(g) Last menstrual flow period :

(h) Premenstrual spotting :

(i) Intermenstrual spotting :

(j) Menopause :
   (a) Age
   (b) Post menopausal bleeding
   (c) Post menopausal spotting
   (d) Post menopausal discharge

**Present Complaints:**

(a) Presenting complaints with duration.

(b) Vaginal discharge: Watery/White/Blood stained.

(c) Foul smelling/Purulent.

(d) Pain in Back/Abdomen.

(e) Urinary symptoms: Burning micturition/Frequency of micturition.

(f) Local symptoms: Ulceration/Itching/Something coming out per vaginum.

(g) Other constitutional symptoms.

**Personal History:**

(a) Smoking habit : Present/Absent

(b) General Hygiene :

(c) Diets : Vegetarian/Non-vegetarian

(d) Others :
Past History:

Relevant past History:

Physical Examination:

(i) General Examination:

- Position :
- Appearance :
- Oedema :
- Built :
- Pallor :
- Clubbing :
- Jaundice :
- Cyanosis :
- Neck glands :
- Koilonychia :
- Teeth & Gums :
- Pulse :
- Blood Pressure :
- Breast :

(ii) Systemic examination:

- Respiratory system :
- CVS :
- Alimentary system :
- Nervous system :
- Excretory system :
(iii) Local examination:
   (a) Inspection of vulva/vulval dystrophy/prolapse/normal.
   (b) Speculum examination:
       - Vagina: Normal/Cervicitis/Erosion/Ulceration
       - Nature of discharge, if any
       - Cervix: Normal/ Cervicitis/ Erosion/ Ulceration
       - Discharge: Serous and seropurulent, blood stained or foul smelling/ Growth of ectocervix or endocervix.

(iv) Inflammatory cells: Conclusion (cytological findings).

Bimanual Examination:

(a) Uterus:
   (i) Size: Normal / Atrophied / Enlarged
   (ii) Shape:
   (iii) Position: Anteverted/Retroverted
   (iv) Mobility: Present/Absent
   (v) Tenderness: Present/Absent
   (vi) Prolapse: Present/Absent

(b) Cervix: Mobility/Consistency/Friability/ Bleeding on touch/ Tenderness.

(c) Fornices: Mobility/Tenderness/Induration.

Rectal examination:
- Rectal mucosa: Free/Fixed
Laboratory Investigation:

(a) Urine :
(b) Blood : TC/DLC/ESR/HB%
(c) VDRL :
(d) HIV :
(e) RBS :

Cervical Cytology (Papanicolaou's stain):

- Cytology No. :
- Date of collection :

(a) Type of preponderant cells :
   (i) Superficial cells ____________________________%
   (ii) Intermediate cells ____________________________%
   (iii) Parabasal cells ____________________________%
   (iv) Basal cells ____________________________%

(b) Nuclei : Cytoplasmic ratio - Normal / Mildly / Moderately / Markedly (increased) / Cytoplasmic maturity.

(c) Cytoplasm : Eosinophil / Basophil / Organophilic

(d) Nucleus :
   (i) Nuclear membrane
   (ii) Chromatin :
      (a) Coarseness
      (b) Angularity
      (c) Hyperchromatism
      (d) Clearity in between.

(e) Nucleolus :

(f) Mitotic figures :

(g) Dyskaryotic cells :
   (i) Tadpole
   (ii) Fibre cells
   (iii) Third type cells
Histological Examination:

- Date of Biopsy:
- Description of specimen:
- Histopathological diagnosis:
- Final diagnosis:
- Follow-up Record:

Collection of specimen:

- Coplin jar.
- Clean glass slides with serial number (H/N) marked for identification and attached paper clip to separate the slides.
- Pencil for marking.
- Cusco's Bivalve speculum without any lubricant.
- Fixative - equal parts of ether and 95% alcohol.

The histopathological technique:

The tissue for histopathological examination were obtained either from cervical punch biopsy (acetic acid (4.5%) is applied to the cervix and punch biopsy is taken from the acetowhite areas) or from whole hysterectomy specimen. The processing of the tissue was carried out as follows:

**Step I**: A trimmed piece of tissue was kept in 10 percent formal saline for overnight for fixation. For identification of specimen label was attached with patients name.

**Step II**: The formal saline was then washed thoroughly with running water and tissue was cleaned & usually processed as follows.
Step III : Dehydration was carried out in the ascending grades of alcohol e.g. 50%, 2 changes, 2 hours each.

Step IV : Kept in absolute alcohol 2 changes (2 hours each).

Step V : Clearing was done in benzene (2 changes) 30 minutes each.

Step VI : Impregnation was done in paraffin both at 57.5°C for 2 hours.

Step VII : Tissues was taken out of the paraffin bath and block was made in ‘Leuikhart’s ‘L’ pieces by putting melted paraffin and allow to cool in cold water.

Step VIII : Serial sections were cut at 3 to 5 micron from the paraffin block. The ribbon obtained was placed in water bath at 56°C and 2 sections were lifted on albuminished slide from each specimen.

Staining procedure for haematoxylin and eosin stain:

Step I : The paraffin section was melted by keeping the slides in hot air over at 56°C

Step II : The slide was placed in xylol (3 changes) for clearing.

Step III : The section was then taken out of xylol and passed through descending grades of alcohol (absolute alcohol, 90 percent, 70 percent, and 50 percent) each step 1-2 minutes.

Step IV : Rinsed in running tap water for 5 minutes.

Step V : Stained with Harris haematoxylin for 3-5 minutes.

Step VI : Washed in running tap water for 5 minutes.
Step VII: Differentiation was done with acid alcohol, (1% HCL acid, 70% alcohol).

Step VIII: Blued in running tap water for 5 minutes.

Step IX: Dehydration in ascending grades of alcohol, 50 percent, 70 percent, 90 percent, absolute alcohol (1-2 minutes in each).

Step X: Stained with 1 percent alcoholic solution of eosin for half minutes.

Step XI: 3 changes was given in xylol for clearing and mounted in DPX and labelled.

Step XII: Treated with absolute alcohol in 2 changes.

Result of staining:
The nuclei of the cells takes bluish violet colour and cytoplasm stains orange to pink.

Microscopic examination of H & E stained Histopathological sections:

(a) Cellularity: It is noted as pleomorphic, benign or malignant, differentiated (well/moderate/poorly) or undifferentiated types of cells (small, round, small spindle, anaplastic and other specific cells).

(b) Characteristic of stroma: This is noted as regular and uniform stromal pattern or not, loose or compact, oedema, fibrosis, invasion by neoplastic process etc.

(c) Vascularity: Whether the vessels were well formed or ill formed lined by tumour cells or endothelium, vascular tumour, degeneration of blood vessels are noted.
d) Presence/absence of giant cells:
e) Presence/absence of mitotic figures:
f) Any ulceration, infiltration by inflammatory cells of specific granulomas are noted.
g) Infiltration of tumour into surrounding tissues are noted.
h) Malignant lesions are subtyped as well differentiated, poorly differentiated (anaplastic) specially the squamous cell (epidermoid) variant of carcinoma of the cervix.

Procedure for collection of materials: (for cytological study)

The material was collected from the gynaecological out patient department of Silchar Medical College & Hospital, Silchar. After evacuation of urinary bladder, the patient was kept either lithotomy or in left lateral position. The material was collected prior to bimanual examination. The vaginal introitus was opened up with the help of a cusco's bivalve speculum. The material was collected from the whole circumference of squaocolumnar junction, by means of Ayer's spatula, under direct vision. The cellular material was rapidly spread on two clear glass slides and care was taken that material from each side of spatula was present on each slide. The smear was then dipped immediately in a coplin jar containing equal volume of ether & 95% alcohol. The slides were kept in the solution for at least half an hour to ensure fixation.

Staining of smears:

The staging technique standardized by Papanicolaou (1941) was followed in this study.
Principle:

The alcohol wet fixation produces distinctive artifacts, stain results in well stained nuclear chromatin, differential cytoplasmic counterstaining of acidophilic and basophilic cells and cytoplasmic transparency due to alcoholic solutions.

Advantage:

(i) Gives a sharp nuclear staining
(ii) Differential cytoplasmic stain for hormonal study.
(iii) Imparting transparency to the cytoplasm which enables the observer to interpret cell underlined. This is preferably used for gynaecological and non-gynaecological specimen.

Characters:

1. Haematoxylin: This is only for nuclear staining.
2. OG. 6 & EA – 36: These are for cytoplasmic staining.

Reagents:

Harri’s haematoxylin:

Prepared as follows:

- Haematoxylin : 1 gm
- Absolute alcohol (95%) : 10 ml
- Potassium alum : 20 gms.
- Distilled water : 200 ml
- Mercuric oxide : 0.5 gm

The haematoxylin is dissolved in 95% alcohol and potassium alum in distilled water with the aid of heat. The two solutions are mixed
together. The mixture is just boiled at a temperature of 95°C. As soon as it starts boiling it is removed from the flame and mercuric oxide red is added bit by bit. The flask containing the solution is then immersed into cold water bath. After cooling it is filtered and stored in coloured bottle.

**Orange G - 6**

Orange G -6 solution is prepared as follows:

- Orange G (S. S.) : 0.5gm
- 95% Ethyl alcohol : 100 ml
- Phosphotungstic acid : 0.015 gm.

**Eosin Azure - 6 (E.A - 36):**

The stock solution of (a) light green S.F., yellowish, (b) Eosin yellow, (c) Bismark brown are prepared as follows:

(A) **Light green S.F.**:
- Light green S. F. yellowish (S. S.) : 0.5 gm
- 95% alcohol (Ethyl) : 100 ml

(B) **Eosin yellow**:
- Eosin yellow (S. S.) : 0.5 gm
- 95% Ethyl alcohol : 100 ml

(C) **Bismark brown**:
- Bismark brown (S. S.): 0.5 gm
- 95% Ethyl alcohol : 100 ml
From the stock solution, the working solution of E. A. - 36 is prepared as follows:

- Light green SF yellowish (A) : 45 ml
- Eosin yellow (B) : 45 ml
- Bismark brown (c) : 10 ml
- Phosphotungstic acid : 0.200 gm
- Saturated solution of lithium carbonate - 1 drop (aqueous)

All these solutions are kept in the refrigerator when not in use.

**Staining steps:**

The fixed slides are transformed directly from the fixative into the following solution:

- 80% Ethyl alcohol : 10 dips
- 70% ethyl alcohol : 10 dips
- 50% ethyl alcohol : 10 dips
- Distilled water : 10 dips
- Harris Haematoxylin : 3-5 minute
- Running tap water : 1 minute
- Hydrochloric acid (0.5%) : 5 dips
- Running tap water : 1 minute
- Distilled solution of lithium carbonate : 1 minute

(solution saturated 30 drops in 100ml of water)

- Running tap water : 1 minute
- 50% ethyl alcohol : 10 dips
- 70% ethyl alcohol : 10 dips
- 80% ethyl alcohol : 10 dips
- 95% ethyl alcohol : 10 dips
• Orange G – 6 : 1 minute
• 95% ethyl alcohol : 10 dips
• 95% ethyl alcohol : 10 dips
• E. A - 36 : 2 minutes
• 95% ethyl alcohol : 10 dips
• 95% ethyl alcohol : 10 dips
• Absolute alcohol : 4 minutes
• Xylin : 5 minutes

Slides are then mounted with D.P.X.

Examination of cytology slides:

The slides for examination was placed on the stage of microscope. Care was taken to place the slide in such a way that the identification number and name of the patient remain on the right hand side of the slide.

Result of the staining:

(a) *Epithelial cells*:

• Nuclei - Bluish violet
• Cytoplasm of superficial cell- Pink
• Cytoplasm of intermediate cell- Bluish green
• The least mature deeper cells take basophilic (cyanophilic) stain, while the mature superficial cells usually takes eosinophilic stain (acidophilic).

(b) *Red blood cells*:

These appear bright red.

(c) *Leucocytes*:

It stains pale blue with dark blue black nuclei.
(d) **Bacteria:**
These appear grey.

(e) **Trichomonad:**
These appear ovoid with faint grayish blue cytoplasm. They possess small elliptical nuclei situated at the pole.

(f) **Monilia:**
Hyphae appears pink, spores brilliant red.

(g) **Mucus:**
These appear pale blue or as pinkish strands.

**Microscopic examination:**

The smear stained by papanicolaou method were examined under low power first to verify the staining result. The smear which did not stain properly were discarded and they were carefully restrained from excess storage slide.

The smears were examined thoroughly. A total number of 200 cells were calculated by the help of blood cells calculator and classified. The classification of cells was based on morphological and staining characteristics.

The smears were also examined to detect the presence of atypical cells and were graded as mild, moderate and severe dysplasia depending upon the morphological changes in the cells. Particular interest was given to nuclear cytoplasmic ratio. The presences of malignant cells were also carefully examined. The classification of different grades of dysplasia was followed according to the criteria laid down by the international reference centre for nomenclature in exfoliative cytology (*Luthra, 1969*).
Cervical Cancer Screening: Detection of HPV

The direct detection of HPV in cervical specimens may offer an alternative or complement to population-based cytological screening. Recent studies have demonstrated that HPV test results are more sensitive (although they are less specific) than Pap smears in detecting high-grade dysplasia in older women (Schiffman et al., 2000). In most scenarios, women with positive HPV tests still have Pap tests or a diagnostic procedure to provide cytological or histological confirmation of their disease. Several technologies exist for the molecular detection of HPV infection. Most of these technologies, while sensitive and specific, are too costly and cumbersome to incorporate into large scale screening programs. Recently, molecular diagnostics kits have been tested in developed and developing countries through several large research projects incorporating HPV screening into cervical dysplasia detection programs (Serwadda et al., 1999). Currently, two commercially available molecular diagnostic kits have been approved by the United States Food and Drug Administration (US FDA) and field tested in the developing world. Both kits, the Hybrid Capture Tube test and the Hybrid Capture II test (HC II), are produced by the Digene Corporation (Gaithersburg, Maryland).
Table 5.2: Commonly used HPV screening tests:

<table>
<thead>
<tr>
<th>Test</th>
<th>Technique</th>
<th>Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAP Smear test</td>
<td>Cervical smear cytology study</td>
<td>Cell morphology study of stained smear under a microscope</td>
</tr>
<tr>
<td>Hybrid Capture Assays</td>
<td>Signal Amplification technique</td>
<td>It is a nucleic acid hybridization assay primarily used for detection of HPV</td>
</tr>
<tr>
<td>Polymerase Chain reaction (PCR)</td>
<td>Target Amplification Technique</td>
<td>A chemical reaction that results in synthesis of a large no. of target strands</td>
</tr>
</tbody>
</table>

1. Peri-nuclear halo
2. Enlarged nucleus
3. Hyper chromatin
4. Peripheral condensation of cytoplasm

Fig. 5 (c) Characteristics of Koilocytes (HPV infected cell) detected by Pap smear
Molecular-based HPV diagnostics remain largely untested in broad screening programs, however. Research and pilot projects are under way in several countries to clarify the diagnostic, clinical and programmatic implications of HPV screening for cervical cancer prevention. HPV cannot be cultured reliably in a laboratory setting; therefore, HPV diagnostics rely on molecular technologies that detect HPV DNA in cervical/vaginal samples.

Molecular techniques can be broadly divided into those technologies that are not amplified, such as nucleic acid probe tests and those that utilize amplification, such as polymerase chain reaction (PCR). Amplification techniques can be further divided into three separate categories: (1) target amplification, in which the assay amplifies the target nucleic acids (for example, PCR); (2) signal amplification, in which the signal generated from each probe is increased by a compound-probe or branched-probe technology; and (3) probe amplification, in which the probe molecule itself is amplified (for example, ligase chain reaction). To date, target and signal amplification techniques, in addition to non-amplified techniques, have been applied to the detection of HPV. Because there are many HPV types with different oncogenic potential, diagnostic tests must not only detect HPV DNA, they also must determine the types(s) present in each specimen. Several diagnostic technologies also are able to estimate a specimen's viral load, which approximates the average number of viral genomes in the cervical cells sampled. It has not been determined whether such semi-quantitative data yield clinically relevant information. Some studies have found no association between viral load and disease progression; (Nindl et al., 1999, Clavel et al., 1999) research is ongoing to further define this issue. Signal-amplified techniques for detecting HPV
include hybrid capture and branched DNA approaches. The most widely used technique is the hybrid capture technology as described below. Hybrid Capture Technology (HC), developed by the Digene Corporation, detects nucleic acid targets directly, using signal amplification to provide sensitivity comparable to target amplification methods. Digene has developed two products for the detection of HPV: the first generation Hybrid Capture Tube (HCT) test and the more recent Hybrid Capture II (HCII) assay. Both assays detect “high-risk” HPV types. The HCT test detects the following high-risk types (as initially defined by Digene and supported by epidemiological studies); 16, 18, 31, 33, 35, 45, 51, 52 and 56. HCT was granted US FDA approval in May 1995. In March 1999, the US FDA approved Digene’s second-generation HPV detection kit (HC II). Four additional viral types were added to the high-risk category in the HC II test: 39, 58, 59 and 68. The level of detection of the second-generation HC II is rated at 5,000 viral copies per sample, or one picogram of HPV DNA per sample (in contrast to HCT, which detects 10 picograms). To perform the HC assay, cervical or vaginal clinical specimens-collected through self-sampling or obtained by a health care provider during a pelvic examination-are combined with an extraction buffer to release and denature the target HPV DNA. The released target DNA then combines with specific RNA probes to create RNA-DNA hybrids, which are captured onto a solid phase by an antibody specific for the hybrids. These captured RNA-DNA hybrids are then tagged with antibody reagents linked to alkaline phosphatase. A chemiluminescent substrate then produces light that is measured on a luminometer in relative light units (RLUs). The amount of light generated is proportional to the amount of target DNA in the original specimen (Digene, Inc. Hybrid
capture technology.

Another 100 patients were selected randomly for HPV virus DNA test and evaluation. HPV DNA test was done by enzymatic methods of HPV DNA PCR in our series as follows:

**Polymerase Chain Reaction (PCR) - based assays:**

PCR allows the in vitro replication of specific DNA target sequences in order to generate sufficient copies for subsequent detection and analysis. The first step in this process requires the separation of the double-stranded target DNA into single strands (denaturation), which is accomplished by heating the sample to about 95°C. At this temperature, the hydrogen bonds between the complementary DNA bases break and the strands separate. The next step (annealing) involves cooling the reaction to 40-60°C, at which temperature short synthetic single stranded DNA molecules, called oligonucleotides will hybridise with their complementary sequences on the target strands. These oligonucleotides then act as primers for the last step in the reaction (extension) in which a thermostable DNA polymerase enzyme catalyses the formation of two new double stranded DNA molecules (amplicon) using each of the original target DNA single strands as templates.

By repeating this cycle of denaturation, annealing and extension, each newly synthesized double-stranded DNA molecule can serve as a template for the next cycle, and the number of molecules increases in an exponential fashion. PCR can theoretically produce 10^6 identical copies from a single double stranded DNA molecule after 30 cycles of amplification and thereby achieve its exceptionally high sensitivity.
5.5: ELECTRON MICROSCOPE

We examined cervical carcinoma tissue under electron microscope to see ultramicroscopic morphological alterations of carcinoma tissue in different grades of the disease.

For this purpose the cervical tissue was collected from operation theater and were preserved in 10% buffered formalin (in 0.1 M phosphate buffer, pH 7.2) for 24 hours at room temperature just after the tissue were removed from the patient either by minor or major operation. The tissue were dissected and prepared for SEM examination. The SEM examination was done under a LEO 1430 VP electron microscope (Carl Zeiss, Carl Zeiss-Str. 56, 73447 Oberkochen, Germany) in Jashlok Hospital, Mumbai.
5.6: RESULTS AND DISCUSSION

Table 5.3: Distribution of the study subjects according to the cervical Cytology Report

<table>
<thead>
<tr>
<th>Cervical cytology</th>
<th>No. of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>150</td>
<td>30%</td>
</tr>
<tr>
<td>Inflammatory</td>
<td>198</td>
<td>39.6%</td>
</tr>
<tr>
<td>ASCUS</td>
<td>80</td>
<td>16%</td>
</tr>
<tr>
<td>LSIL</td>
<td>50</td>
<td>10%</td>
</tr>
<tr>
<td>HSIL</td>
<td>20</td>
<td>04%</td>
</tr>
<tr>
<td>Cancer Cervix</td>
<td>02</td>
<td>0.04%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>500</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

Fig 5.3: The Bar Diagram showing distribution of the study subjects according to the Cervical Cytology Report
5.6: DISCUSSION

Usha Saraiya 1984-1998 had cancer cervix detection by cytology of 1.36% which is very high in compared to our series might be the fact that they had a more selective screening than our series. She also reported 0.38% of HSIL whereas our series it was only 04% which again much lower in our series.

5.7: CONCLUSION

Pap smear were taken from all the 500 subjects; reports were normal in 150 cases (30%), presence of inflammatory cells in 198 cases (39.6%), ASCUS in 80 cases (16%), LSIL in 50 cases (10%), HSIL in 20 cases (04%) and only 2 cases (0.4%) was found in cervical cancer.
Fig. 5 (d) : LSIL showing cellular architecture of nuclear and cytoplasm both in low power and high power field
Fig. 5 (e) : HSIL showing cellular architecture of nuclear and cytoplasm both in low power and high power field
Fig. 5 (f) : HSIL (Moderate dysplasia) as looked on in histopathological examination
Fig. 5 (g) : Pap smears showing inflammatory smears on both the photographs
Table 5.4: Distribution of study subjects according to age and Cytology Reports

<table>
<thead>
<tr>
<th>Types of Cytology</th>
<th>30-35</th>
<th>35-40</th>
<th>40-45</th>
<th>45-50</th>
<th>50-55</th>
<th>55-60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>70</td>
<td>30</td>
<td>30</td>
<td>00</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>38.46%</td>
<td>20%</td>
<td>30%</td>
<td>00%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Inflammatory</td>
<td>80</td>
<td>50</td>
<td>40</td>
<td>20</td>
<td>00</td>
<td>08</td>
</tr>
<tr>
<td></td>
<td>43.95%</td>
<td>33.33%</td>
<td>40%</td>
<td>50%</td>
<td>00%</td>
<td>44.44%</td>
</tr>
<tr>
<td>ASCUS</td>
<td>20</td>
<td>30</td>
<td>20</td>
<td>10</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td></td>
<td>10.98%</td>
<td>20%</td>
<td>20%</td>
<td>25%</td>
<td>00%</td>
<td>00%</td>
</tr>
<tr>
<td>LSIL</td>
<td>10</td>
<td>20</td>
<td>10</td>
<td>10</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td></td>
<td>05.49%</td>
<td>13.33%</td>
<td>10%</td>
<td>25%</td>
<td>00%</td>
<td>00%</td>
</tr>
<tr>
<td>HSIL</td>
<td>00</td>
<td>20</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td></td>
<td>00%</td>
<td>13.33%</td>
<td>00%</td>
<td>00%</td>
<td>00%</td>
<td>00%</td>
</tr>
<tr>
<td>Cancer Cervix</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>02</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td></td>
<td>00%</td>
<td>00%</td>
<td>00%</td>
<td>0.10%</td>
<td>00%</td>
<td>00%</td>
</tr>
<tr>
<td>Total</td>
<td>182</td>
<td>150</td>
<td>100</td>
<td>40</td>
<td>10</td>
<td>18</td>
</tr>
</tbody>
</table>

5.6: DISCUSSION

From the above table it is evident that the grades of lesion increases with the age of the patients (LSIL 13.33% in 35-40 years of age so also HSIL). Similar results were obtained by Wahi et al., 1962; Upreti, 1980 and Chauhan et al., 1987.
5.7: CONCLUSION

When distributed according to age, 25% of the study subjects in the 45-50 year age group had ASCUS, whereas another 25% in the same age group had LSIL. 20% of subjects in both the 35-40 and 40-45 year age groups had ASCUS, whereas it was only 10.98% in the 30-35 year age group. 13.33% of the subjects in the 35-40 year age group had LSIL and another 13.33% in the same age group had HSIL.

The table indicates that the number of subjects considered under the different types of Cytology differ significantly as indicated by the corresponding p value 0.0015 (<0.05).

In the similar manner one can also conclude that the number of subjects considered under the different ‘Age Groups’ differ significantly as indicated by the corresponding p value 0.0016 (<0.05).
Table 5.5: Prevalence of HPV infection out of random 100 cases of HPV study

<table>
<thead>
<tr>
<th>HPV status</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV positive</td>
<td>16%</td>
</tr>
<tr>
<td>HPV negative</td>
<td>84%</td>
</tr>
</tbody>
</table>

5.6: DISCUSSION

Though our series shows high prevalence of HPV infection but Shin et al., 2003 showed a prevalence of 10.4% in his South Korean series.

5.7: CONCLUSION

Prevalence of HPV infection is 16% in general population in reproductive years which is quite high in compare to other studies in our series.
Fig. 5 (h): Human papillomavirus (HPV)16/18 detection by type-specific PCR

Fig. 5 (i): Detection of HPV 16 by PCR
HPV typing with enzyme restriction.

Fig. 5 (j) Detection of HPV-16 PCR product by agarose gel electrophoresis.
Table 5.6: Distribution of high-risk HPV positive cases in the study sample according to age

<table>
<thead>
<tr>
<th>HPV status</th>
<th>30-35</th>
<th>35-40</th>
<th>40-45</th>
<th>45-50</th>
<th>50-55</th>
<th>55-60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>06</td>
<td>02</td>
<td>04</td>
<td>02</td>
<td>02</td>
<td>00</td>
</tr>
<tr>
<td></td>
<td>16.67%</td>
<td>6.6%</td>
<td>20%</td>
<td>25%</td>
<td>100%</td>
<td>00%</td>
</tr>
<tr>
<td>Negative</td>
<td>30</td>
<td>28</td>
<td>16</td>
<td>06</td>
<td>00</td>
<td>04</td>
</tr>
<tr>
<td></td>
<td>83.33%</td>
<td>93.33%</td>
<td>80%</td>
<td>75%</td>
<td>00%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Fig 5.6: The Bar Diagram showing distribution of high-risk HPV positive cases in the study sample according to age

5.6: DISCUSSION

In our series it is clearly indicates that the HPV infection is common in younger age group but persistence of HPV infection in higher age groups is generally high risk HPV virus. Similar results were also found by other authors, Baken et al., (1995); Burk et al., (1995).
5.7: CONCLUSION

16.67% of subjects in the 30-35 year of age group tested positive for high-risk HPV, 6.6% in the 35-40 year of age group, 20% in the 40-45 year of age group, 25% in the 45-50 year of age group and 100% in the 50-55 year age group were positive for high risk HPV. So, this clearly indicates persistence of HPV infection in higher age groups are mostly high risk HPV infection.
Table 5.7: Distribution of high-risk HPV among different cytology reports

<table>
<thead>
<tr>
<th>HPV status</th>
<th>Cervical cytology (LBC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>Positive</td>
<td>04</td>
</tr>
<tr>
<td></td>
<td>13.3%</td>
</tr>
<tr>
<td>Negative</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>86.67%</td>
</tr>
</tbody>
</table>

5.6: DISCUSSION

The similar result of high association around 40-50% between abnormal cytology and high risk HPV virus is also found Carvalho et al., (2003) is their Brazilian study.
5.7: CONCLUSION

Of the 100 samples tested, only 16 were positive for high-risk HPV. When co-related with cervical, only 4 of the 30 normal cytology were HPV positive (13.33%), 6 of the 16 cases of AUCUS (37.50%), 4 of the 10 cases of LSIL (40%) and 2 of the 4 cases of HSIL (50%) were positive for high-risk HPV. So, it clearly indicates an upward trend of abnormal cytology with high risk positive virus.
Detection of HPV-16 PCR product by agarose gel electrophoresis

Fig. 5 (k): Agarose gel electrophoresis of PCR products of HPV type 16

Fig. 5 (l): Detection of HPV 18
Table 5.8: Distribution according to high-risk HPV status and age at marriage

<table>
<thead>
<tr>
<th>Age at marriage (in years)</th>
<th>HPV status</th>
<th>Percentage</th>
<th>Negative</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;18 years</td>
<td>Positive</td>
<td>10</td>
<td>33.3%</td>
<td>20</td>
</tr>
<tr>
<td>≥ 18 years</td>
<td>Negative</td>
<td>06</td>
<td>8.6%</td>
<td>64</td>
</tr>
</tbody>
</table>

5.6: DISCUSSION

Thus, younger age at marriage is significantly associated with HPV positive status (< 18 years) more than marriage at higher age (>18 years). Similar result was also found by Prussia et al., (2002).
5.7: CONCLUSION

10 subjects of the total 30 who were married at less than 18 years of age tested positive for high-risk HPV (33.3%), whereas, only 6 of the 70 subjects who were married at 18 years or more of age tested positive for the same (8.6%).

Thus, younger age at marriage was significantly associated with positive HPV status among the study subjects.

Analysis: The $\chi^2$ test for contingency tables is performed. This test is used to test if two attributes are independent of each other. The $\chi^2$ statistic value is 9.58 and the corresponding p-value is 0.002 (< 0.05). This indicates that there is a relation between HPV status and Age at Marriage.
Table 5.9: Distribution according to high-risk HPV status and number of partners

<table>
<thead>
<tr>
<th>HPV status</th>
<th>No. of partners</th>
<th>Positive</th>
<th>Percentage</th>
<th>Negative</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≥ 2</td>
<td>06</td>
<td>60%</td>
<td>04</td>
<td>40%</td>
</tr>
<tr>
<td></td>
<td>01</td>
<td>10</td>
<td>11.1%</td>
<td>80</td>
<td>88.9%</td>
</tr>
</tbody>
</table>

Fig 5.9: The Bar diagram showing distribution according to high-risk HPV status and number of partners

5.6: DISCUSSION

In our series 60% of the cases shows high risk HPV positive having more than 1 or 2 sexual partner (more than 1 or 2 marriage). Similar result was also found by Wheeler et al., (1993).
5.7: CONCLUSION

6 out of 10 subjects who had 2 or more partners tested positive for high-risk HPV (60%), whereas, only 10 out of 90 subjects were positive for high-risk HPV (11.1%).

Thus, there was a significant association between HPV positive status and more than one partner (marriage) among the study subjects.

Analysis: The $\chi^2$ test for contingency tables is performed. This test is used to test if two attributes are independent of each other. The $\chi^2$ statistic value is 16.01 and the corresponding p-value is 0.000. This indicates that there is a relation between HPV status and Number of partners.
Table 5.10 (a) : Distribution according to high-risk HPV status and smoking status

<table>
<thead>
<tr>
<th>HPV status</th>
<th>Smoking status</th>
<th>Positive</th>
<th>Percentage</th>
<th>Negative</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>04</td>
<td>40%</td>
<td>06</td>
<td>60%</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>12</td>
<td>15%</td>
<td>68</td>
<td>85%</td>
</tr>
</tbody>
</table>

➢ Out of 100 patients 10 patients were occasional smokers so not included in the study.

5.6: DISCUSSION

Cancer Epidemiological Biomarkers Prev. 2006, also clearly indicates a strong association of HPV infection and smoking.
5.7: CONCLUSION

4 of the 10 subjects who were smokers tested positive for high-risk HPV (40%), whereas, only 15% who did not smoke tested positive for the same.

Thus, there was no significant association between smoking and HPV positive status among the study subjects.

Analysis: The $\chi^2$ test for contingency tables is performed. This test is used to test if two attributes are independent of each other. The $\chi^2$ statistic value is 3.8 and the corresponding p-value is 0.051 (more than 0.05). This indicates that HPV status and Smoking status are independent but closely associated.
Table 5.10 (b) : Distribution according to high-risk HPV status & education

<table>
<thead>
<tr>
<th>Educational status</th>
<th>HPV positive</th>
<th>HPV negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary or less</td>
<td>08 (50%)</td>
<td>32</td>
</tr>
<tr>
<td>Upto secondary</td>
<td>04 (25%)</td>
<td>30</td>
</tr>
<tr>
<td>More than secondary</td>
<td>04 (25%)</td>
<td>22</td>
</tr>
</tbody>
</table>

5.6: DISCUSSION

Franceschi et al., 2009 also indirectly mentioned about associations of HPV infection in lower educated groups of people.

5.7: CONCLUSION

50% of the high-risk HPV positive study subjects had just received primary education or were illiterates. 25% of the high-risk
HPV positive study subjects were educated up to secondary level while the remaining 25% were educated more than secondary level.

**Analysis:** The $\chi^2$ test for contingency tables is performed. This test is used to test if two attributes are independent of each other. The $\chi^2$ statistic value is 0.937 and the corresponding p-value is 0.626. This indicates that HPV status and Educational Status are independent.
Table 5.10 (c): Distribution according to high-risk HPV status and per capita income

<table>
<thead>
<tr>
<th>Per capita income (In rupees)</th>
<th>HPV positive</th>
<th>HPV negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1,000</td>
<td>04 (25%)</td>
<td>20</td>
</tr>
<tr>
<td>1,000-2,500</td>
<td>10 (62.5%)</td>
<td>44</td>
</tr>
<tr>
<td>&gt; 2,500</td>
<td>02 (12.5%)</td>
<td>20</td>
</tr>
</tbody>
</table>

Fig 5.10 (c): The Pie Chart showing distribution according to high-risk HPV status and per capita income

5.6: DISCUSSION

Schiffman et al., 1993 also found a similar association in lower socio economic group and HPV infection.
5.7: CONCLUSION

25% of the high-risk HPV positive population in the study belonged to the relatively lower socio-economic status than the 62.5% of the high-risk HPV positive study subjects who belonged to the relatively better privileged socio-economic group. 12.5% of the high-risk HPV positive study subjects belonged to the relatively well-privileged socio-economic status.

Analysis: The $\chi^2$ test for contingency tables is performed. This test is used to test if two attributes are independent of each other. The $\chi^2$ statistic value is 1.044 and the corresponding p-value is 0.593. This indicates that HPV status and Per capita income are independent of each other.
5.8: BIBLIOGRAPHY


factor for persistent cervical dysplasia. Journal of the National Cancer Institute; 87 (18): 1365-1371.


