PREVALENCE OF HIGH RISK HUMAN PAPILLOMA VIRUS IN SYMPTOMATIC WOMEN ATTENDING OUR GYNAECOLOGY OPD

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CERTIFICATE

*This is to certify that the dissertation on "**Prevalence of High Risk Human Papiloma Virus in Symptomatic Women Attending our Gynaecology OPD"** is a bonafide work done by **Dr.Vijayalakshmi Palanisamy**, in Institute of Obstetrics & Gynaecology, Egmore, Chennai - 8, Madras Medical College, Chennai - 3 during 2007 - 2010 under my supervision and guidance in partial fulfillment of the regulation laid down by the Tamil Nadu Dr.M.G.R.Medical University for MD (Obstetrics & Gynaecology) Branch - II Degree Examination to be held in March 2010.*

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DECLARATION

I. ***Dr.Vijayalakshmi Palanisamy***, solemnly declare that the dissertation titled **"PREVALENCE OF HIGH RISK HUMAN PAPILLOMA VIRUS IN SYMPTOMATIC WOMEN ATTENDING OUR GYNAECOLOGY OPD**" has been prepared by me.
This is submitted to the Tamilnadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the rules and regulations for the M.D. Degree Examination in Obstetrics and Gynaecology. This has not been submitted previously by me for the award of any degree or diploma from any other university.

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INTRODUCTION

Invasive cancer of cervix is considered as a preventable disease because it has a long preinvasive state, cervical cytology screening programs are currently available and the treatment of preinvasive lesions is effective. Normally the lifetime probability for developing cervical cancer is 1:128.Although screening programs in US are well established, it is estimated that 30% of cervical cancer cases will occur in women who have never had a PAP test. In developing countries, this percentage approaches sixty.(1)
Human Papilloma Virus is the major aetiological agent for cervical cancer, the second common cancer among the women everywhere in the world (Munoz et al 1994). This virus is also implicated in other anogenital cancers.HPV is among the most important viruses in causation of cancer. A large number of epidemiological, biological and chemical studies are underway to get to know the nature of this infection and its outcome. The prospects of HPV vaccine in preventing cervical cancer make it the most suitable target for studies in low resource settings with high morbidity and mortality from cervical cancer.
Cervical cancer is the second most common cancer among women worldwide. The majority of the cases occur in the developing world, where in most countries, it is the leading cause of cancer mortality in women. (2)
An estimated 470,000 new cases of cancer cervix are diagnosed each year worldwide and 80% of these occur in the developing world. A quarter of the global burden is experienced in India, where about 126,000 new cases and 71000 deaths attributable to cervical cancer are estimated to occur each year(3,4). Cancer cervix constitutes15-51% of all female cancers and rates of age-standardised incidence range from 17.5 to 55% per 100,000 women in different regions of India(4,5). More than 80% are diagnosed at an advanced clinical stage and 5 year survival is less than 40%(6,7). In many developed countries a decline in the incidence and mortality caused by cervical cancer has been observed in the past 30 years as a result of screening by cytology(8,9).
India has the burden of having the largest number of women with cervical cancer contributing to18% of world's total number. One out of every 5 women in world suffering from cervical cancer is from India (10).
Tamilnadu has the large incidence of cervical cancer with very high number of 28.6 per 100,000 women from Tiruvallur district (ICMR) (11). Chennai PBCR has had the highest incidence rate of cervical cancer among the Indian PBCRs. This AAR is somewhat lower than that seen in the registries in Africa and Brazil (Parkin et al 2002). The district wise MAARS indicate a belt of high incidence rates even higher than that in Chennai PBCR in North-eastern districts of Tamilnadu including Pondicherry which had the highest MAAR of 39.2/100,000, closely followed by Villupuram with a MAAR of 31.1/100,000, and Cuddalore with MAAR 29.9/100,000 (cancer atlas india.org).
Worldwide HPV 16 and 18 cause approximately 70% of cervical cancer, AIS,CIN 3,VIN2 and VaIN 2/3and 50% CIN 2-the eight most common high risk genotypes(HPV16,18,45,31,33,52,58 &35) account for 90% of cervical cancer cases. Apart from HPV 16/18, each individual genotype causes a small proportion(less than 5%)of cases(12-16).
HPV 6,11,16 & 18 cause 35-50 % of all CIN1,VIN1 &Va1N1cases(17).It is now universally accepted that nearly all cervical cancers of high grade intraepithelial neoplasia are associated with high risk HPV types. Owing to the strong association, it has been suggested that high risk HPV detection might be used as a tool to identify women at risk for subsequent development of cervical cancer. Guidelines need to be formulated for HPV testing in cervical cancer screening and for vaccination. For this, age prevalence of high risk HPV in cytologically normal cervical smear needs to be determined.
With this aim, we undertook the present study to determine the prevalence of high risk HPV infections in women with symptoms of chronic discharge per vaginum attending our Gynaecology OPD and those with cancer cervix.

CERVICAL INTRAEPITHELIAL NEOPLASIA

The concept of pre invasive disease of cervix was introduced in 1947, when it was recognised that epithelial changes could be identified that had the appearance of invasive cancer but were confined to the epithelium (17.1).Subsequent studies showed that if these lesions are not treated, they can progress to cervical cancer (18).Improvement in cytological assessment led to the identification of early precursor lesions called dysplasia, which signalled possible development of future cancer. The concept of CIN was introduced in 1968 when Richard indicated that all dysplasias have the potential of progression (19).
It is now recognised that most CIN 1 & some CIN 2 lesions regress spontaneously if treated (20); Nevertheless CIN refers to a lesion that may progress to invasive carcinoma. This term is equivalent to the term dysplasia, which means abnormal maturation; consequently proliferating metaplasia without mitotic activity should not be called dysplasia. Squamous metaplasia should not be diagnosed as dysplasia or CIN because it does not progress to invasive cancer.

SQUAMOCOLUMNAR JUNCTION

The squamocolumnar junction rarely remains restricted to the external os. Instead it is a dynamic point that changes in response to puberty, pregnancy, menopause and hormonal stimulation. Lactobacilli act on the glycogen to lower the pH after menarche, stimulating the sub columnar reserve cells to undergo metaplasia.
Metaplasia advances from the original squamocolumnar inward, toward the external os and over the columnar villi. This process establishes an area called the transformation zone. The transformation zone extends from the original squamocolumnar junction to the physiologically active squamocolumnar junction.
In most cases, CIN is believed to originate as a single focus in the transformation zone at the advancing squamocolumnar junction. The anterior lip of the cervix is twice as likely to develop CIN as the posterior lip, and the CIN rarely originates in the lateral angles. The entire SCJ with early metaplastic cells is susceptible to oncogenic factors, which may cause these cells to transform into CIN. Therefore, CIN is most likely to begin either during menarche or after pregnancy when metaplasia is most active. Conversely, after menopause there is little metaplasia and is at a lower risk of developing CIN.

COLUMNAR EPITHELIUM

It has a single layer of columnar cells with mucus at the top and a round nucleus at the base. The glandular epithelium is composed of numerous ridges, clefts and infoldings, and when covered by squamous metaplasia, leads to the appearance of gland openings.

METAPLASTIC EPITHELIUM

Metaplastic epithelium found at the SCJ, begins in the subcolumnar reserve cells. Under stimulation of lower vaginal acidity, the reserve cells proliferate, lifting the columnar epithelium. The immature metaplastic cells have a large nuclei and a small amount of cytoplasm without glycogen. As the cells mature normally, they produce glycogen, eventually forming the 4 layers of epithelium. The metaplastic process begins at the tips of the columnar villi, which are exposed first to the acid vaginal environment. As the metaplasia replaces the columnar epithelium, the central capillary of the villus regresses and the epithelium flattens out, leaving the epithelium with its typically vascular network. As

metaplasia proceeds into the cervical clefts, it replaces the columnar epithelium and similarly flattens the epithelium. The deeper clefts, however, may not be completely replaced by metaplastic epithelium, leaving mucus secreting columnar epithelium trapped under the squamous epithelium. Some of these glands open onto the surface; others are completely encased with mucus collecting in nabothian cysts. Gland openings and nabothian cysts mark the original SCJ and the outer edge of the original transformation zone.

NORMAL TRANSFORMATION ZONE

The original squamous epithelium of vagina and exocervix has 4 layers

1. The basal layer is a single row of immature cells with large nuclei and a small amount of cytoplasm.
2. The parabasal layers include 2-4 rows of immature cells that have normal mitotic figures and provide the replacement cells for the overlying epithelium.
3. The intermediate layers include 4-6 rows of cells with larger amounts of cytoplasm in a polyhedral shape separated by an intercellular space. Intercellular bridges, where differentiation of glycogen production occurs, can be identified with a light microscopy
4. Superficial layers include 5-8 rows of flattened cells with small uniform nuclei and a cytoplasm filled with glycogen. The nucleus becomes pyknotic, and the cells detach from the surface. These cells form the basis for Pap testing.

CIN

CIN is graded according to the proportion of epithelium occupied by undifferentiated cells. In CIN 1 (Mild dysplasia) only the basal third or less of the epithelium is occupied by the undifferentiated cells. A lesion is classified as CIN 2 (Moderate dysplasia) if the cells occupy between 1/3 and 2/3rd of the epithelium. If more than 2/3rds of the epithelial thickness is occupied by undifferentiated cells, a diagnosis of CIN 3 is made .In most severe form of CIN 3 ,atypical cells occupy the full thickness of the epithelium and mitotic figures, often abnormal, are seen at all times(21).
It takes 1-17 years for an in situ lesion to become invasive, with an average of 10 years. So the mean age at which carcinoma in situ is found is 35 years, the comparable figures for micro invasive carcinoma and invasive cancer being 44 and 53 respectively. Recently in developed countries there is second peak of invasive carcinoma at between 30 and 40 years and in these younger women the change from an in situ to invasive carcinoma appears to be much faster(weeks or months rather than years).Coexisting HPV infection may accelerate the pace of the disease.

COLPOSCOPY OF THE CERVIX

It is the examination of the vagina and the cervix in vivo with a binocular microscope (the colposcope) using an external white light for illumination. Currently, screening methods for cervical cancer include cervical cytology (Pap smear), visual inspection after application of cetic acid (VIA), or Lugols iodine (VILI) and the high risk HPV DNA tests. Colposcopy is used to obtain directed cervical biopsies in screen positive women for the histopathologic diagnosis of cervical intraepithelial (preinvasive) or invasive cancer.

INDICATIONS FOR COLPOSCOPY

1. Evaluation of the women with squamous or glandular cell abnormalities on Pap smear, with no gross lesions on cervix or vagina.

2. Persistence of inflammatory cells despite adequate treatment.
3. Persistence of keratinized cells
4. Evaluation of women who test positive for the visual screening tests: VIA and VILI
5. Evaluation of women who test positive for screening high risk HPV DNA test
6. Evaluation of women with postcoital bleeding, metrorrhagia and postmenopausal bleeding
7. Naked eye examination reveals unhealthy cervix or vagina suspicious of malignancy
8. Monitoring of women treated for CIN
9. Evaluation of women exposed to DES in utero

The most important part of colposcopy is identifying and visualising the SCJ in its entirety. The cervix is swabbed with 5% acetic acid solution followed by 50% Lugols iodine.

The colposcopic examination is unsatisfactory if the SCJ is not visualised in its entirety, the procedure is not possible because of constant bleeding from the friable epithelium, or the cervix is not visualized completely.(22)

COMBINED COLPOSCOPIC INDEX

Reid and Scalzi proposed a scoring system to predict the histologic diagnosis on the basis of four colposcopic features. In RCI, colposcopic signs, i.e., margins, colour , vascular patterns and iodine staining are graded into two objective categories; low grade CIN 1 or high grade CIN2 and CIN 3, combined into a single index called the Reids colposcopic index. (23)

REIDS COLPOSCOPIC INDEX (24)

Colposcopic sign	0	1	2
margin	Condylomatous or micropapillary Contour flocculated, or feathered Jagged, angular, satellite lesion beyond TZ	Regular lesion with smooth borders	Rolled indistinct peeled edges
colour	Shiny snow white, semi-transparent opaque	Shiny grey-white intermediate	Dull oyster-white grey
vessels	Uniform fine calibre, non-dilated capillary loops	Absence of surface vessels	Definite punctuation and mosaic
iodine	Positive iodine uptake	Partial iodine uptake	Yellow staining lesion

Colposcopy score
0-2 HPV OR CIN 1
3-4 CIN1 OR CIN2
5-8 CIN 2 OR CIN 3

HUMAN PAPILLOMA VIRUS

Beral (1974) postulated that exposure to sexually transmitted infection is an important determinant of cervical cancer .Zur Hausen(1991)suggested that HPV infection and HPV viral gene expression have emerged as necessary but not significant factors for cancer induction. Reviewing the epidemiological evidence linking HPV to cervical cancer, Bosch et al (1997) concluded that over 90% of cervical cancer could be attributed to certain HPV types. The central role of HPV in cervical carcinogenesis has far reaching implications in prevention of this cancer.

MOLECULAR VIROLOGY OF HPV

HPV is a small double stranded DNA virus that is a member of the Papova virus group .The subtype of HPV are not serotype viruses but are genotypes in which the typing scheme is based on the similarity of one HPV type to the other known HPV types at the DNA level .The central HPV DNA repository is in Heidelberg and this facility assigns a new type if HPV after adequate studies .The viral genome of HPV consists of approximately 7900 nucleotides and all viral gene transcription occurs off one strand.

The HPV genome may be divided into three parts based on the function of the encode genes :the early E region E6,E7,E1,E2,E4,and E5 and the late L region.L1 L2and L3 and non –coding region which harbours the origin of replication and transcription control signals essential for the regulatory functions of the genome.

HPV genome is approximately 7900 bp in length The early (E) region of the genome is separated from the late (L) region by the long control region (LCR) that contains sequences involved in the regulation of expression of HPV proteins.

The viral genome DNA in fully formed viral particles is surrounded by a protein coat known as the viral capsid that consists of the (L) regions L 1 and L 2; The (E) region proteins are associated with cell transformation and viral gene regulation and are most critical in the pathogenesis of invasive cancer. Between (E) and (L) lies the LCR, which contains promoter and enhancer DNA sequences critical to viral gene transcription by both viral and cellular genes.

The specific HPV types exhibit a degree of tissue tropism. Some types such as HPV 1 and 2 are most found in the keratinized skin of the palms and soles in the form plantar and palmar warts .The types such as 6, 11, 16 and 18 are most found in the keratinized skin and the mucosal surfaces of the anogenital region including the cervix .Types 16 and 18 are considered to have a malignant phenotype as they exhibit a strong association with invasive cancer (Palafsky and Holly 1995).

DETECTION OF HPV INFECTION AND HPV GENOTYPES

Reliable and reproducible measurement of exposure is an important aspect of epidemiological investigations .In studying the relationship between HPV and cervical neoplasia ,it is not always possible to obtain tissue biopsies .A cytological finding of koilocytosis represents HPV infection but cytology cannot detect the larger percentage of infections at the DNA level .The limit of the earlier epidemiological studies was the lack of an appropriate method for assessing type specific HPV infection.

Serological methods were also used to study the HPV infection. Seroreactivity to HPV infection is a reflection of the cumulative exposure to HPV. It is also useful to use serological methods to study the transmission of HPV. High risk of seropositivity to HPV 16 with multiple sexual partners has been shown in studies (25).

The techniques of Southern, dot-blot and filter in situ hybridisation have suffered from problems of sensitivity and specificity, particularly when DNA extracted from cervical smears was examined (Munoz et al1988).The polymerase chain reaction (PCR) is an in vitro method for primer directed enzymatic amplification of specific target DNA sequences (Saiki et al 1988).PCR generates millions of copies of a specific DNA fragment in a few hours by in vitro enzymatic synthesis. HPV-PCR consists of amplification of a targeted portion of the viral DNA and identification of the amplified product, PCR has been extensively used for HPV identification from exfoliated cells and biopsy tissues and for facilitating cloning and sequencing of HPV genomes. Stringent measures have to be adopted to avoid contamination of the specimen for PCR diagnosis. PCR has been shown to be more sensitive than filter in situ hybridisation and Southern blot analysis in the detection of HPV in cervical scrapes (Melchers et al 1989).

ETIOLOGICAL ROLE OF HPV IN CANCER CERVIX

Experimental studies have provided strong evidence that HPV is the long sought venereal cause of cervical neoplasia .A series of epidemiological studies with adequate exposure measurement in different settings have confirmed this finding.(Munoz et al 1994).
Prevalence of HPV DNA ranging from 22% to 100% have been reported in case series from different settings(IARC 1995).Bosch et al (1995) carried out a world wide prevalence study of HPV in cervical cancer in 1995.More than 1000 specimens from 22 countries were studied .HPV DNA was detected in 93% of tumours with no significant variation in HPV positivity among countries .HPV 16 was present in 50% of the specimens and HPV 18 in 14% of the specimens .These results confirmed the role of genital HPV in causation of cervical cancer .The etiological role of HPV and other risk factors in precancerous lesions of the cervix have been reviewed by Moorthy and Mathew(2000)

THE NATURAL HISTORY OF CERVICAL HPV INFECTION

Cervical carcinogenesis passes through well-defined entities as the changes occur in the epithelium .The age specific incidence of cervical dysplasia shows that it was most often diagnosed among women in their 20s, carcinoma in situ among those aged 30-39 years and invasive cancer after the age of 40 years (Canadian Task Force 1976)
Basal cells in the cervical epithelium rest on the basement membrane which is supported by the dermis. HPV is thought to access the basal cells through micro-abrasions in the cervical epithelium. Following infections the early HPV genes (E1, E2, E4, E5, E6 and E7) are expressed and the viral DNA replicates from the episomal DNA. In the upper layers of the epithelium (the mid-zone and the superficial zone) the viral genome is replicated further and the late L1 , and L2 and E4 are expressed.L1 and L2 encapsidate the viral genomes to form progeny virions in the nucleus. The shed virus can then initiate a new infection. Low grade intraepithelial lesions support productive viral replication. An unknown number of HPV viral infections progress to high grade intraepithelial neoplasia. The progression of untreated lesions to micro-invasive and invasive cancer is associated with the integration of HPV genome into the host chromosome with associated loss of disruption of E2 and subsequent up-regulation of E6 and E7 oncogene expression in LCR(long control region) (26).

PERSISTENT HPV INFECTION

It is now widely believed that a persistent infection with a high risk HPV type is necessary for the development of high grade CIN and invasive cancer.

HPV INTEGRATION

HPV can be found in cervical material in episomal forms, integrated forms or in mixed forms that contains both viral integration into the host cell genome occurs downstream of the early genes E6 and E7., often in the E1 or E2 region .This disruption results in the loss of a negative feedback control of oncogene expression by viral regulator E2 proteins .Integrant derived transcripts are more stable than those derived from episomal viral DNA and HPV 16 integration has been associated with selective growth advantage for affected cells (27).

HPV VIRAL LOAD

It is now clear that the relationship between the viral load and the disease varies with HPV types .For example cross sectional studies show that HPV 16 viral load increases with increasing disease severity ,whereas that of HPV 18 does not (28,29).
If the cytopathic effect observed in the exfoliated cells is a reflection of the viral load, then this might explain why the cytological changes detected after HPV 18 infection underestimate the severity of the underlying histological abnormality, unlike those detected after HPV 16 infections(30).

EPIGENETIC CHANGES IN CERVICAL NEOPLASIA

Both viral and the host gene can be targeted by the cellular DNA methylation machinery .The pattern of methylation of the HPV genes varies with the viral life cycle, the presence of disease and possibly the viral type (31, 32, 33, 34).The denovo methylation of HPV DNA could be host defence mechanism for subsequent transcription of foreign DNA or a strategy that the virus uses to maintain a long term infection or both (35, 36) .

STAGES LEADING UPTO CERVICAL CANCER AFTER HPV INFECTION

HPV infection usually clears within a few months ;about 90% of the infections clear within 2 years(37).Persistence of infection beyond 12 months is associated with increased risk of cancer .It can lead to moderate or severe CIN 2 or CIN3 or to adenocarcinoma in situ often grouped together as CIN2 or CIN 3 or AIS; which if untreated has a high probability of progressing to cancer(38). HPV infects the basal layer of the epithelium. Most infections of the cervix are asymptomatic and the virus is cleared without treatment, median time for clearance is 8 months (39).More than 90% of the infections are cleared within 2 years (39,40,41,42). Early HPV infections may be accompanied by mild changes in the epithelium .An abnormal growth of the squamous cells of the cervix ,detected by cytological examination of the cervical smears is called a squamous intraepithelial lesion .The changes in cells are described as low grade (LSIL) or high grade(HSIL),depending on how much of the cervical epithelium is affected and how abnormal the cells appear .Equivocal changes seen on cervical smears are called atypical squamous cells or atypical glandular cells .Abnormal cells of the cervix detected by histological examination of the cervical biopsies are classified as cervical intraepithelial neoplasia (CIN).;They are graded from CIN1 to CIN3 according to the proportion of the cervix affected. The majority of LSIL or CIN1 lesions disappear within a few months without treatment .If HPV infection persists however, it can lead to moderate or severe CIN2 orCIN3,or to adenocarcinoma in situ, which if untreated has a high probability of progressing to cancer.

CERVICAL CANCER

It refers to the malignant condition of the cervix.

SIGNS AND SYMPTOMS OF CANCER CERVIX

The early stages of cervical cancer may be completely asymptomatic. Patients are commonly 30 years and above. In some developing countries like Uganda, it has been noted that younger women are reporting to health facilities with advanced disease of cervix compared to previous years .This has been attributed to immune suppression from HIV or AIDS .The common symptoms for cancer

of cervix include:

- Intermenstrual bleeding
- Postcoital per vaginal bleeding
- Abnormal per vaginal discharge which tends to be foul smelling
- Lower abdominal pain ,backache are symptoms for advanced diseases due to the infiltration of cancer to involve nerves of the sacral plexus
- Leakage of urine or stool incontinence may occur when the cancer has advanced to stage 4 disease to involve the urinary bladder and rectum respectively.

NB: It is very important to do a vaginal and a speculum examination to be able to look at the cervix .Its important to have a good source of light when looking at the cervix.

CAUSES

HPV infection

The most important risk factor in the development of cervical cancer is infection with a high risk strain of HPV. The virus cancer link works by triggering alterations in the cells of the cervix. More than 150 types of HPV are acknowledged to exist. Of these 15 are classified as high risk types (16,18,31,33,35,39,45,51,52,56,58,59,68,73,and 82), 3 as probable high risk (26,53,66) and 12 as low risk (6,11,40,42,43,44,54,61,70,72,81,CP6108) (43), but even those may cause cancer. Types 16 and 18 are generally acknowledged to cause about 70% of the cervical cancer cases. Together with type 31, they are the prime risk factors for cervical cancer (44).

OTHER FACTORS THOUGHT TO BE ASSOCIATED WITH CANCER CERVIX

Marital and sexual factors

The epidemiologists have noted that risk of cervical cancer is strongly influenced by sexual behaviour. This has led to the discovery of role HPV infection. Studies have shown increased risk due to marriage at young age, onset of regular sex at an early age<20 years, multiple lifetime no. of sexual partners(45).These risk factors remain significant especially among those women without apparent HPV infections.

Role of male sexual partners

In most studies the husbands of cervical cancer patients were found to report more sexual partners, history of various genital infections like venereal warts, gonorrhoea and herpes simplex genitalis compared to husbands of control subjects. Frequent use of condoms was associated with a lower risk for cancer cervix (46).

Gynaecological and obstetric events

Multiparity with short intervals between pregnancies(<2 years) has been consistently shown to increase the risk of SIL and cervical cancer(47).There is little evidence to show that the risk of cervical cancer is affected by age at menarche and menopause, characteristics of menses and personal hygiene(48).

Contraceptive methods

Recent research is showing that long term users of oral contraceptives are at excess risk for cervical cancer, even after adjusting for sexual and social factors. The risk may be stronger for adenocarcinoma than squamous cell neoplasm (48) Regular users of barrier methods of contraception (condom or diaphragm) have been found to have lower risk of cervical cancer (49).

Genetic factors

Although some reports suggest that a familial tendency does exist, but there is still little attention to it (50).

Dietary factors

Micronutrients are thought to have a protective effect on cervical cancer, by promoting the regression of low grade intraepithelial lesion. Some components of fruits and vegetables have been suggested to be protective too (51).

Smoking

Some case control studies and a cohort investigation have demonstrated increased risk of cervical cancer and SIL among smokers even after controlling for most other risk factors. However the smoking effect is restricted to squamous cell carcinoma and not among other histological types (52).

Infections other than HPV

HSV-2 and chlamydiae have been shown to increase the risk (53).One of the studies conducted in Uganda showed an increased risk of cervical cancer with multiple and concurrent infections, thus addressing the hypothesis that chronic cervicovaginal infections may increase the risk of HPV (54).

COMPLICATIONS OF CANCER CERVIX

The common ones include:

- severe anaemia as a result of severe or chronic on and off bleeding from the cervix
- renal failure due to obstruction of the ureters by the infiltrating cancer
- lymphoedema due to the blockage of lymphatic drainage leading to swelling of the lower limbs
- vesicovaginal and rectovaginal fistula
- severe pain as a result of infiltration of the sacral nerves
- mortality is commonly due to anaemia and uraemia

DIAGNOSIS

Biopsy procedures

Confirmation of the diagnosis of cervical cancer or pre-cancer requires biopsy of the cervix. This is done through colposcopy, using VIA to highlight the abnormal cells on the surface of the cervix or a punch biopsy in a frank carcinoma (55).

PATHOLOGIC TYPES

Histologic subtypes of invasive cervical carcinoma include the following (56, 57).Though squamous cell carcinoma is the cervical cancer with the most incidence ,the incidence of adenocarcinoma of the cervix has been increasing in the recent decades(57).

1. Squamous cell carcinoma (about 80-85%)
2. Adenocarcinoma(about 15% of cervical cancers in UK)
3. Adenosquamous carcinoma
4. Small cell carcinoma
5. Neuroendocrine carcinoma

STAGING

Cancer cervix is staged by the International Federation of Gynaecology and Obstetrics (FIGO) staging system, which is based on clinical examination, rather than surgical findings. It allows only the following diagnostics tests to be used in determining the staging: inspection, palpation, colposcopy, endocervical curettage, hysteroscopy, cystoscopy, proctoscopy, intravenous urography, and X ray examination of the lungs and skeleton and cervical conisation.
The TNM staging system for cervical cancer is analogous to the FIGO staging

Stage 0-full thickness involvement of the epithelium without invasion into the stroma (carcinoma in situ)

Stage 1-limited to cervix

1A-diagnosed only by microscopy; no visible lesions
1A1- stromal invasion <3mm in depth and 7mm or less in horizontal spread
1A2- stromal invasion between 3 and 5 mm with horizontal spread of 7 mm or less
1B –visible lesion or microscopic lesion with more than 5 mm of depth or horizontal spread of more than 7 mm
1B1-visible lesion 4cm or less in greatest dimension
1B2- visible lesion more than 4cm

Stage 2- invasion beyond the cervix

11A- Without parametrial invasion, but involves the upper 2/3rd of the vagina
11A1-clinically visible lesion less than or equal to 4 cm in greatest dimension
11A2-clinically visible lesion more than 4 cm in greatest dimension
11B- with parametrial invasion

Stage 111-extends to pelvic wall or lower 1/3 of vagina

111A-involves lower third of vagina
111B- extends to pelvic wall or causes hydronephrosis or non-functioning kidney

Stage 1V-spread to pelvic organs or distal metastasis

1VA- invades mucosa of bladder or rectum and or extends beyond true pelvis
1VB-distant metastasis

TREATMENT

Microinvasive cancer cervix (stage 1A) is usually treated by hysterectomy. For stage 1A2, the lymph nodes are removed as well. An alternative for patients who desire to remain fertile is a local surgical procedure such as loop electrical excision procedure (LEEP) or cone biopsy (58).If cone biopsy does not produce clear margins one more possible option is a trachelectomy (59).
Early stages (1B1 and 11A <4cm) can be treated with radical hysterectomy with removal of the lymph nodes or radiation therapy. Radiation therapy is given as external beam radiotherapy to pelvis and brachytherapy (internal radiation)
Larger early stage tumours (1B2 and 11A >4cm) may be treated with radiotherapy and cisplatin based chemotherapy, hysterectomy, or cisplatin chemotherapy followed by hysterectomy.
Advanced stage tumours (11B-1VA) are treated with radiation therapy and cisplatin based chemotherapy.

PREVENTION

Awareness

According to the US National Cancer Institute s 2005 Health Information Trends survey, only 40% of American women surveyed had ever heard of HPV infection only 20% have heard of its link to cervical cancer (60).

SCREENING

The widespread introduction of the Papanicolaou Test for cervical cancer screening has been credited with dramatically reducing the incidence and mortality of cervical cancer in developed countries.(61).Recommendations for how often a pap smear should be done varies from once a year to once every 5 years. The ACS recommends that cervical cancer screening should begin approximately 3 years after the onset of vaginal intercourse and or no later than 21 years of age (62).Guidelines vary on how long to continue screening ,but well screened women who have not had abnormal smears can stop screening at the age of 65 to 70 (ACS).
Liquid based cytology has been incorporated within the UK national screening programme. Its main advantage has been to reduce the number of inadequate smears from 9% to around 1% (63).
The HPV test is newer technique for cervical cancer triage which detects the presence of HPV infection in the cervix. It is more sensitive than Pap smear, but less specific and its role in routine screening is still evolving. Since more than 99% of invasive cervical cancers worldwide contain HPV, some researchers recommend that HPV testing be done together with routine cervical screening (64).

PREVENTIVE VACCINATION

Gardasil, licensed and manufactured by Merck and Co. is a vaccine against HPV types 6, 11, 16, and18. Gardasil is upto 98% effective (65).It is now on the market after receiving approval from the USFDA on June 8, 2006(66). Gardasil has also been approved in the EU (67).
Glaxo Smithkline has developed a vaccine called Cervarix which has shown to be 92% effective in preventing HPV strains 16 and18 and is more effective for more than 4 years (68).
HPV vaccines are targeted at girls and women of age 9-26 because the vaccine only works if given before infection occurs. The high cost of this vaccine has been a cause for concern.

CONDOMS

They offer some protection against cervical cancer (69).

SMOKING AVOIDANCE

Carcinogens from tobacco increase the risk for many cancers including cervix cancer and women who smoke have double the risk of a non-smoker to develop cervical cancer.

NUTRITION

Fruits and vegetables

High levels of vegetable consumption were associated with a 54% decreased risk of HPV persistence (70).

Vitamin E

HPV clearance time was significantly shorter among women with the highest compared to lowest serum levels of tocopherols, but significant trends in these association were limited to infections of about 120 days(71).

Folic acid

Improving folate status in subjects at risks of getting infected or already infected with high risk HPV may have a beneficial impact in the prevention of cervical cancer (72, 73).

PROGNOSIS

Prognosis depends on the stage of the cancer. With treatment, the 5 year survival rate for the earliest stage of invasive cancer is 92% and the overall (all stages combined) 5 year survival rate is about 72% (74).

With treatment, 80 to 90% of women with stage 1 cancer and 50 to 65 % of those with stage 11 are alive 5 years after diagnosis. Only 25 to 35% of women stage 111 cancer and 15% or fewer of those with stage 1V cancer are alive after 5 years (75).

According to the FIGO, survival improves when radiation therapy is combined with cisplatin based chemotherapy (76).

AIM OF THE STUDY

The aim of the study is to determine the prevalence of high risk human papilloma viruses 16 and 18 in women with symptoms of chronic discharge per vaginum and those with cancer cervix attending our Gynaecology OPD.

MATERIALS AND METHODOLGY

SETTING OF THE STUDY

The study was conducted at the Institute of Obstetrics and Gynaecology, Egmore in collaboration with the Department of Biotechnology, Cancer Biology Lab, IIT, Chennai between 2008-2009.

STUDY DESIGN AND POPULATION

It is a hospital based study .300 Consenting women attending the Gynaecology OPD at our Institute were enrolled with 100 cases belonging to the symptomatic group and 100 belonging to cancer cervix and 100 cases who came to the OPD for other complaints.

INCLUSION CRITERIA

The women with the following criteria were included in the symptomatic group in our study

1. Age 16 to 70 years
2. Patients with complaints of persistent vaginal discharge.
3. Patients with complaints of intermenstrual or postcoital bleeding
4. Unhealthy cervix per speculum

Simultaneously 100 women with frank carcinoma cervix and 100 women who attended the Gynaecology OPD for other complaints who underwent total Hysterectomy due to complaints other than uterine cervical lesions such as uterine fibroid, endometriosis and so on were also studied for HPV prevalence.

EXCLUSION CRITERIA

The women fulfilling the following criteria were excluded from the study

1. Pregnant women and puerperium
2. Women with bleeding at the time of examination
3. Clinical evidence of acute infection
4. Unsatisfactory colposcopy
5. Unmarried women
6. Women undergoing radiotherapy or chemotherapy for cancer cervix
7. Women with cancer cervix recurrence

CONSENT AND ETHICAL CONSIDERATIONS

A written informed consent in local vernacular was obtained from each patient prior to enrolment in the study .Ethics Committee of the Institution approved the study protocol.

METHODOLOGY

Relevant socioeconomic, obstetric, gynaecological, and medical history was obtained in a pre-tested, semi structured questionnaire.

SPECIMEN COLLECTION IN SYMPTOMATIC WOMEN

Women with chronic discharge were examined under illumination per speculum, gross naked eye findings were noted and cases selected after considering the Pap smear report of inflammatory smear. Pap smear was taken by Ayre's spatula by rotating through 360 degrees across the squamocolumnar junction so as to obtain the representative samples. The smear was fixed in absolute alcohol. Cervical abnormalities identified by visual inspection with acetic acid or abnormal cytology were referred to colposcopy. Visible lesions identified by colposcopy were biopsied. A final diagnosis of CIN was assigned to each woman based on the review of cytology and histology.

Colposcopic index method: Using the colposcope the cervix was visualised under low power to note any abnormal findings. Capillaries and surface blood vessels of the cervix were examined with green filter. Freshly prepared 5% glacial acetic acid was gently applied twice over the cervix to ensure acetowhite reaction followed by visual inspection with Lugols Iodine using 50% iodine. Transformation zone was defined both at the proximal and distal ends. Colposcopy was considered unsatisfactory if the squamocolumnar junction was not visualised completely and advised endocervical curettage. In case of satisfactory colposcopy, the cervix was divided into 4 quadrants by an imaginary line passing through the centre from 6o clock to 6o clock and 3o clock to 3o clock. Examination of each quadrant was done in clockwise direction beginning from right upper quadrant. Acetowhite reaction was seen in the transformation zone; the margin, colour, vessels, and colposcopy signs were scored using RCI Scoring method and recorded. Colposcopic guided biopsies were taken with punch biopsy forceps from the site with the highest score. The tissue was immediately transferred to a vial containing 10% formaldehyde and sent for histopathological examination and a part of it was collected in RNA Later solution for HPV testing. Pressure was applied to the sites to control bleeding. Women were counselled for follow-up and genital hygiene. They were reviewed with cytology and histopathological reports were compared and further management was advised according to guidelines for CIN or CIS.

EVALUATION OF CERVICAL INFLAMMATION

Cervical inflammation was assessed by counting the number of neutrophils observed in microscope fields on Pap slides from each study subject. Enrolment Pap-stained smears were evaluated at x400 initially to identify cervical mucus. Smears having no identifiable cervical mucus were considered invalid for evaluation because of uncertainties related to sampling adequacy. Valid slides were observed at x1000 to identify neutrophils, identifiable by their multilobed nuclei. The numbers of neutrophils were counted in five nonadjacent fields not contaminated by squamous epithelial cells, and were averaged and scored as no inflammation (0–5 neutrophils/field), intermediate inflammation (6–30 neutrophils/field), and cervicitis (More than 30 neutrophils/field).

Importantly, to minimize bias, readings of Pap slides for cytopathology were done independently of the blinded assessments for Nugent score/ neutrophils by collaborators who are not trained to evaluate cytopathology of cervical cells

SPECIMEN COLLECTION IN WOMEN WITH FRANK CERVICAL CANCER

All 100 cases underwent the standard procedures to confirm the diagnosis of cervical cancer and establish the stage of the disease as per standard of care in the centre. Staging was based on International Federation of Obstetrics and Gynaecology criteria. During clinical examination, two small pieces of tissues were taken from the tumour using punch biopsy forceps, one sample sent for histopathological examination and the other sample collected in RNA later solution for HPV DNA

testing.

SPECIMEN COLLECTION IN WOMEN WITH OTHER COMPLAINTS

In Women with other complaints the cervical tissue samples were obtained from theatres posted for fractional curettage, after verifying the basic screening report.

DEFINITION OF A POSITIVE RESULT

Cytology reports were reported as positive when more than 30 neutrophils were detected per high power field. VIA was considered to be positive if definite acetowhite lesions were visualized closed to the squamocolumnar junction or if the entire cervix or growth of the cervix turned acetowhite. VILI was considered to be positive if yellow, iodine non-uptake areas were visualized close to the squamocolumnar junction, or if the entire cervix or growth on the cervix turned yellow.

HPV DNA TESTING

On enrolment, cervical tissue samples of the selected participants were assayed for HPV DNA using the Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). All samples were assayed for HPV DNA using a more sensitive PCR-based L1 consensus primer HPV test **(18, 19**). cDNA synthesized were tested for HPV by PCR using MY09/MY11 L1 consensus primers with primers for GAPDH as the internal PCR control. Valid PCR results were obtained from 300 samples. Amplified DNA was separated by electrophoresis, and HPV DNA was detected by Ethidium bromide staining.

RNA ISOLATION:

Trizol reagent from GIBCO BRL

It is a ready to use reagent for the isolation of total RNA from the cells and tissues. During sample homogenization or lysis, it maintains the integrity of the RNA, while disrupting cells and dissolving cell components._____

REAGENTS REQUIRED

- Chloroform
- Isopropyl alcohol
- 75% Ethanol (in DEPC-treated water)
- RNAse-free water or 0.5% SDS solution [To prepare RNAse-free water, draw water into RNAse-free glass bottles. Add diethylpyrocarbonate (DEPC) to 0.01% (v/v). Let stand overnight and autoclave. The SDS solution must be prepared using DEPC-treated, autoclaved water.

1. HOMOGENIZATION

Tissues

Homogenized tissue samples in 1 ml of TRIZOL® Reagent per 50-100 mg of tissue using a glass-Teflon® or power homogenizer (Polytron, or Tekmar's TISSUMIZER® or equivalent). The sample volume should not exceed 10% of the volume of TRIZOL®

Reagent used for homogenization.

2. PHASE SEPARATION

Incubated homogenized samples are kept for 5 minutes at 15 to 30°C to permit the complete dissociation of nucleoprotein complexes. 0.2 ml of chloroform per 1 ml of TRIZOL® Reagent is added. Tubes were shaken vigorously by hand for 15 seconds and incubated at 15 to 30°C for 2 to 3 minutes. The samples are centrifuged at no more than 12,000 × *g* for 15 minutes at 2 to 8°C. Following centrifugation, the mixture is separated into a lower red, phenol-chloroform phase, an interphase, and a colourless upper aqueous phase. RNA remains exclusively in the aqueous phase. The volume of the aqueous phase is about 60% of the volume of TRIZOL® Reagent used for homogenization.

3. RNA PRECIPITATION

The aqueous phase was transferred to a fresh tube, and precipitated the RNA from the aqueous phase by mixing with isopropyl alcohol. Use 0.5 ml of isopropyl alcohol per 1 ml of TRIZOL® Incubated samples at 15 to 30°C for 10 minutes and centrifuged at no more than 12,000 × *g* for 10 minutes at 2 to 8°C. The RNA precipitated, often invisible before centrifugation, forms a gel-like pellet on the side and bottom of the tube.

4. RNA WASH

The supernatant was removed and the RNA pellet was washed once with 75% ethanol, adding at least 1 ml of 75% ethanol per 1 ml of TRIZOL.The sample is mixed by vortexing and centrifuged at no more than 7,500 × *g* for 5 minutes at 2 to 8°C.

5. REDISSOLVING THE RNA

At the end of the procedure, the RNA pellet was briefly dried (air-dry or vacuum-dry for 5-10 minutes). It is important not to let the RNA pellet dry completely as this will greatly decrease its solubility. Partially dissolved RNA samples have an A260/280 ratio < 1.6. Dissolve RNA in RNAse-free water or 0.5% SDS solution by passing the solution a few times through a pipette tip, and incubating for 10 minutes at 55 to 60°C.

SYNTHESIS OF cDNA USING REVERSE TRANSCRIPTASE ENZYME

- 10µL of RNA was taken in a 0.5ml PCR tube and RT-PCR reaction mixture was added as follows:

REACTION MIX

5X Buffer	:	10 µL
MgCl2	:	2 µL
dNTP	:	4 µL
oligo dT	:	2 µL
MMLV RT	:	1 µL
RNA	:	10 µL
Water	:	15 µL
RNAse Out	:	0.4 µL

- The above reaction was run using following programme

Table 2: Represents the Reverse Transcriptase Program

Add RNA+Primer+Water mix	
T=70^0C	5 minutes
Pause → Add dNTPs, DTT, RT Buffer (5X), RNAse inhibitor, Water, MMLV RT reaction mix	
T=37^0C	5 minutes
T=37^0C	1 hour
T=95^0C	5 minutes
Hold 4^0C forever	

POLYMERASE CHAIN REACTION

Polymerase Chain Reaction was set up in an Eppendorf thermocycler (Mastercycler Personal) as follows:

10X buffer	: 1.2 µL
MgCl2	: 0.4 µL
dNTP	: 0.2 µL
Taq polymerase	: 0.2 µL
Forward primer	: 0.8 µL
Reverse primer	: 0.8 µL
Water	: 12 µL

The above reaction was run using the following PCR program:

Step 1:	95ºC	:	5min

Step 2:	94 ºC	:		1 min.
Step 3:	58 ºC	:		1 min (appendix)
Step 4:	72 ºC	:		1 min
Step 5: Go to	:	Step 2	:	Repeat the cycle 35 times.
Step 6:	72 ºC	:		10 min

The PCR product was checked on 1% Agarose gel in TAE.

Table 1: Primers Used

PRIMER/ Predicted Base Pair Size (bp)		***SEQUENCE***	***Annealing temperature***
GAPDH (588)	Sense Antisense	CCA CCC ATG GCA AAT TCC ATG GCA TCT AGA CGG CAG GTC AGG TCC ACC	62°C
HPV16 (450)	Sense Antisense	AAA CAG ATG AAG TGC TCC TTC CAG G TGG AGA ACA CCA CTT GTT GCT CCA	58°C
HPV18 (450)	Sense Antisense	CGG GAC GTG GAG CTG GCC GAG GAG CAC CAG CTG GTT ATC TCA CAG CTC	58°C

RESULTS

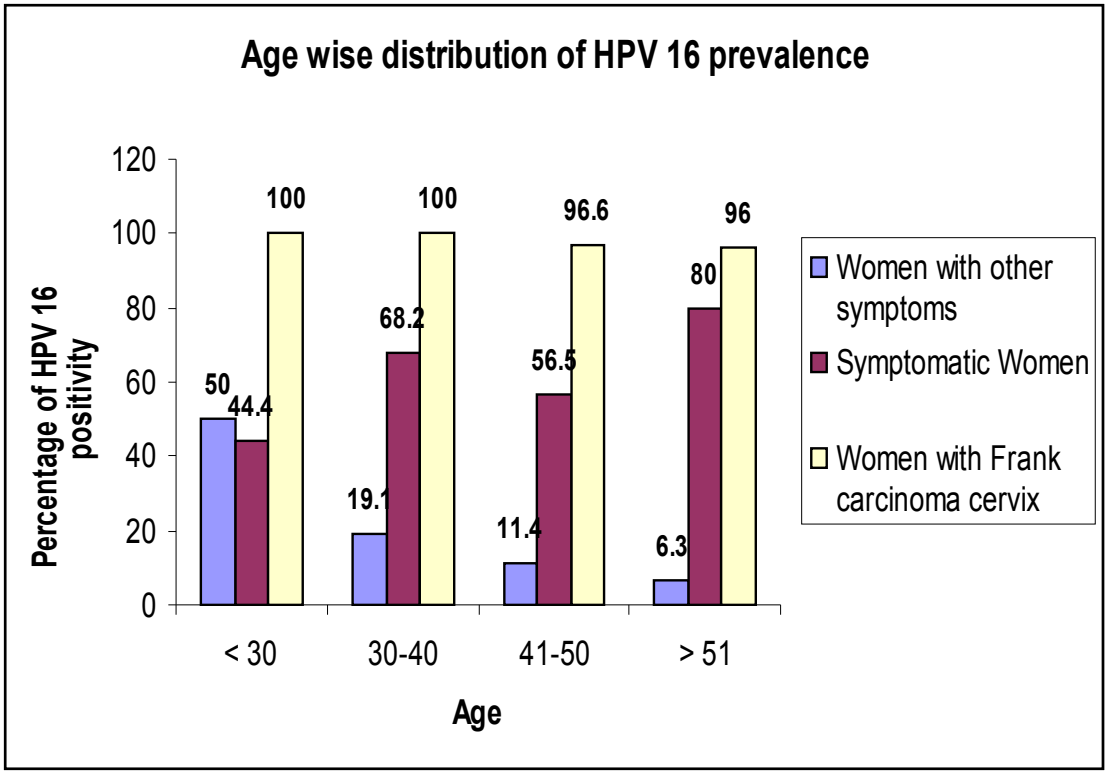
Total Population

This study had a total population of 300 women who attended our gynaecology OPD with 100 women belonging to the symptomatic group, 100 women belonging to group of women who attended the op for other complaints and 100 women who had frank carcinoma cervix.
Per speculum examination was done and Pap smear examination was done followed by coloposcopy guided biopsy in the symptomatic group while in women with frank carcinoma cervix a punch biopsy was taken from the cervical growth in women who attended the OPD for other complaints and who where posted for fractional curettage, the cervical biopsy sample was collected from the theatre .All these samples were tested for high HPV 16 and 18 using RTPCR
The mean age of the study population was 44.77 years with a standard deviation of 10.2

DISTRIBUTION OF HPV PREVALENCE IN EACH GROUP

SOCIO DEMOGRAPHIC CHARACTERISTICS OF WOMEN, CORRELATED WITH HIGH RISK HPV TYPE 16 PREVALENCE

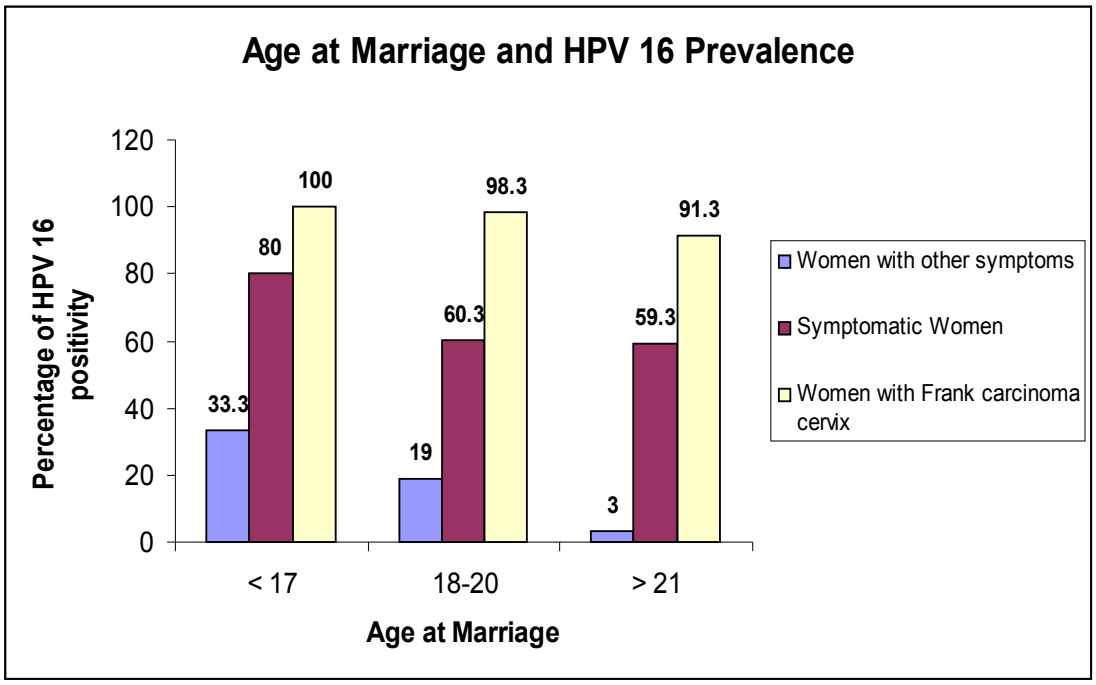
1. AGE WISE DISTRIBUTION OF HPV 16 PREVALENCE



HPV 16 prevalence in symptomatic group was maximum in the age group of 31 to 40 years and with a prevalence of 68% in the same .In women with other symptoms the over all HPV prevalence was

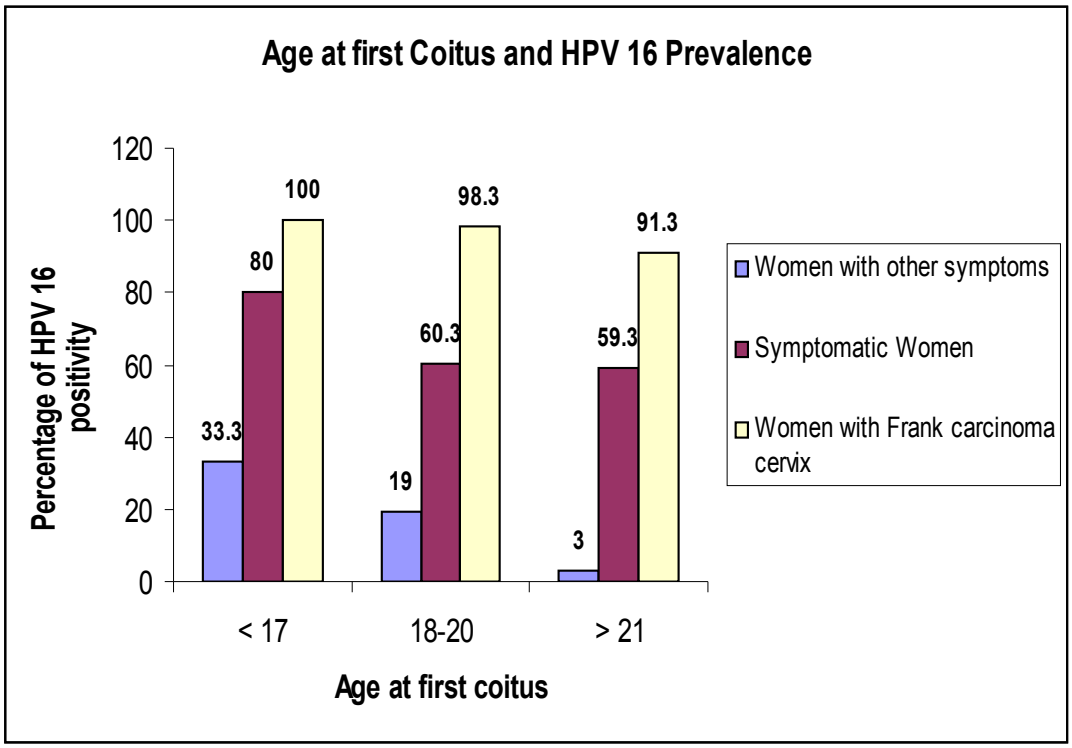
19% .The prevalence in CA cervix group did not show significant variation with age

2. AGE AT MARRIAGE AND HPV 16 PREVALENCE



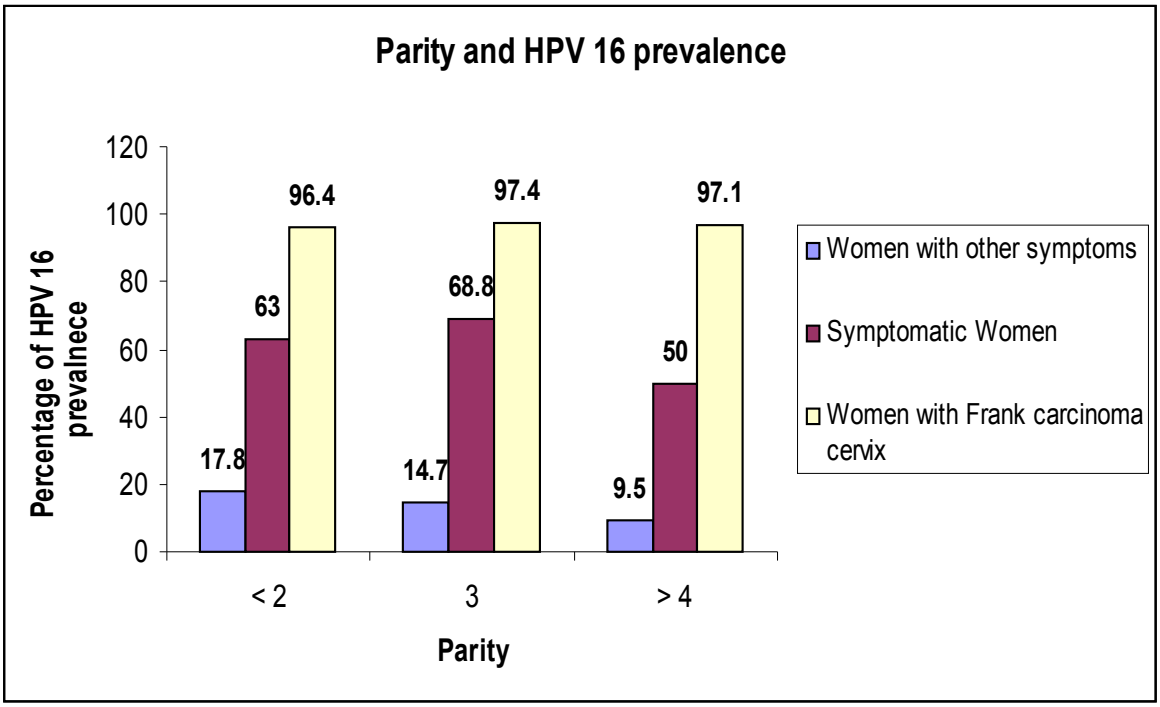
The median age at marriage was 19 years with a standard deviation of 1.85. Women with marriage at an early age had a higher prevalence of HPV infection. In symptomatic women, the prevalence was found maximum in the age group of less than 17 years which was 80% whereas that in women with other symptoms was 33.3%.The prevalence in ca cervix group did not show significant variation with age at marriage.

3. AGE AT FIRST COITUS AND HPV 16 PREVALENCE



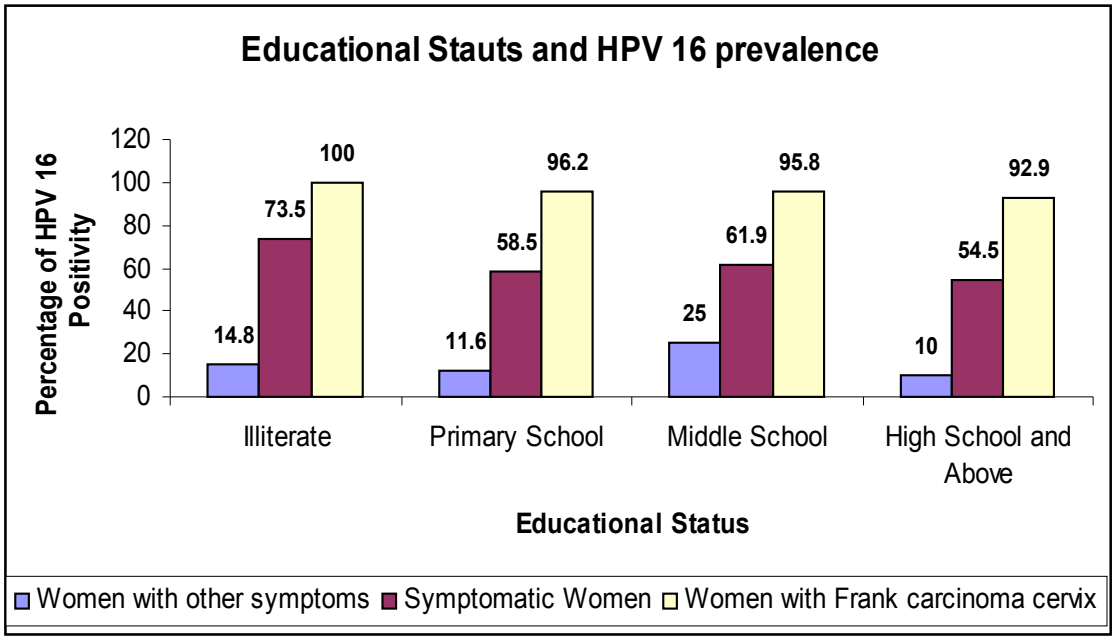
The median age at first coitus was 19 years with a standard deviation of 1.85. Women with coitus at an early age had a higher prevalence of HPV infection. In symptomatic women, the prevalence was found maximum in the age group of less than 17 years which was 80% whereas that in women with other symptoms was 33.3%.The prevalence in ca cervix group did not show significant variation with age at first coitus.

4. PARITY AND HPV 16 PREVALENCE



Number of pregnancies among the 3 study groups was unrelated to HPV 16 positivity

5. EDUCATIONAL STATUS AND HPV 16 PREVALENCE

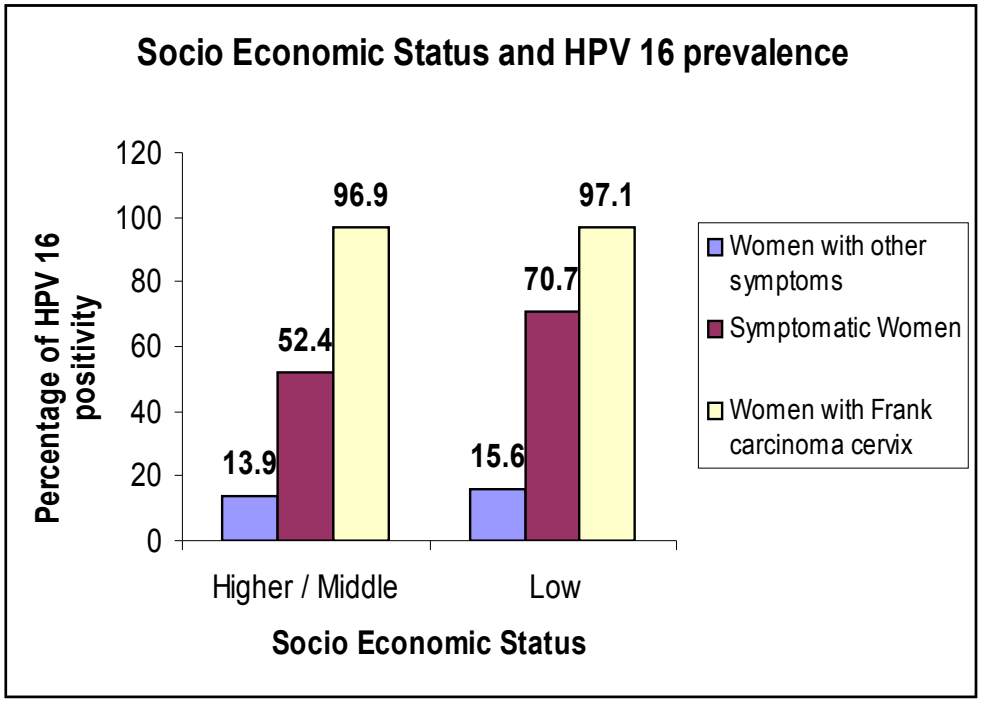


The distribution of educational status of the 300 women is given the following table.

Educational status	*Symptomatic women*	*Cancer cervix*	*Women with Other symptoms*
Illiterate	34%	36%	27%
Primary school	12%	36%	43%
Middle school	21%	24%	20%
High school and bove	33%	14%	10%

70% of the study population were illiterate or had only primary level education. The prevalence of HPV 16 was higher in illiterate and primary education group when compared to those who had middle or higher education. In index population the prevalence of HPV 16 in illiterate group was 73.5% while in women with other symptoms it was 14%.In illiterate women with ca cervix the same was found to be 100%.

6. SOCIOECONOMIC STATUS AND HPV 16 PREVALENCE



Women belonging to lower socioeconomic status had a higher risk of HPV 16 infection than those from medium or high socioeconomic group in the study.

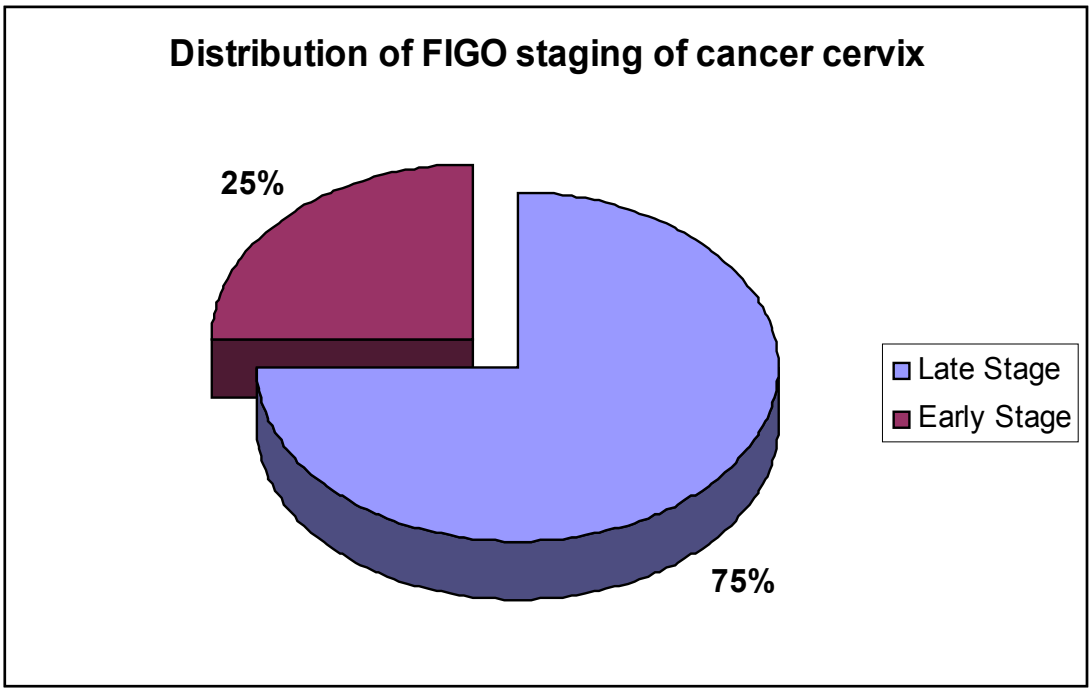
CARCINOMA CERVIX AND HPV 16 AND 18 PREVALENCE

The prevalence of hpv 16 and 18 in cancer cervix was 97% and 32% respectively. The prevalence of hpv 18 was more with early age at marriage and early age at first coitus. It was also high in the women belonging to lower socioeconomic status

Age at marriage(years)	*HPV 16 positivity*	*HPV 18 positivity*
<17	100%	17.6%
18-20	98.3%	40%
>=21	91.3%	21.7%

STAGEWISE DISTRIBUTION OF CANCER CERVIX

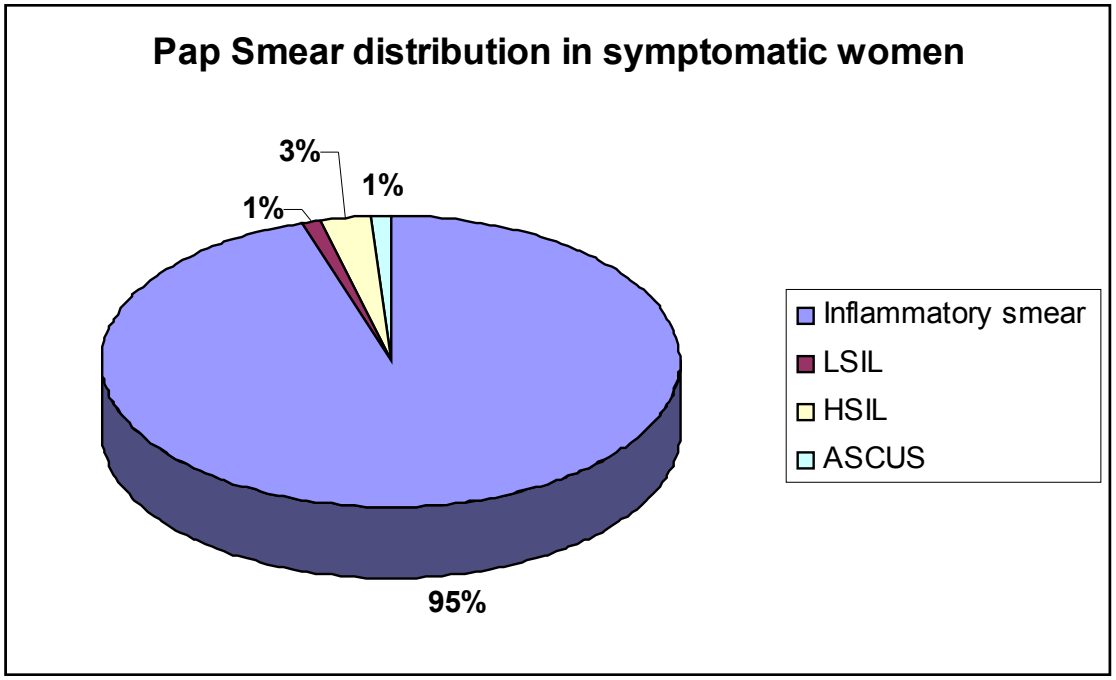
Most of the women with ca cervix in the OPD presented with late stage, 11B and above. Women with early stage comprise 25% of the total group and those with late stage comprise 75% of the total group.



STAGEWISE HPV 16 PREVALENCE IN CANCER CERVIX

Prevalence of hpv infection in early stage was 96% and in the late stage was 97%

PAP SMEAR AND HPV 16 PREVALENCE

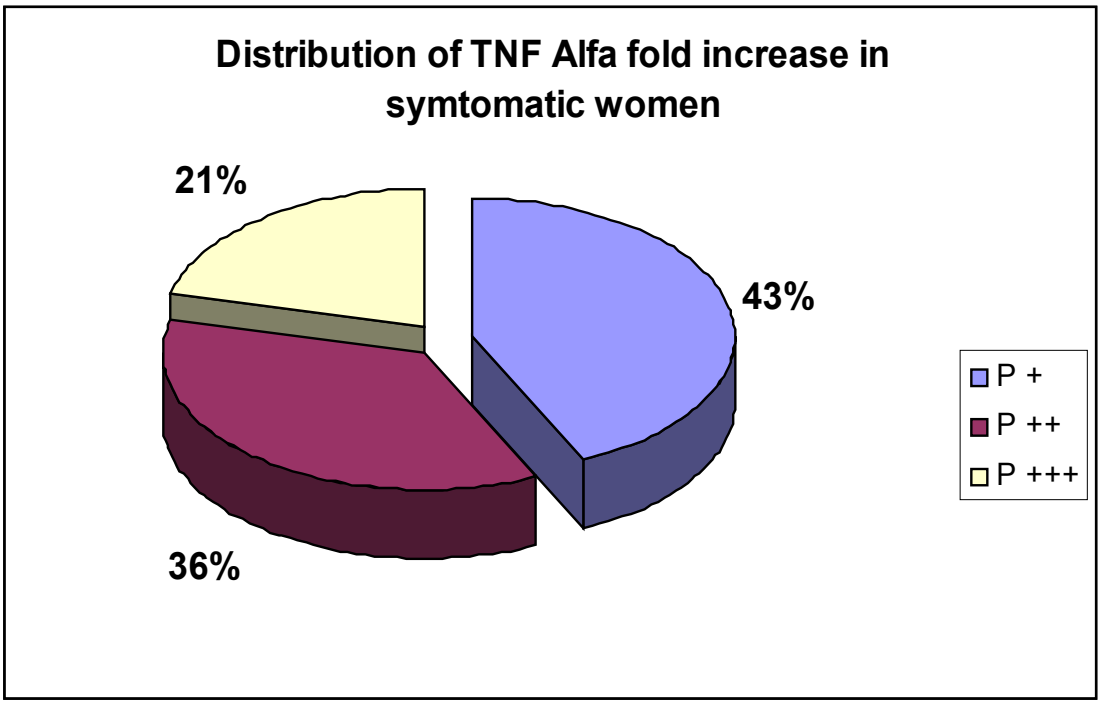


S.No	*Report*	*Hpv 16 Prevalence*
1	inflammatory	64%
2	LSIL	100%
3	HSIL	100%

4	ASCUS	100%

HPV 16 prevalence in women with inflammatory smear was 64% while dose with pap smear report showing LSIL, HSIL and ASCUS showed a 100% positivity.

TNF ALFA IN SYMPTOMATIC WOMEN



It was found to be increased in symptomatic women and 100% positive in HPV 16 infection .The increase in expressions of TNF alpha was not associated with intensity of HPV infection .In this study we attempted to analyse TNF levels in peripheral blood mononuclear cells (PBMC) of chronic cervicitis and squamous cell carcinoma patients. Our results implicated increased TNF levels in chronic cervicitis patients representing their hyper immune response whereas SCC patients showed a low/completely absent TNF expression indicating their immunocompromised status when compared to normal levels.

DISCUSSION

HPV infection is recognised as a public health problem for its role as a critical factor in the pathogenesis of various cancers. Cervical cancer is a preventable disease. It develops following the progression of uncleared HPV infection to high grade and eventually to invasive disease. Women with normal cervical cytology who are infected with high risk HPV, have an approximately 100 fold increased risk of developing CIN 3, compared with uninfected women. Persistence of oncogenic HPV appears essential for the development of cervical neoplasia. With the advent of molecular techniques particularly PCR, it is possible to detect very low quantities of HPV and to subtype the commonly occurring HPV in cervical specimens. The cytologic features of the conventional Pap smears have restricted value in identifying women destined to develop cervical neoplasia. The ALTS study group (Atypical Squamous cells of Unknown Significance- low grade squamous intraepithelial triage study) aimed at resolving the issue of management of low grade cervical lesions. They concluded that women with less than cervical intraepithelial neoplasia2(CIN2) status at initial colposcopy remain at risk for subsequent CIN2 +.Follow-up HPV testing is significantly more sensitive than cytology for detecting missed prevalent cases. In the same study, few cases of CIN3 had a negative HPV testing, which reinforces the fact that even the most sensitive test cannot provide perfect assurance. Thus HPV testing should be used as an adjunct to Pap smear.

There is ample data on prevalence of HPV in women with cervical cancer, however data on HPV 16 and 18 prevalence in symptomatic women with clinically abnormal cervix from India is sparse. HPV 16 and 18 are more prevalent in this part of the world. Type of HPV in primary screening depends upon the population being screened. Clifford et al (77) have suggested that cost effective test could include subset of high risk HPV, which are most likely to progress to cancer. The prevalence of HPV 16 in the index population in our study was found to be 64%.Aggarwal et al (78) reported a HPV prevalence in the same group as 14.2%.Dutta gupta et al (79) also reported a prevalence of 8.8% in the index study. Two large population based studies in cytologically normal women, Sankaranarayanan et al (80) reported 10.3% high risk HPV detection using HC2 in Osmanabad district in West India. Somewhat similar estimate was reported by HC2 in a separate multicentric study in Mumbai (6.3%) and Trivandrum 7.8 %(81) .Soujanya et al (82) in their study showed a HPV 16 prevalence of 10. 4%.The higher prevalence in women attending our Gynaecology OPD may be plausibly attributed to the fact that our Institute is the highest referral centre for the surrounding Government hospitals and primary health centres and that most subjects come here after having taken treatment at several centres and that we targeted women in the high risk group viz, women with chronic vaginal discharge. The probable incrementing factor for the high prevalence of HPV in our study might be the illiteracy and low socioeconomic status of most of the subjects who attended our Gynaecology OPD.

Most prevalent HPV genotype in the symptomatic women was HPV 16. However screening of a larger sample in our hospital will give a better picture on the distribution of HPV genotypes.

In women with other symptoms, the prevalence of HPV 16 was only 15%.Aggarwal et al had reported an estimated prevalence of 7.4% in the same group in their study.

Women with cancer cervix showed a HPV 16 prevalence of 97% and HPV 18 prevalence of 32%.High risk HPV infection was demonstrated as an indicator of malignant lesions like cancer cervix with a magnitude of 84% by Riou et al and 90% by Zur Hausen.Berlin (83, 84, 85) grace et al observed a highly significant association between high risk HPV 16 and 18 infection and development of cervical cancer (P=0.0001). Their data showed that HPV 16 was the most prevalent type in cervical lesions , especially cancer cervix.Soujanya Jain et al in their study showed that the most prevalent HPV types found in invasive cancer cervix in Andhra Pradesh was HPV 16(66.7%),followed by hpv 18 (19.4%). In another case control study in Southern India, it is reported HPV prevalence as high as 99.4% in their invasive cancer group. The difference in the results may be due to the fact that this is a hospital based

study with facilities for the treatment of cancer cervix by radiotherapy and that women in our study mostly presented to us in the late stage of cancer cervix. Therefore we can confirm, that a vaccine targeting HPV 16 could eliminate >50% of the cervical cancer burden in Tamilnadu, as well as South India. More comprehensive genotyping of cervical cancer tissues from North, West, and Northeast India will be needed to justify a single national vaccine strategy for the Indian subcontinent.

We found that the prevalence of HPV 16 in the symptomatic women was highest in the age group between 31-40 years. This coincides with the peak incidence of invasive cancer cervix at 35 years, the next peak at about 50-55 years. But no significant age related difference was noted in Aggarwal et al study. The HPV prevalence in the same age group was reported as 7.1%. Dutta Gupta et al also made similar observations of HPV 16 prevalence among Muslim women. Chaouki et al (86) also reported no significant age related difference .This difference in our study can be attributed to the fact that our Institute is the highest referral centre with the women coming to us after getting treatment at various centres.

Women who got married at an early age showed a higher prevalence of HPV 16(80%) in the symptomatic group. The age specific incidence of cervical dysplasia shows that it was most often diagnosed in women in their 20s (Canadian Task Force).Women are prone to HPV infection with the beginning of sexual intercourse. HPV 16 is the most common type in women with normal cytology. The results in our study showed that most women were infected by HPV as they started their sexual activity earlier. Aggarwal et al reported a prevalence of 8.6% in their study. Dutta gupta et al did not find any association of HPV 16 with the age of consummation of marriage.

Women who were illiterate or had <6 years of education had a significantly higher prevalence of HPV 16, 73% and 56% respectively in the index population. Aggarwal et al also reported a higher rate of HPV with a positivity of 13.1%.This very well supports the fact that cervical cancer is more prevalent in the less educated population reflecting their practices regarding genital hygiene.

Women belonging to a lower socioeconomic status had a higher prevalence of HPV 16 in the index study about 70.7% and in women with cancer cervix, it was 97%. Aggarwal et al also reported a higher rate of high risk HPV infection of 11.8% than those from medium or high socioeconomic group, although the difference was not statistically significant. Franceschi et al (87) has recognised low socioeconomic status as a risk factor for cervical carcinoma as well.

There was no significant parity related difference in the index study in the distribution of HPV. Aggarwal et al reported that more number of women 10.8% with 3 or more children were positive for high risk HPV as compared to those with less than 3 children (6%). The difference was however not statistically significant. Dutta gupta et al and Laczano et al (88) too did not observe any significant association of HPV 16 or 18 with parity.

SUMMARY

This study was conducted in our Institute which comprises women attending the Gynaecology OPD for various reasons. 300 consenting women were enrolled with 100 belonging to the group of women who came with complaints of persistent vaginal discharge, 100 women who came for other complaints and 100 women with frank cancer cervix. A Pap smear was obtained in the index study and colposcopic guided biopsy of the cervical tissues was also obtained. Cervical tissue samples in subjects who underwent total hysterectomy due to complaints other than uterine cervical lesions such as uterine fibroids, endometriosis and so on, were obtained from theatres posted for fractional curettage after verifying the basic screening report .Cervical punch biopsy samples were taken in 100 women who came with frank cancer cervix.

Cervical tissue samples were stored and Reverse Transcriptase Polymerase chain reaction (RT-PCR) was done to assay HPV DNA16 and 18. The prevalence of HPV 16 and 18 in each of the groups was analysed. We found that the prevalence of HPV 16 in the index study, in women with other symptoms and those with cancer cervix was 63%, 15%, and 97% respectively. HPV 18 prevalence in cancer cervix was 32%.HPV 16 prevalence was highest in the age group 30 – 40 years It was also high in women who got married at an early age, women belonging to the lower socioeconomic strata, and in the illiterate group. We did not observe any significant parity related difference in HPV 16 prevalence. The index study generated the prevalence data of subclinical high risk HPV infection in the symptomatic women. The limitation of the study is that being a hospital based study, the women enrolled visited the hospital with varied ailments and thus were not true representations of the community. In addition, the prevalence of high risk HPV, other than hpv 16 AND 18 was not evaluated.

Cancer cervix screening practices are inconsistent in India. Use of Pap smear, as a sole indicator for screening has limitations. The cytological interpretation becomes faulty if the smear is inflammatory, a situation not infrequent among women from the lower socioeconomic background. In a scenario of infrequent screening, screening with a test of high sensitivity provides greater reassurance, that potential disease has not been missed in women who screened negative. It is an irony that the middle and high socioeconomic women, who can afford HPV screening by molecular techniques require it the least, owing to the low prevalence. High risk HPV DNA screening appears to be a valid option in mass cervical screening programmes in the developed countries. In a resource poor country, it is not feasible to offer a universal molecular testing for high risk HPV, till HPV screening is made cheaper. Identification of population at risk will enable focused screening, with a greater cost effective utilization of resources. Screening preferentially should be directed to the target population for the optimal utilization of resources. Needless to say, health education, promotion of condom usage, and need to follow healthy hygienic practices is the most cost effective approach in reducing the incidence of cervical carcinoma in resource-crunched societies.

CONCLUSION

The study generates data of prevalence of sub-clinical HPV in women visiting a tertiary care institution.

1. Prevalence of HPV 16 in symptomatic women attending our OPD is 64% which is high.
2. HPV 16 prevalence is more in women who start their sexual activity at a younger age
3. HPV 16 prevalence is high in women belonging to the lower socioeconomic strata.
4. HPV 16 prevalence is more in illiterate population.

Identification of population at risk will enable focussed screening, with a greater cost effective utilization of resources. The data generated will be useful for laying guidelines for mass screening of HPV, treatment and prophylaxis in the local population and vaccination against HPV.

The implications of the study are that women belonging to the lower socioeconomic strata must be counselled regarding the following

1. Need for frequent follow-up to identify cancer cervix in early stages
2. Patients with persistent HPV positivity and those who have completed their family can be given the option of hysterectomy to avoid the progression to cancer cervix.
3. Emphasis on vaccination against HPV

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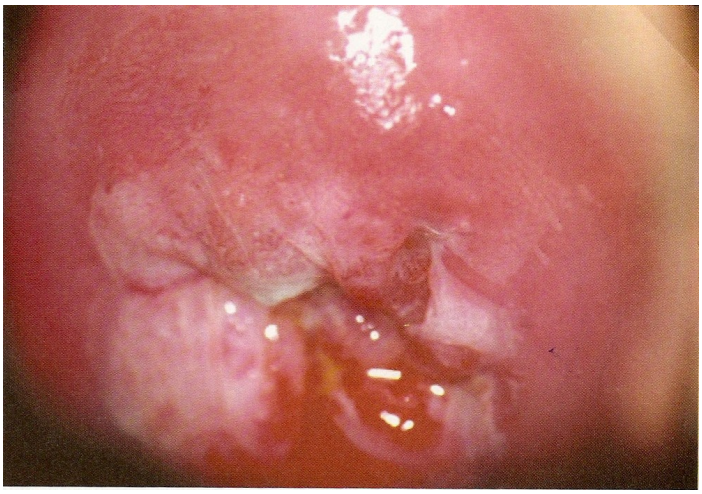
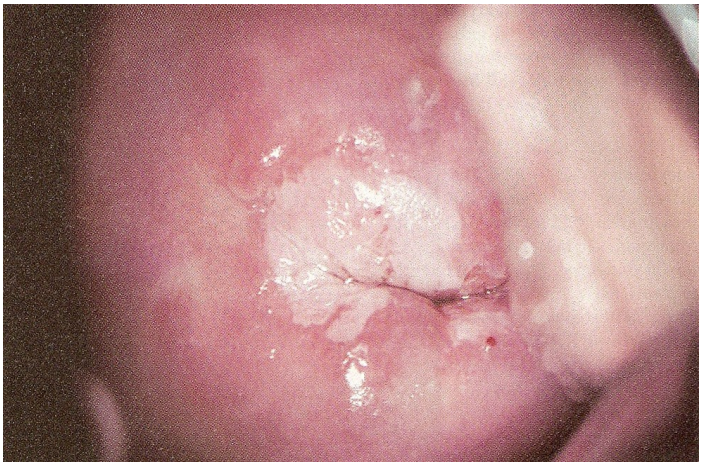
PROFORMA

1. Date:
2. Name:
3. Op. no:
4. Age:
5. Social economic status:
6. Literacy:
7. Marital status:
8. Age at marriage:
9. Age at first coitus:
10. Age at first child birth:
11. Parity:
12. No. of live children:
13. Pap smear:
14. Colposcopy(symptomatic women):
15. Cervix biopsy report:
16. FIGO staging(women with frank carcinoma cervix) :
17. HPV 16 positivity:
18. HPV 18 positivity:
19. Fold increased or decreased of TNF alpha expression (symptomatic women):

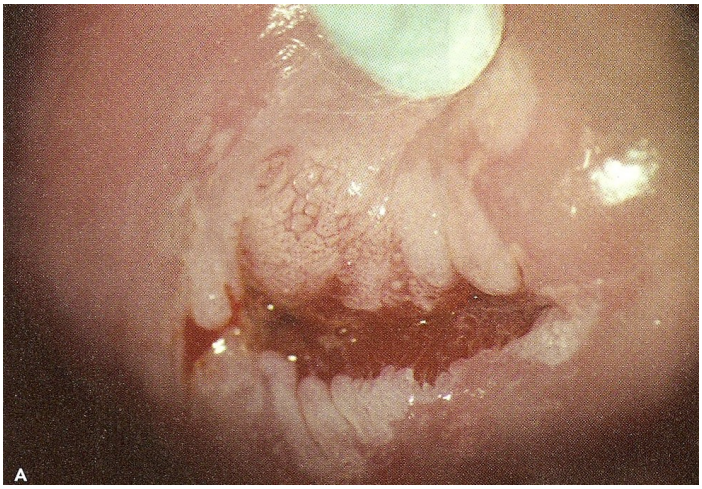
COLPOSCOPY OF CIN 2 ASSOCIATED WITH HPV INFECTION OF THE CERVIX



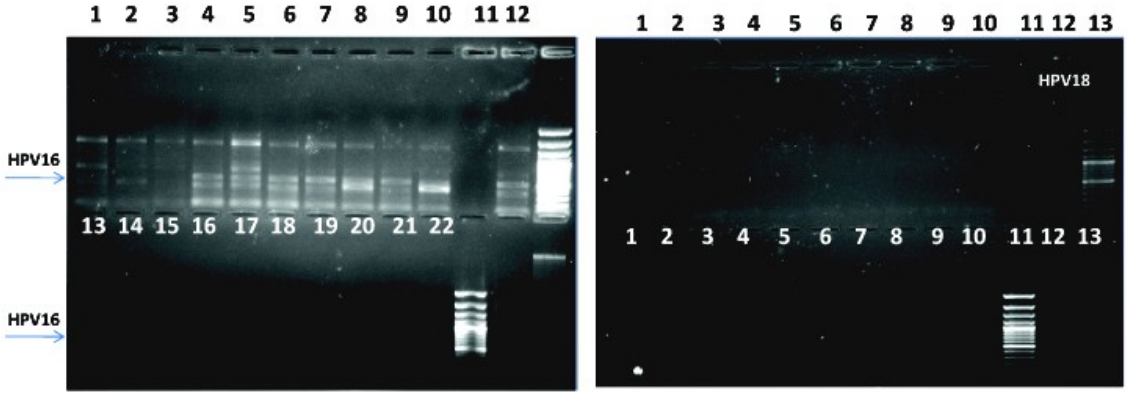
CIN2 - WHITE LESION WITH SURFACE SPICULES



MOSAIC PATTERN AND PUNCTATION



PCR for HPV in Chronic cervicitis tissue samples



Lanes,		
	1-CT1	12-CT51
	2-CT5	13-CT52
	3-CT8	14-CT55
	4-CT38	15-CT56
	5-CT39	16-CT57
	6-CT40	17-CT58
	7-CT41	18-CT59
	8-CT42	19-CT60
	9-CT43	20-CT61
	10-CT47	21-CT62
	11-CT49	22-CT63

ETHICAL COMMITTEE CERTIFICATE

I, Dr.Vijayalakshmi Palanisamy apply for the ethical committee certificate for the project "PREVALENCE OF HIGH RISK HUMAN PAPILLOMA VIRUS IN SYMPTOMATIC WOMEN ATTENDING OUR GYNAECOLOGY OPD" under the guidance of **Prof.Dr.Kalaiselvi, M.D., D.M.,** Institute of Obstetrics and Gynaecology, Egmore, Chennai - 8.

I understand the implications of doing research with human subjects and will fully comply with the regulations and keep the dignity and protect the health of subjects at all costs.

P. Vijaya

Signature of the Postgraduate student

I have no objection to guide this postgraduate student in the project mentioned above. I shall supervise that all the human rights are protected and research is carried on with utmost humanitarian principles.

Signature and seal of the guide

DR. K. KALAICHELVI, M.D., D.M.
Reg. No: 31135
ADDL. PROFESSOR

I certify that this project has been presented in front of the ethical committee, duly formatted in this institution and that all members of the Ethical committee have given permission to conduct this research.

CHAIRMAN ETHICAL COMMITTEE

Professor & Head
Dept. of Community Medicin
Stanley Medical College
Chennai-600 001.

Place :

Date :