INCIDENCE OF HPV INFECTION IN HIGH AND LOW GRADE LESIONS OF CERVIX

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CERTIFICATE

This is to certify that the dissertation titled "**INCIDENCE OF HPV INFECTION IN HIGH AND LOW GRADE LESIONS OF CERVIX "** is the bonafide work done by **Dr.B. SHANTHA PERUBA** between September 2008 to August 2009 during her M.D.,O.G., course at ISO - KGH, MMC Chennai.

DEAN

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CONTENTS

INCIDENCE OF HPV INFECTION IN HIGH AND LOW GRADE LESIONS OF CERVIX

INTRODUCTION

Carcinoma of the uterine cervix is the most common cancer in South Indian women and occupies the top rank among cancers in women in most developing countries, constituting 34% of all women's cancers. To an estimated annual global incidence of 500,000 cervical cancers, India contributes 100,000, ie. 1/5 of the world burden.[23] The magnitude of the problem is thus more than evident. The world pattern of cervical cancer, together with the age adjusted rate and ranking, clearly indicate that cervical cancer is predominantly a problem of poorer socio-economic societies.[1]

On the other hand, uterine cervical cancer is a favourable site for an effective control program. It is easily accessible and there is usually a long latent period of intraepithelial neoplasia which is easily recognizable by the Pap smear. Furthermore, treatment at this stage is very effective.

The burden of cervical cancer in India, taken in the context of the additional problems of advanced disease at presentation, the country's limited resources and health infrastructure, and the paucity of trained personnel emphasize the urgent need for a control program.

Cancer of the cervix has been the most important problem in women in India over the past two decades.

The first IARC-sponsored case control study on HPV and cervical cancer in India, which was carried out at the Cancer Institute, Chennai, documented that 99% of uterine cervical cancers were HPV-positive compared to only 22% in the controls.

Preventive vaccination is under intensive study.

Accepted methods for early cervical cancer detection and control for a developing environment include:

1. Education; access to health care
2. Unaided visual inspection and clinical downstaging
3. Aided Visual Inspection (VIA)[24]
4. VIA with magnification (VIAM)
5. HPV testing

Ref Cytology Uni. of Zimbabwe/JHPIEGO Cervical Cancer Project Visual inspection with acetic acid for Cervical Cancer Screening Lancet 1999, 353, 869-73

Four of the five districts in India were Cancer Cervix is very much prevelant are concentrated in the north eastern

region of Tamil Nadu state and Puducherry.

Ref. *Annual Report, 1982, National Cancer Registry*, New Delhi: Indian Council of Medical Research; 1985.

WHO has stated in studies that " Quite often,a considerable discussion is focused on which screening test to use ¾ cytology or alternatives to cytology, such as VIA or HPV testing or which combinations/sequence of screening tests should be used for screening in developing countries. Choosing a suitable screening test is only one aspect of a screening programme"32

Screening for cancer cervix forms an important way of preventing the disease.HPV DNA assay is the most sensitive test among the screening methods.This study is the detection of the HPV infection among low and high grade lesions**.**HPV infection has a long latency period even before the visible precancerous lesions which makes the test the best screening method.

In developing countries like India, there is a lack of effective screening programs for cervical cancer. In these countries, no clinically significant reduction in the incidence of cervical cancer has occurred during the past three

decades.[19,20,21,22] In developed countries, by contrast, there has been a major decline in cervical-cancer mortality after the introduction of large-scale cytologic testing. The limited success of such screening in developing countries has stimulated evaluation of testing for human papillomavirus (HPV).

Sensitivity = 93.0% 95% CI = 84.3 to 97.7

Specificity = 61.1%

95% CI = 57.7 to 64.4

Disease Prevalence = 8.0%

Assay Positive Predictive Value = 17.2%

Assay Negative Predictive Value = 99.0%

The Utility of HPV DNA Testing for Triage of Women with

Borderline Pap Smears was conducted in 1996 under the direction of the Kaiser Foundation Research Institute and the Kaiser Permanente Medical Group.

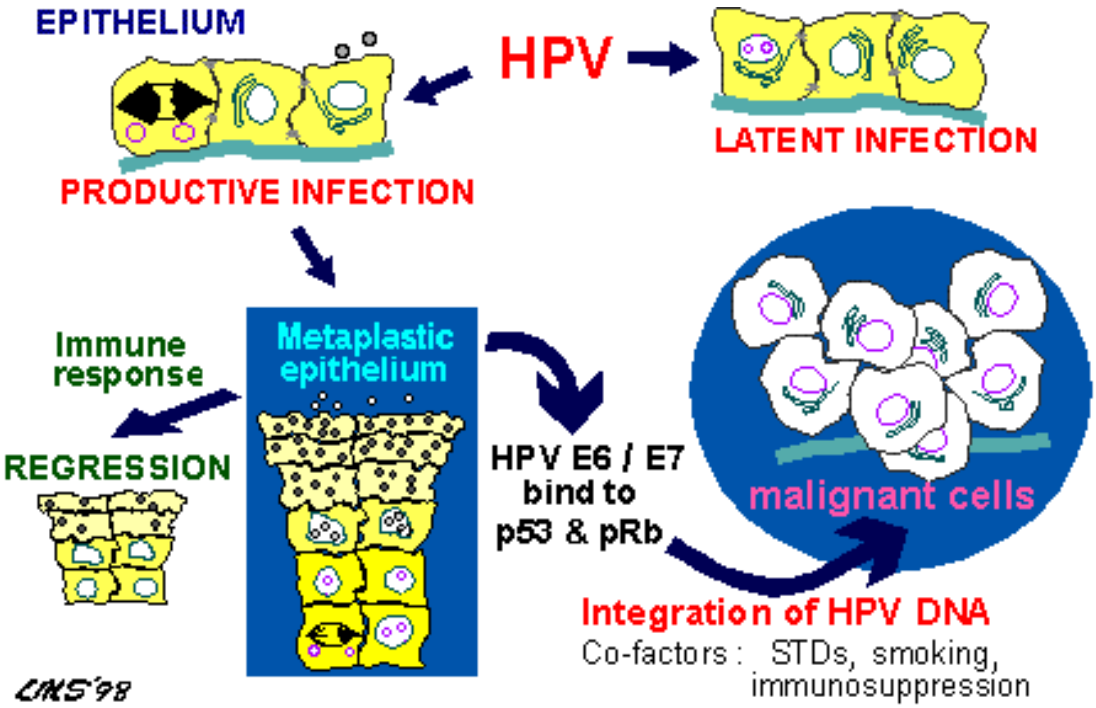
HPV VIRUS:

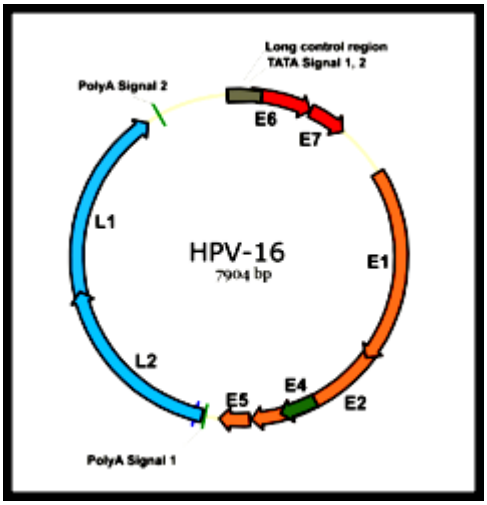
Human papillomaviruses are composed of an 8000 base pair double-stranded circular DNA molecule surrounded by a protein capsid.

LIFECYCLE OF HPV:

The HPV lifecycle strictly follows the differentiation program of the host keratinocyte. The HPV virion infects epithelial tissues through micro-abrasions, whereby the virion associates with putative receptors such as alpha integrins and laminins, leading to entry of the virions into basal epithelial cells through clathrin-mediated endocytosis and/or caveolin-mediated endocytosis depending on the type of HPV. At this point, the viral genome is transported to the nucleus by unknown mechanisms and establishes itself at a copy number between 10-200 viral genomes per cell. A sophisticated transcriptional cascade then occurs as the host keratinocyte begins to divide and become increasingly differentiated in the upper layers of the

epithelium.

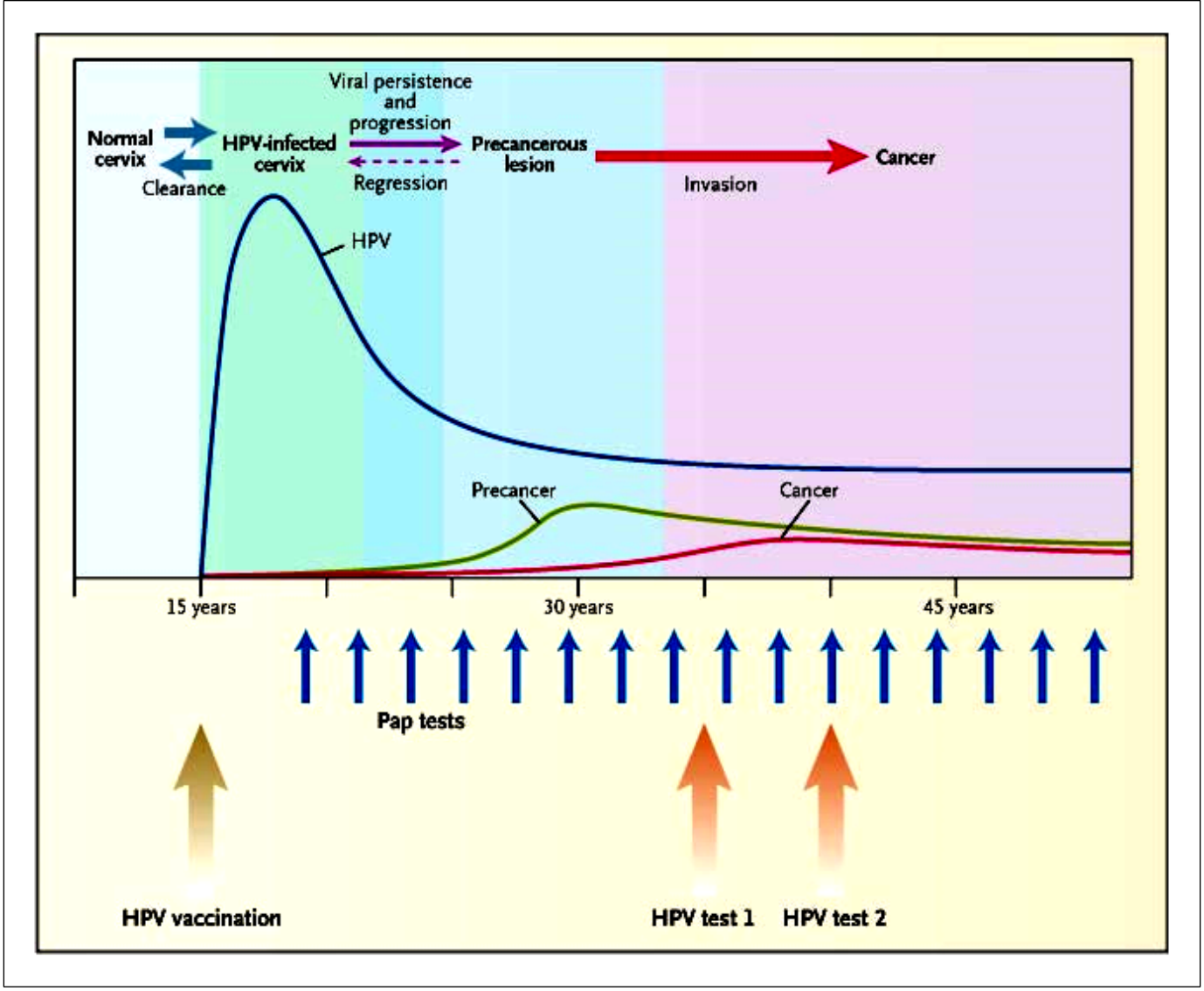




E6/E7 proteins

The viral oncogenes, E6 and E7 modify the cell cycle so as to retain the differentiating host keratinocyte in a state that is favorable to the amplification

of viral genome replication and consequent late gene expression. E6 in association with host E6 AP (associated protein), which has ubiquitin ligase activity act to ubiquitinate p53 leading to its proteosomal degradation. E7 (in oncogenic HPVs) acts as the primary transforming protein. E7 competes for retinoblastoma protein (pRb) binding, freeing the transcription factor E2F to transactivate its targets, thus pushing the cell cycle forwards. All HPV can induce transient proliferation, but only 16 and 18 can immortalize cell cycle (in vitro). In the upper layers of the host epithelium, the late genes L1 and L2 are transcribed/translated and serve as structural proteins which encapsidate (Encapsidation is the process of incorporating a nucleic acid sequence (e.g., a vector, or a viral genome) into a viral particle) the amplified viral genomes. Once the genome is encapsidated, the capsid appears to undergo a redox-dependent assembly/maturation event which is tied to a natural redox gradient that spans both suprabasal and cornified epithelial tissue layers. This assembly/maturation event stabilizes virions, and increases their specific infectivity. Virions can then be sloughed off in the dead squames of the host epithelium and t the viral lifecycle continues.



HPV TO CANCER LATENCY PERIOD

Once an HPV virus invades a cell, an active infection occurs, and the virus can be transmitted. Several months to years may elapse before squamous intraepithelial lesions (SIL) develop and can be clinically detected.

Carcinogenic and possibly carcinogenic types of HPV

Carcinogenic types

Species 9: 16, 31, 33, 35, 52, and 58

Species 7: 18, 39, 45, 59, and 68

Species 5: 51 and 82

Species 6: 56

Species 11: 73

Possibly carcinogenic types

Species 11: 53

Species 5: 26

Species 6: 66

HPV types and associated diseases

Notable HPV types and associated diseases

Over 100 different HPV types have been identified and are referred to by number. Types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 are "high-risk" sexually transmitted HPVs and may lead to the development of cervical intraepithelial neoplasia (CIN) and vulvar intraepithelial neoplasia (VIN).

Disease	HPV type
Common warts	2, 7
Flat warts	3, 10
Anogenital warts	6, 11, 42, 44 and others
Genital cancers	• Highest risk : 16, 18, 31, 45 • Other high-risk: 33, 35, 39, 51, 52, 56, 58, 59 • Probably high-risk: 26, 53, 66, 68, 73, 82

HPV types 16 and 18 together cause about 70 percent of cervical cancers. It is important to note, however, that the great majority of high-risk HPV infections go away on their own and do not cause cancer.

In 2006, the U.S. Food and Drug Administration (FDA) approved Gardasil, a vaccine that is highly effective in preventing infection with types 16 and 18, two “high-risk” HPVs that cause most (70 percent) cervical cancers and types

6 and 11, which cause most (90 percent)

HPV testing for cervical HPV infection

HYBRID CAPTURE TEST:

In March 2003, the US FDA approved the Digene HPV HC2 DNA Test; a "hybrid-capture" test manufactured by Qiagen, as the primary screening tool for detecting HPV cervical infection as an adjunct to Pap testing, and may be performed during a routine Pap smear. It can detect the DNA of the 18 HPV types that most commonly affect the cervix and distinguish between "low" and "high-risk" HPV types, but it cannot determine the specific HPV types.

It is performed by collecting cells from the cervix and then sending them to a laboratory for analysis. The test can detect high-risk types of HPV even before there are any conclusive visible changes to the cervical cells.

The collection technique is similar to that of a Pap smear: remove excess mucous, insert a brush 1 to 1.5 cm into the cervical os, make three full turns in a counter-clockwise direction,insert the brush to the bottom of the transport tube, snap off the shaft, carefully cap the tube, and ship the tube at room temperature within two weeks.

THE HC2 High-Risk HPV DNA Test using capture 2 technology is a nucleic acid hybridization assay with signal amplification that utilizes microplate chemiluminescent detection. Specimens containing the target DNA hybridize with a specific HPV RNA probe. The resultant RNA:DNA hybrids are captured onto the surface of a microplate well coated with antibodies specific for RNA:DNA hybrids.several alkaline phosphatase molecules are conjugated to each anti body. As the substrate is cleaved by the bound alkaline phosphatase, light is emitted which is measured as relative light units on a luminometer.

An RLU measurement equal to or greater than the cutoff value indicates the presence of high-risk HPV DNA sequences in the specimen. High volume sample-throughput testing with the hc2 High-risk HPV DNA Test can be performed utilizing the rapid capture system (RCS). To enable high volume sample-throughput testing, all the procedural steps of the assay are performed by the RCS, with the exception of specimen denaturation, chemiluminescent signal detection, and result reporting.

HPV DNA can also be detected by PCR with the use of generic or consensus primers. This technique is more sensitive than the Hybrid Capture hc2 assay, allowing identification of specific HPV types and variants.8, 13, 18–20 it

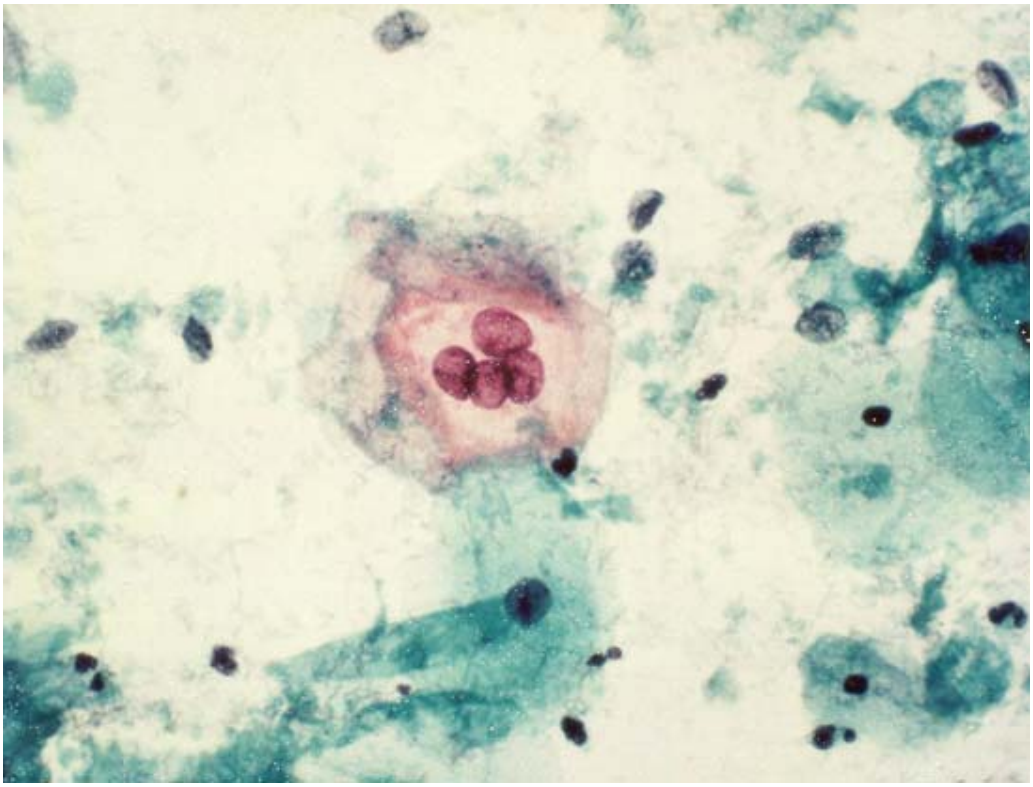
awaits final clinical validation. Both techniques are amenable for high-throughput, rapid, automated testing (e.g., Hybrid Capture can test up to 96 samples in less than 2 h).

The recent outcomes in the identification of molecular pathways involved in cervical cancer provide helpful information about novel bio- or oncogenic markers that allow monitoring of these essential molecular events in cytological smears, histological or cytological specimens. These bio- or onco-markers are likely to improve the detection of lesions that have a high risk of progression in both primary screening and triage settings. E6 and E7 mRNA detection PreTect HPV-Proofer, (HPV OncoTect) or p16 cell-cycle protein levels are examples of these new molecular markers. According to published results these markers, which are highly sensitive and specific, allow to identify cells going through malignant transformation

OTHER METHODS OF SCREENING FOR CANCER CERVIX:

PAP SMEAR

The Pap smear is the mainstay of primary screening for cervical cancer. It should be performed with a cervical broom turned three times in a counter-clockwise fashion or with both an endocervical brush turned three times and along-tipped Ayres's spatula rotated once on the os of the cervix. Excessive mucous on the cervix should be cleaned off before the specimen is taken. The specimen can be put in a liquid specifically designed for LBC or can be smeared on a glass slide. If a broom is used, the secretions should be stroked once across the slide. If a brush and spatula are used, the brush should be unrolled on the upper part of the slide from left to right, and the spatula should be stroked on the lower part of the slide from left to right, without returning on the smeared material.



KOILOCYTE

The major system used to report the results of Pap tests in the United States is the Bethesda System. In this system, samples with cell abnormalities are divided into the following categories:

• ASC—Atypical Squamous Cells. Squamous cells are the thin, flat cells that form the surface of the cervix. The Bethesda System divides this category into two groups:

1. ASC–US—Atypical Squamous Cells of Undetermined Significance. The squamous cells do not appear completely normal, but are uncertain what the cell changes mean. Sometimes the changes are related to HPV infection.

2. ASC–H—Atypical Squamous Cells cannot exclude a High-grade squamous intraepithelial abnormality. ASC–H may indicate a higher risk of being precancerous compared with ASC–US.

• AGC—Atypical Glandular Cells. Glandular cells are mucus-producing cells found in the endocervical canal or in the lining of the uterus.

• AIS—endocervical Adenocarcinoma In-Situ. Precancerous cells are found in the

glandular tissue.

Pap test results may also be described using an older set of categories called the “dysplasia scale.”

There are four degrees of dysplasia: mild, moderate, severe, and carcinoma in situ.Carcinoma in situ is a precancerous condition that involves only the layer of cells on the surface of the cervix, and has not spread to nearby tissues. In the Bethesda System, mild dysplasia is classified as LSIL; moderate or severe dysplasia and carcinoma in situ are combined into HSIL.

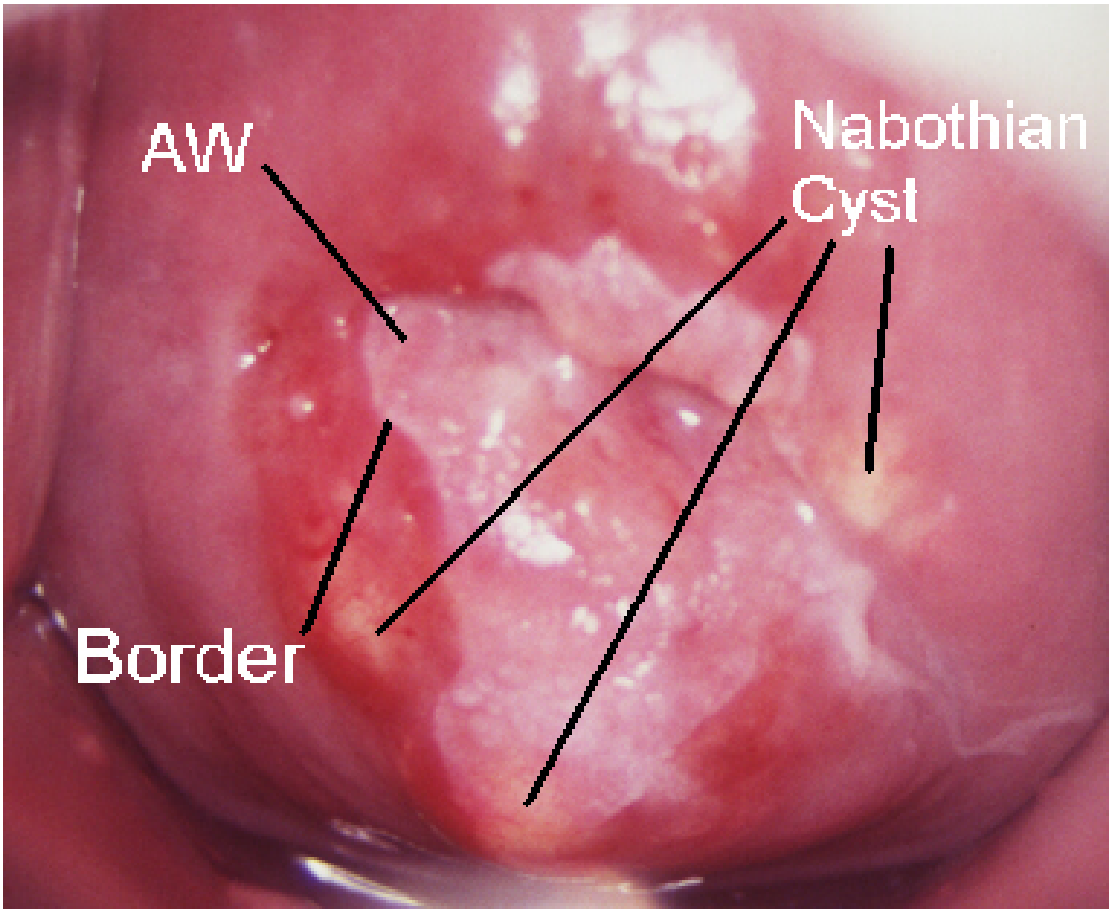
Cervical intraepithelial neoplasia (CIN) is another term that is sometimes used to describe abnormal tissue findings. Neoplasia means an abnormal growth of cells. The term CIN along with a number describes how much of the thickness of the lining of the cervix contains abnormal cells. CIN–3 is considered to be a precancerous condition that includes carcinoma in situ.

Tests used to screen for and diagnose precancerous cervical conditions

A Pap test is the standard way to check for any cervical cell changes. A Pap test is usually done as part of a gynecologic examination**.**

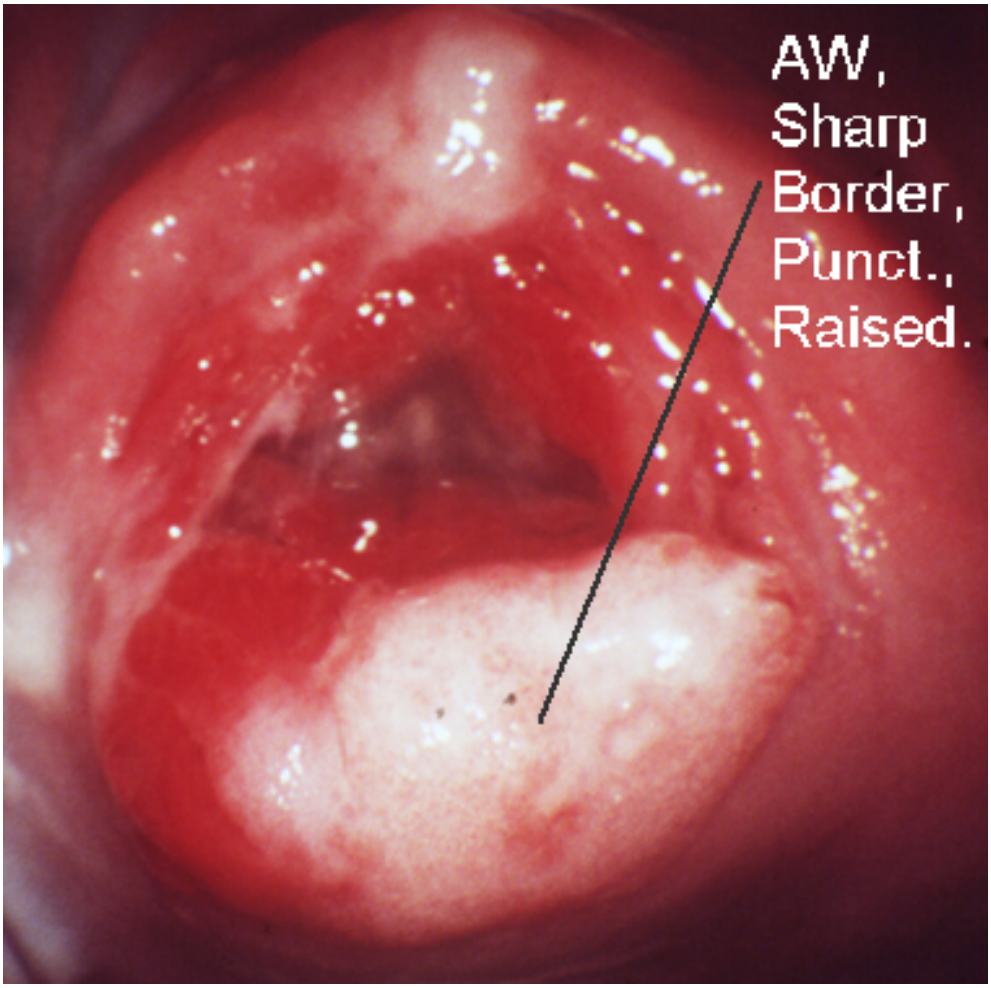
COLPOSCOPIC PICTURE OF LSIL

LSIL is a low grade lesion with a faint acetowhite areas in colposcopy and are classified as mild dysplasia or CIN 1.


AW
Nabothian
Cyst
Border

• HSIL—High-grade Squamous Intraepithelial Lesion. HSILs are more severe abnormalities and may eventually lead to cancer if left untreated.

COLPOSCOPIC PICTURE OF HSIL



REVIEW OF LITERATURE

Cuzick et al 1 evaluated 2009 women with both pap and HPV PCR for HPV SEROTYPES 16, 18, 31, and 33.Women were referred for colposcopy for any degree of dysplasia and/or appositive HPV test.A total of 231 women underwent colposcopy.Using a threshold of mild degree or greater as a

reference standard, the sensitivity of HPV testing for identifying high risk types was 75%.[1]

Clavel et al 2 used the HC 2 assay in conjunction with cytology in studing 1627 samples.For the detection of biopsy- confirmed high grade lesions, the sensitivity of HPV testing was 98.1%[2].

Kuhn et al 3 studied 2944 previously unscreened women using sequential cytology, the HC assay, cervical inspection, and cervicography**.conclusion:**The sensitivity of HPV testing was superior (88.4%) to that of other methods[3].

Elfgren K4, Rylander E, Rådberg T, Strander B, Strand A, Paajanen K, Sjöberg I, Ryd W, Silins I, Dillner J; Swedescreen Study Group conducted a Randomized Controlled trial about the Colposcopic and histopathologic evaluation of women participating in population-based screening for human papillomavirus deoxyribonucleic acid persistence**.conclusion:**70% of women with abnormal colposcopic lesions show positive for HPV infection. However 90% of them regressed over the next 2 years[4].

Kaufman RH5, Adam E, Icenogle J, Lawson H, Lee N, Reeves KO, Irwin J, Simon T, Press M, Uhler R, Entman C, Reeves WC evaluated 1128 women

and studied the Relevance of human papillomavirus screening in management of CIN[5].

Clavel C, Masure M, Bory JP, Putaud I, Mangeonjean C, Lorenzato M, Gabriel R, Quereux C, Birembaut P conducted a preliminary study on 1518 women to detect in routine high-grade cervical lesions human papillomavirus by Hybrid Capture II-based detection[6].

In a study of women with invasive cervical cancer, and matched controls, from four high risk areas in Panama) Mexico, Costa Rica and Columbia HPV 16 or HPV 18 was detected in 62% of 759 case patients and 32% of 1430 controls. The relative risk increased with increasing HPV DNA copy number.68 such case-control studies are complicated by a series of variables, not the least of which is the number of sexual partners[7].

Correlation between HPV presence and coital factors was disputed by Hording et al who compared Greenlandic and Danish women. Greenland has one of the highest incidences of cervical cancer in the world, more than five times higher than Denmark. Greenlandic women commence sexual activity at a significantly younger age and are more likely to have more than 20 lifetime partners (53%) when compared with their Danish counterparts (4%). Despite these behavioural differences, HPV 16 DNA was found in a similar incidence

(55% vs. 45%) in Greenlandic and Danish'women with cervical cancer[8].

Campion et al8 reviewed 26 women who progressed to CIN 3 after initial evidence of mild atypia or dyskaryosis; 85% of those progressing had HPV 16 DNA, compared to the original group[9].

Lorincz et al 9 reported on 398 women, seen in a private gynaecology clinic, who had HPV presence assessed by Southern blot hybridisation of cervical samples obtained from swabs. Women with normal smears were followed up for an average of 2 to 3 years by further smears; among those who originally had HPV 15% developed cytological changes consistent with cervical intraepithelial neoplasia compared with 5% who were HPV negative.Ref Lorincz A T,Schiffman M H , Jauffer W J etal. Temporal association of HPVB with cervical cytologic abnormalities[10].

Similarly Koutsky et al followed a cohort of 241 women with normal smears using dot-filter hybridisation at 4 monthly intervals; the 2 year cumulative incidence of CIN 2-3 from the time of first positive test for HPV DNA was 28% compared with 3% if the smear remained HPV negative[11].

Young et al 10 reported that of 38 women with an abnormal smear 36 had HPV 16 and 22 had both HPV 16 and HPV 11. However, 7 of 10 women with a normal smear also tested positive for HPV 16 or HPV 11 DNA[12].

Tidy et al 11 reported that 67% of dyskaryotic smears contained HPV 16, and, if carcinoma was present the figure rose to 100%. Again, 117 of 140 (84%) of the normal smears were also positive. He detected by PCR[13].

Macnab et al 12 found that 84% of cervical and vulval carcinoma contained HPV 16 DNA, but also found" the virus DNA in 73% of histologically normal control tissue adjacent to the lesion. This control tissue was taken from the uterus at radical hysterectomy or from a strip of abdominal wall skin superior to the transverse abdominal incision at radical vulvectomy and at least 2 cm from the nearest tumour edge. When specimens of ectocervical epithelium taken at hysterectomy performed for non-neoplastic conditions were'examined, only 11% contained HPV 16 DNA[14].

Meanwell et alsl 13 found HPV 16 DNA in 35% of women with normal cervical smears. They, however found no evidence of virus DNA integration, whereas Macnab's group32 detected HPV DNA integrated within host chromosome and surprisingly, sometimes present in higher viral copy

numbers in normal tissue than in the neoplastic tissue[15].

. Kitchener et al" studied a series of sexually active women, recruited at a Family Planning Clinic, with a routine cervical smear in which koilocytosis suggested the presence of HPV infection but the absence of dyskaryosis. 10% of the women had HPV 16 DNA in tissue taken from the transformation zone, and some had HPV 16 in the apparently normal adjacent squamous epithelium[16].

Syrjane et al 15 found progression to CIN 3 from lowgrade lesions in 66 of 508 women (12.9%) during a mean follow up period of 35 months, where HPV 6 and HPV 11 were found as frequently as HPV 16 and HPV18[17].

Portland Kaiser Permanente studies allowed researchers to examine whether testing for specific human papilloma virus (HPV) types—HPV16 and HPV18—were more effective at predicting risk for precancerous conditions or cervical cancer than testing for a broad pool of cancer-causing, or oncogenic, HPV types. The study authors found that women who tested positive for HPV16 or HPV18 had an increased risk for precancerous conditions and cervical cancer compared to those women who tested negative for HPV)[18].

In October 1999, Sankaranarayanan et al 16 initiated a cluster-randomized, controlled trial to evaluate the effectiveness of a single round of HPV testing, cytologic testing, or VIA in reducing the incidence of cervical cancer, as compared with a control group that received usual care in a previously unscreened, high-risk population in the Osmanabad district in the state of Maharashtra, India. They reported the cervical-cancer incidence and mortality in the four groups after 8 years of follow-up. They found that HPV testing was the most objective and reproducible of all cervical screening tests and was less demanding in terms of training and quality assurance. In low-resource settings with no capacity for colposcopy and histopathological analysis (e.g., many countries in sub-Saharan Africa), HPV-positive women without clinical evidence of invasive cancer could receive immediate treatment, such as cryotherapy. However, since most HPV infections in young women regress rapidly without causing clinically significant disease, such an approach raises a legitimate concern. Hence, HPV testing should not be used for primary screening of women under 30 years of age[19].

Against the prevailing view during the 1970s, Harald zur Hausen 17 postulated a role for human papilloma virus (HPV) in cervical cancer. He assumed that the tumour cells, if they contained an oncogenic virus, should harbour viral DNA integrated into their genomes. The HPV genes promoting

cell proliferation should therefore be detectable by specifically searching tumour cells for such viral DNA[20].

Sowjanya 18 AP, Jain M, Poli UR, Padma S, Das M, Shah KV, Rao BN, Devi RR, Gravitt PE, Ramakrishna G. Centre for DNA Fingerprinting and Diagnostics, Hyderabad, A.P. India. conducted a study about the Prevalence and distribution of high-risk human papilloma virus (HPV) types in invasive squamous cell carcinoma of the cervix and in normal women in Andhra Pradesh, India[21].

Cherian Verghese OF Trivandrum Kerala conducted a study about the prevalence of HPV in high risk cervical lesions in Kerala.**conclusion:** is as follows the over all prevalence rate is 7% with higher prevalence among low SES,women of poor education,early age of marriage and husbands of women with multiple sexual partners[22].

STUDY

AIM OF THE STUDY :

To determine the HPV status in high and low grade cervical lesions in women of reproductive age group .

SETTING:

It is a prospective study conducted in the Institute of Social Obstetrics (ISO) & Govt. Kasturba Gandhi Hospital for Women and Children, Triplicane. Chennai attached to the Madras medical College, Chennai.

SAMPLE SIZE:

IT is conducted in a sample of 50 women with high risk cervical lesions during the period 2008-2009 .

Around 350 women in the reproductive age group are screened in the colposcopy clinic and among them 50 are selected for HPV testing.

MATERIALS AND METHODS :

THE HC2 High-Risk HPV DNA Test using capture 2 technology is a nucleic acid hybridization assay with signal amplification that utilizes micro plate chemiluminescent detection.

It is a solution phase hybridization assay which results in HPV DNA signal amplification.

All my tests are done in the Lab of RENU DIAGNOSTICS.

The kit is an in vitro nucleic Acid Hybridization Microplate Assay with Amplification using Microplate chemiluminescene for the Qualitative Detection of 13 High-Risk types of human papillomavirus (HPV) DNA in cervical Specimens. It is a DNA PAP Cervical Sampler.

The Manufacturers are DIEGENE, 1201, Clopper road, Gatheisberg, USA. A R Med Limited.

Samples are collected by cervical brushings in liquid media and subjected to the test

The assay uses RNA probes that react with 13 DNA targets

These RNA-DNA hybrids are captured by monoclonal antibodies bound to the well plate and detected with anti hybrid antibodies by chemiluminesence.

Step 1Denature DNA [~1 hour]To initiate the lab process (labeling tubes for identification, etc.).

1. Cervical specimen is added to sample tube.
2. Denaturation agent is added to tube.

Step 2: Mixing of probes and hybridization [~1.5 hours]

1. Probe B cocktail for the high-risk HPV types is prepared and Added to the Tubes. (Probe B is specific for oncogenic HPV types.)
2. The tubes are placed in a water bath at 65°C for 30 minutes.
3. The samples are washed several times using a standard laboratory reagent.

Step 3: Hybrid capture [~1.5 hours]

1. The processed samples are transferred to a microtiter plate provided in the kit.
2. It is placed on a mechanical shaker for 30 minutes (allowing the RNA-DNA hybrids created in Step 2 to be “captured” by antibodies attached to the walls of the microtiter well).

Step 4: Detection for labeling [~1 hour]

1. Additional antibodies tagged with alkaline phosphate, are added which bind with the captured materials from Step 3. When the alkaline phosphatase separates from the antibody complex, light is produced.

Step 5: Detection, validation, and interpretation [~1 hour]

2. The light released during Step 4 is measured using a luminometer with integrated computer software. Any sample that emits light as bright as or brighter than the light released by a positive control is considered a positive signal for HPV.

INCLUSION CRITERIA:

Women in the age group 25-35 yrs

Women who are leading an active sexual life

Women who have HSIL and HSIL in colposcopic findings .

EXCLUSION CRITERIA:

Post menopausal women

Who are not leading an active sexual life

Invasive cancer cervix

Normal colposcopic findings

Women with gross active PID or cervicitis

FOLLOW UP:

HPV testing is either coded positive or negative.

The results are expressed as a ratio of the specimen strength in relative light units (RLU) to the concurrently tested 1pg/ml HPV DNA controls.

HPV positive patients are followed up with colposcopic examination and repeat testing after 6months.

RESULTS AND ANALYSIS

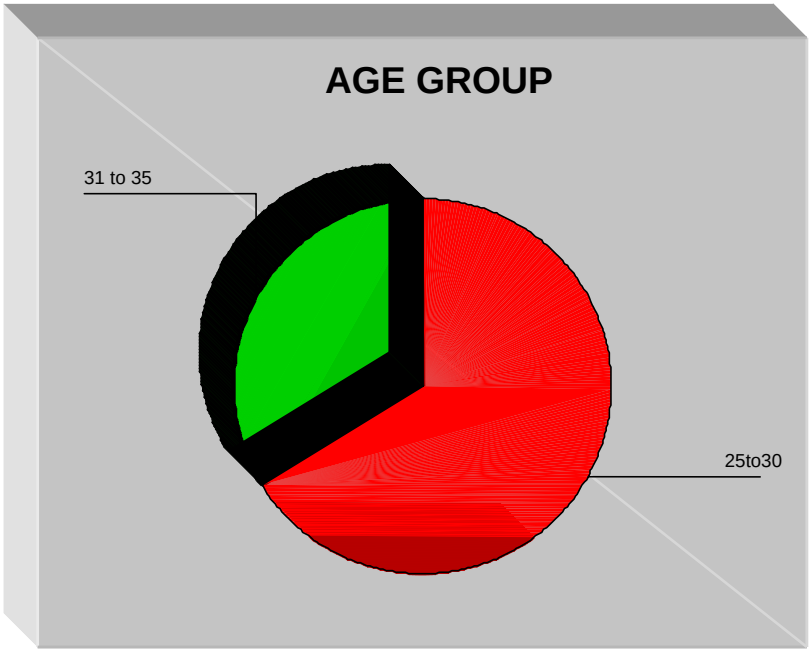


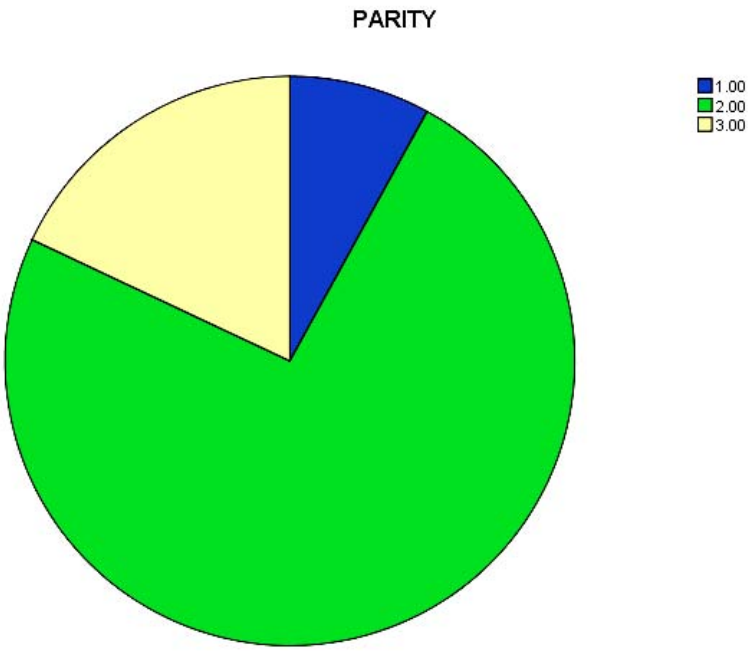
TABLE1

	Frequency	Percent
25 to 30	**33**	**66.0**
31 to 35	**17**	**34.0**
Total	**50**	**100.0**

The study group involves reproductive age group women who are leading an active sexual life.

66% were in 25 to 30yrs age group.

34% were in 31 to 35yrs age group.



1-P1 2-P2 3-P3

TABLE 2

	Frequency	Percent
1.00	4	8.0
2.00	37	74.0
3.00	9	18.0
Total	50	100.0

The distribution of parity among the study group were

P1 and P2 82%

P3 18%.Most of the study population were multi parous women.

CONTRACEPTION

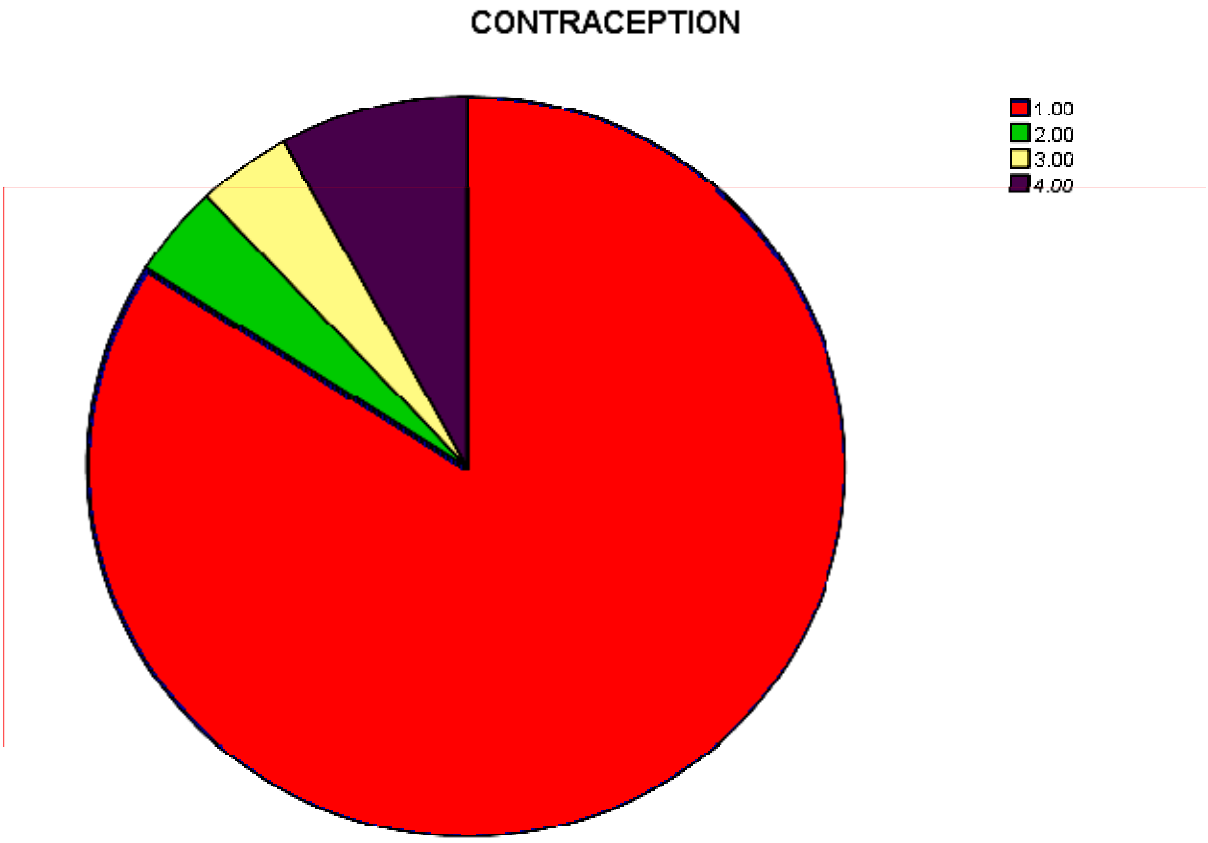
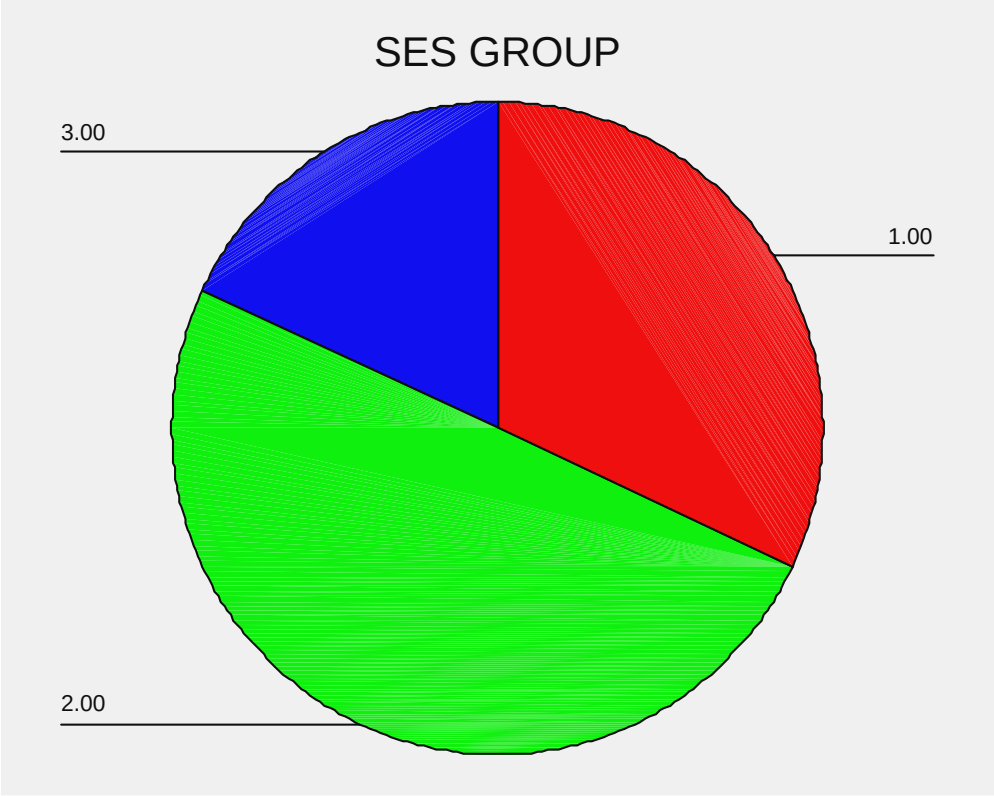


TABLE 3

	Frequency	Percent
1 PS	42	84.0
2 BARRIER	2	4.0
3 OCP	2	4.0
4 IUCD	4	8.0
Total	50	100.0

84% of study population was sterilized by Puerperal Sterilization.

8% used IUCDs. 4% each used barrier and OCPs.



1income Rs1500PM and 2 income Rs2000PM

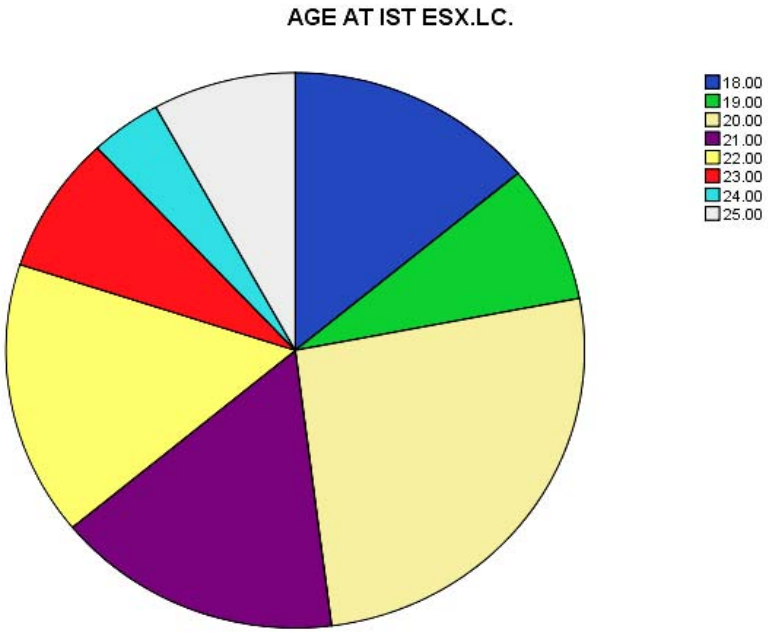
3 income≥ Rs 3000 PM.

82% belong to income ≤ Rs 2000

18% belong to income ≥Rs3000

TABLE 4

	Frequency	Percent
1.00	16	32.0
2.00	25	50.0
3.00	9	18.0
Total	50	100.0



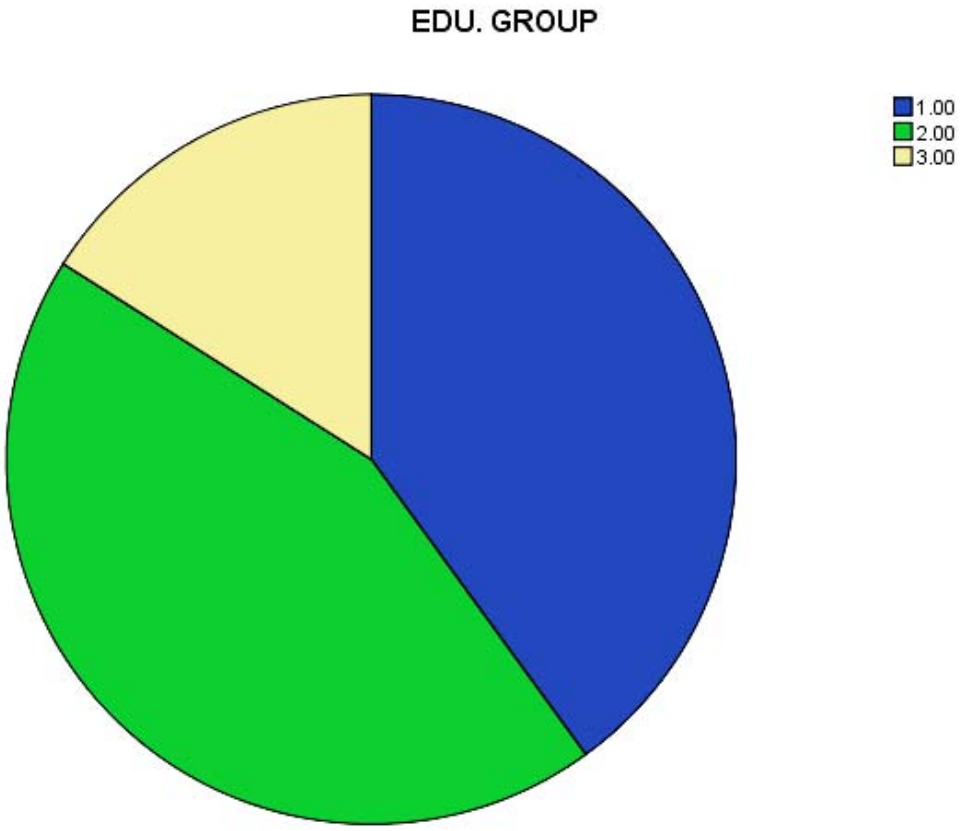
AGE OF MARRIAGE

80% of the study group had consummation of marriage before 22 yrs.

20% were in 23 to 25 yrs age group.

TABLE 5

AGE	Frequency	Percent
18.00	7	14.0
19.00	4	8.0
20.00	13	26.0
21.00	8	16.0
22.00	8	16.0
23.00	4	8.0
24.00	2	4.0
25.00	4	8.0
Total	50	100.0


EDU. GROUP
1.00
2.00
3.00

1-< 5th std

2 -5th to 10th std

3-> 10th std

84% were studied below 10th std.

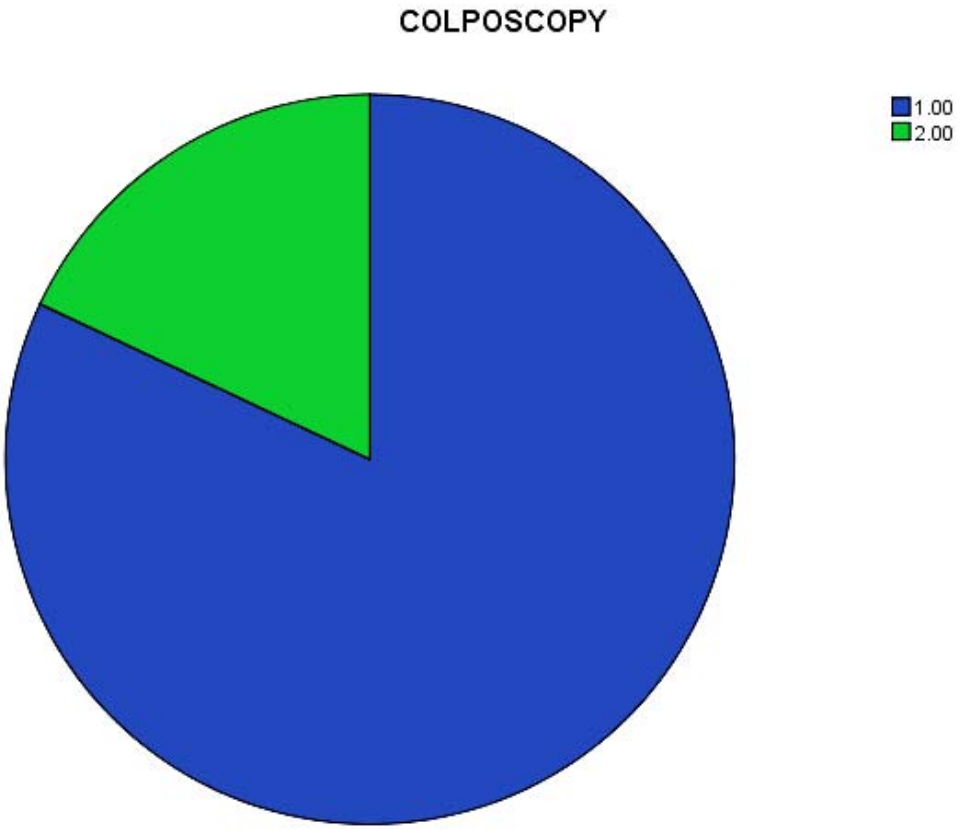
16%studied above 10th std.

EDUCATION

TABLE 6

EDUCATION	VALID	PERCENT
UP TO 5th STD	20	24

5th TO 10th	22	68
ABOVE 10th STD	8	8
TOTAL	50	100



1

-LSIL

2- HSIL

TABLE 7

COLPOSCOPY	FREQUENCY	PERCENT
LSIL	**41**	**82**
HSIL	**9**	**18**
TOTAL	**50**	**100**

82% of study population had LSIL and 18% of study population had HSIL as their colposcopic findings.

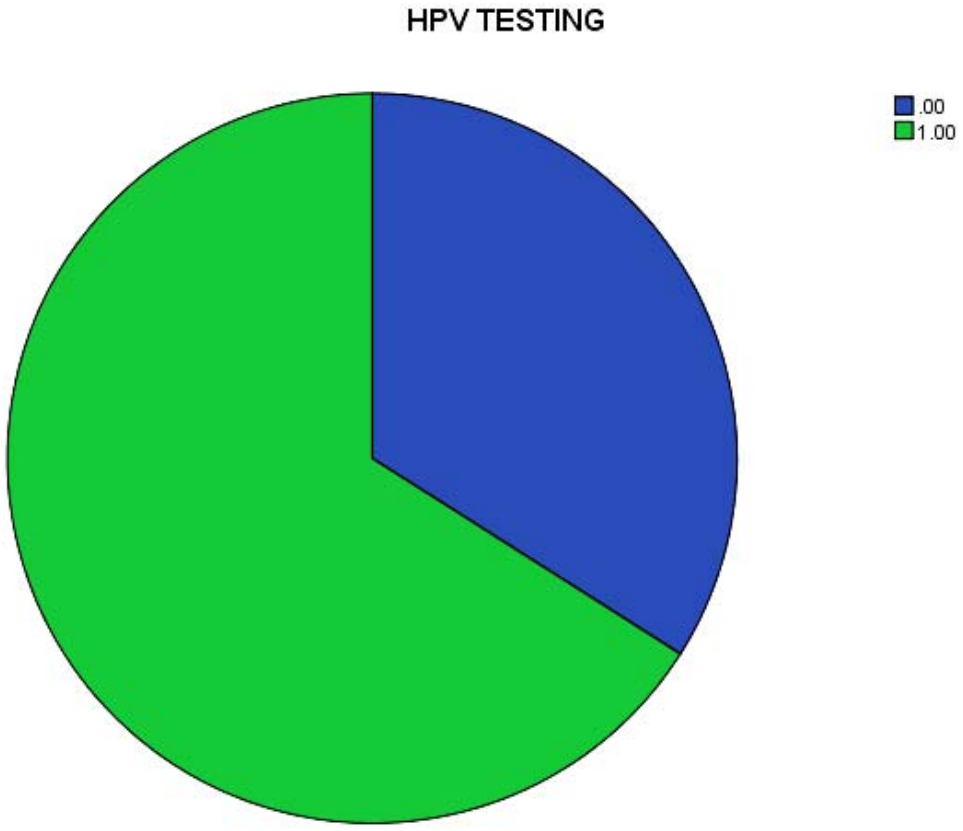
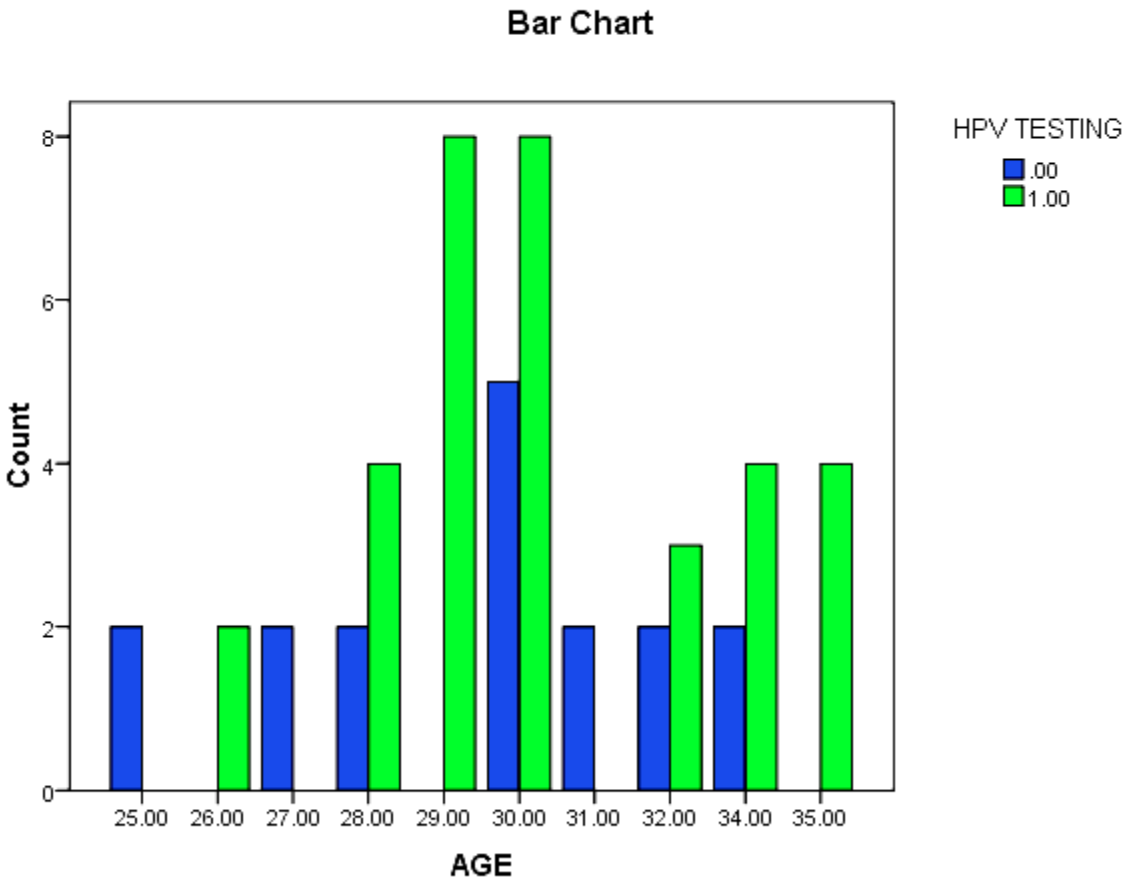


TABLE 8

HPV TESTING	FREQUENCY	PERCENT
POSITIVE	17	34
NEGATIVE	33	66

66% Of colposcopic lesions (LSIL and HSIL)were positive for HPV testing. 34% of lesions were negative for HPV testing.

AGE & HPV TESTING



1-HPV POSITIVE

2-HPV NEGATIVE

TABLE 9

HPV TESTING

AGE GROUP	NEGATIVE	POSITIVE	TOTAL
25 TO 30	11	22	33
31 TO 35	6	11	17
TOTAL	17	33	50

66% of HPV positive population falls in the age group 25 to 30 years.

The peak incidence of HPV is between 28 to 30 yrs of age group.

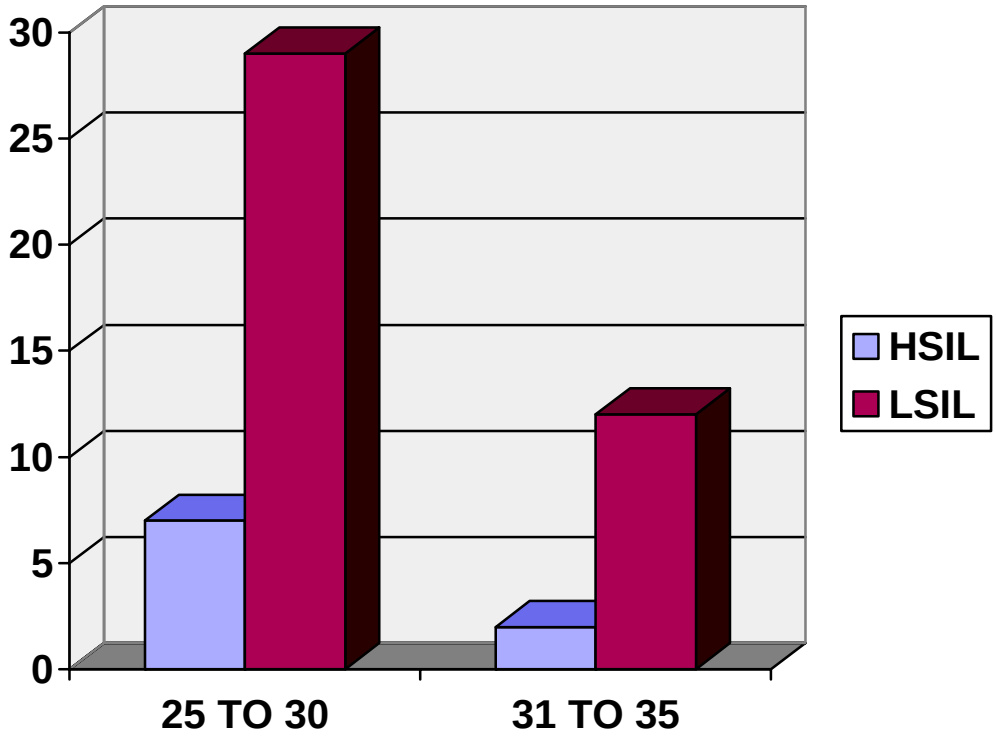
The incidence of HSIL and LSIL among different age groups

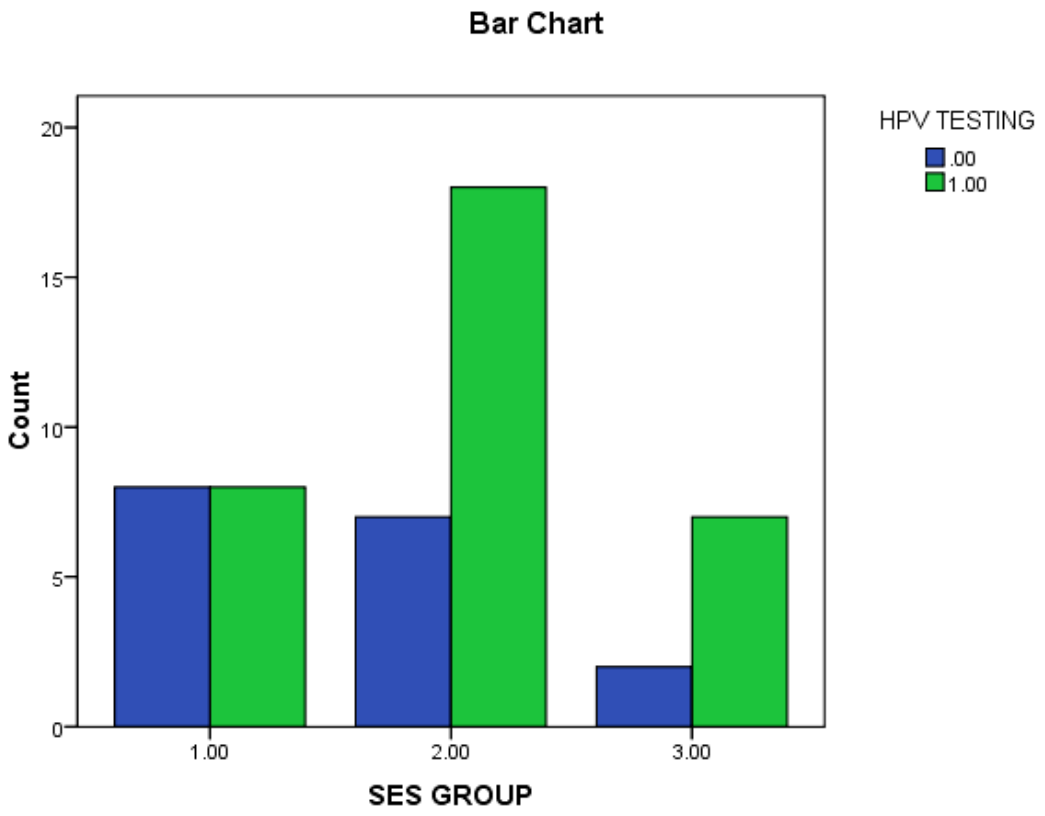
TABLE 10

AGE GROUP	HSIL	LSIL	TOTAL
25 TO 30	7	29	36
31 TO 35	2	12	14
TOTAL	9	41	50

.

The LSIL and HSIL lesions show peak incidence between age groups 25 to 30 years.





1income ≥ Rs 3000 PM . and 2 income Rs2000PM

3 income Rs1500PM

TABLE 11

HPV TEST

SES/INCOME PM	NEGATIVE	POSITIVE
≥ Rs 3000 .	8	8
Rs 2000	7	18
Rs1500	2	7
TOTAL	17	33

The study is conducted mainly in low socio economic group.88% of the study population with income<RS2000 were positive for HPV. Among income group ≥ Rs 3000 PM HPV positivity is 50%.HPV infection is prevalent among low socio economic status.

EDUCATION

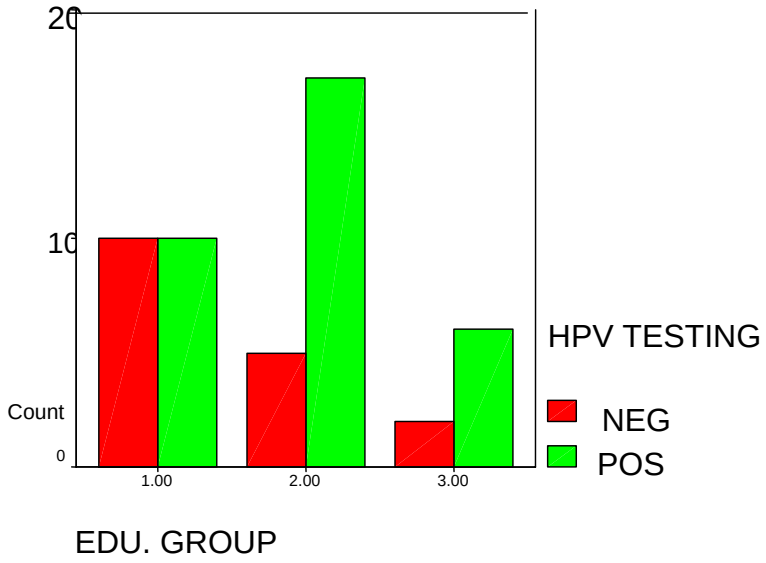


TABLE 12

HPV TEST

EDUCATION	NEGATIVE	POSITIVE
1-NIL	10	10
2-<5th STD	5	17
3- 5th to 10th STD	2	6
TOTAL	17	33

The HPV infection is increased among illiterate women and who are studied less. My study is conducted more on women who are studied less than high school

AGE OF MARRIAGE

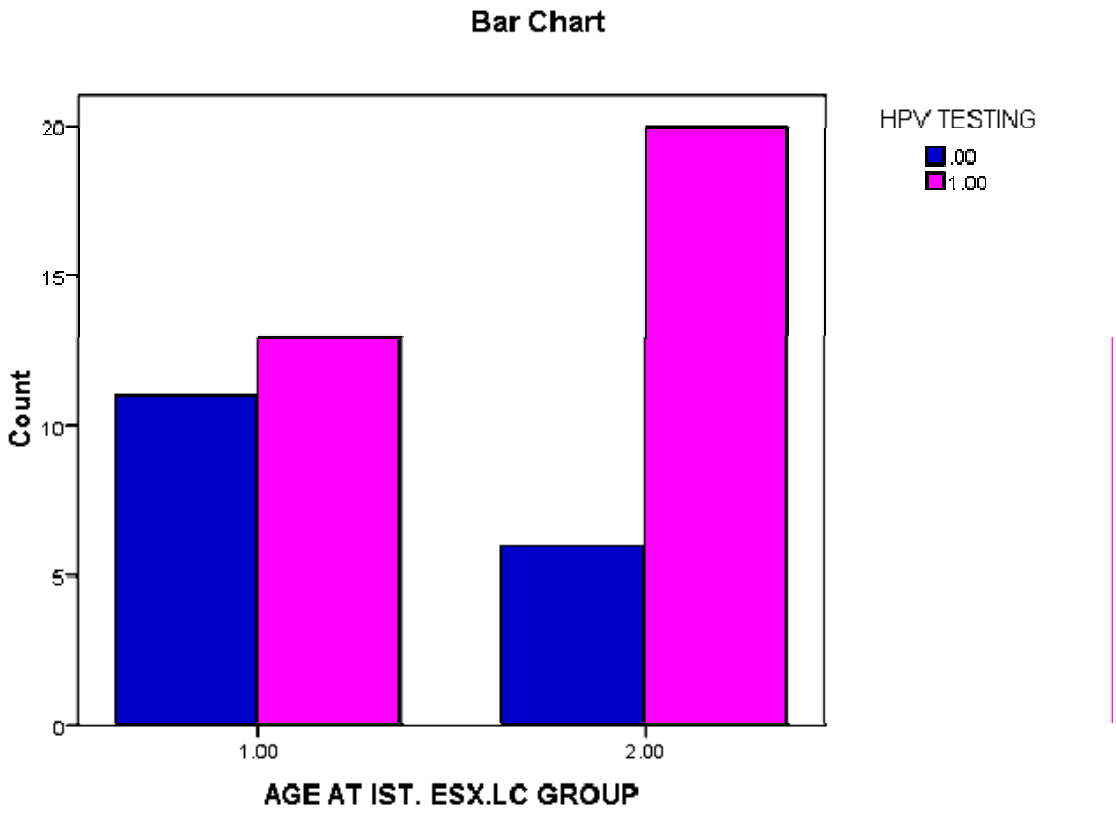


TABLE 13

HPV TEST

AGE OF MARRIAGE	NEGATIVE	POSITIVE
2 < 20 YRS	6	20

1 20 to 25 YRS	**11**	**13**
TOTAL	**17**	**33**

Earlier is the age of marriage the HPV infection rate is high.77% of those who are married < 20 yrs got infected with HPV where as only 50% of those who are married >21 yrs got infected with HPV.

PARITY

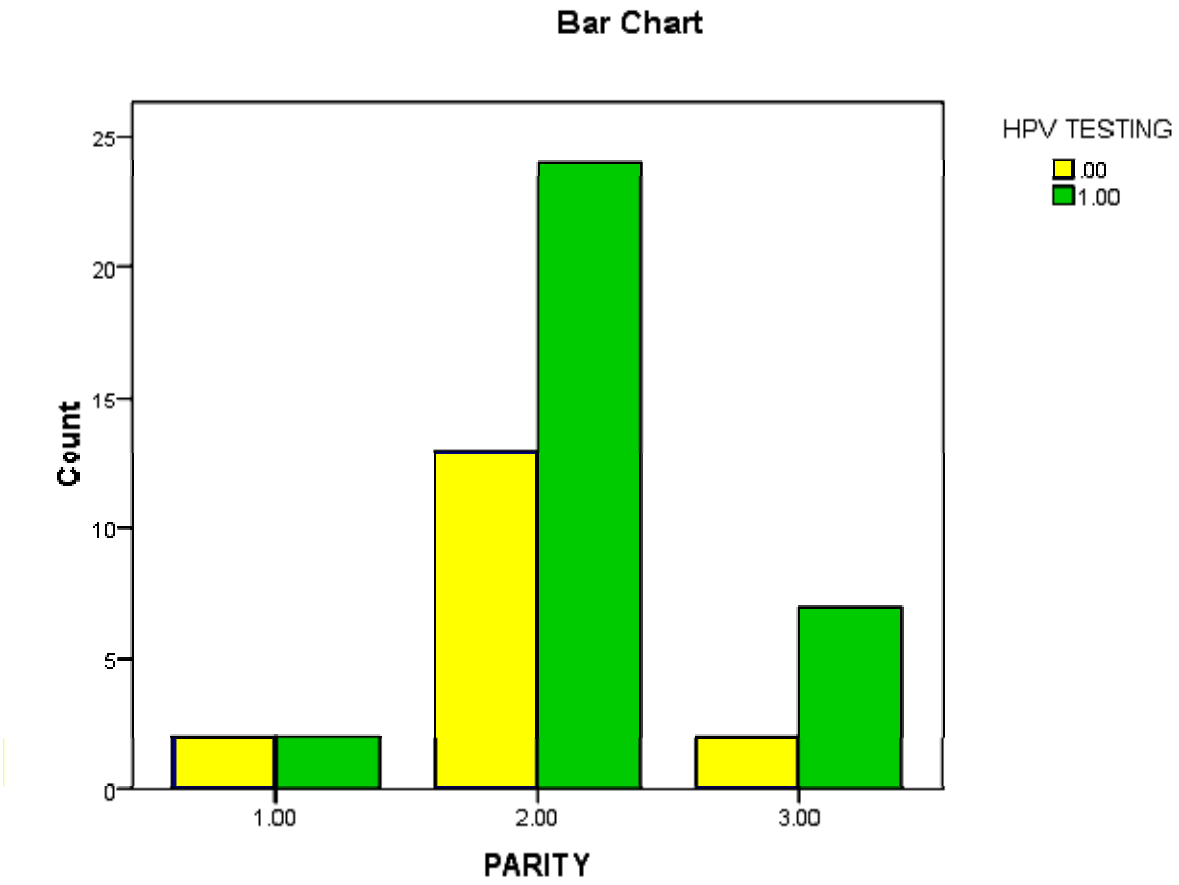
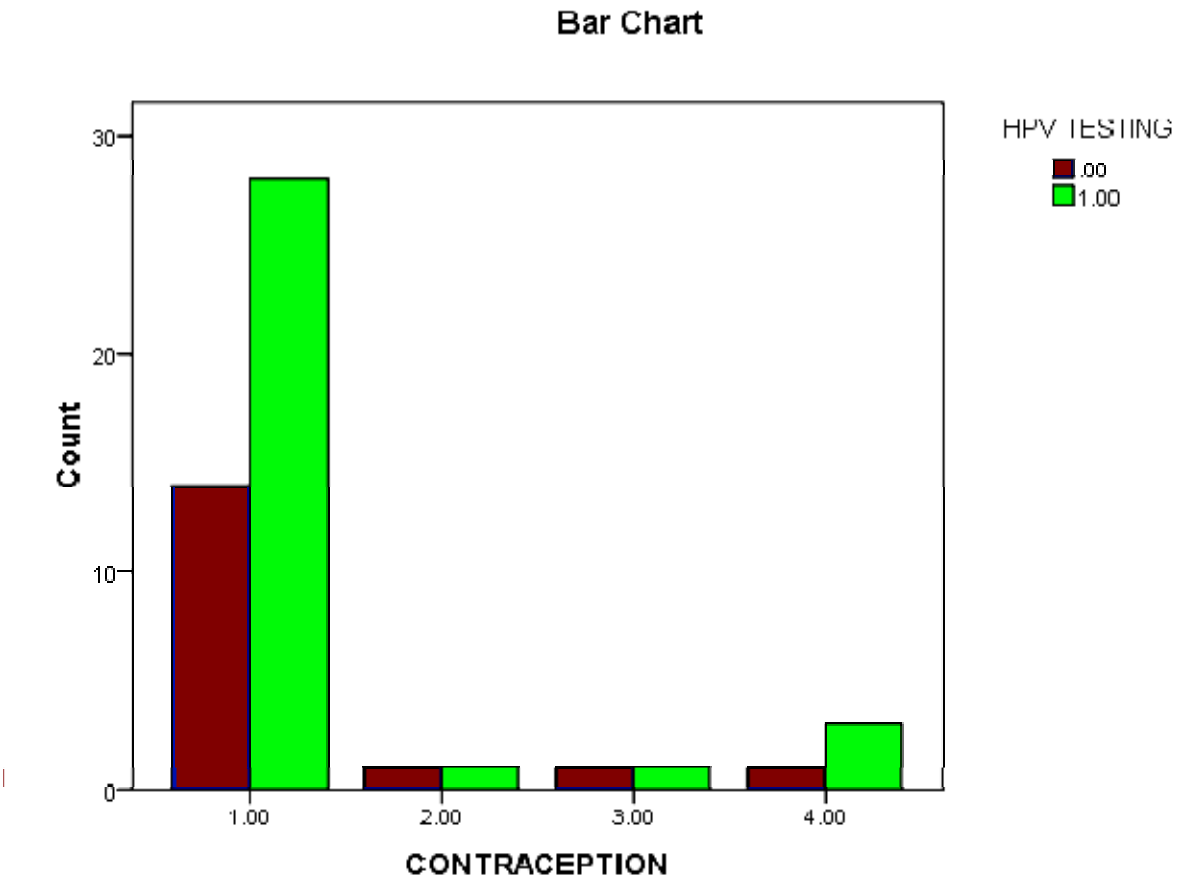


TABLE 14

HPV TEST

PARITY	NEGATIVE	POSITIVE
P1	2	2
P2	13	24
P3	2	7
TOTAL	17	33

The infection rate is high among multiparous women.



1 PS

2

BARRIE

R

3 OCP

4 IUCD

1-HPV POSITIVE

2-HPV NEGATIVE

TABLE 15

HPV TEST

CONTRACEPTION	NEGATIVE	POSITIVE
PS	14	28
BARRIER	1	1
OCP	1	1
IUCD	1	3
TOTAL	17	**33**

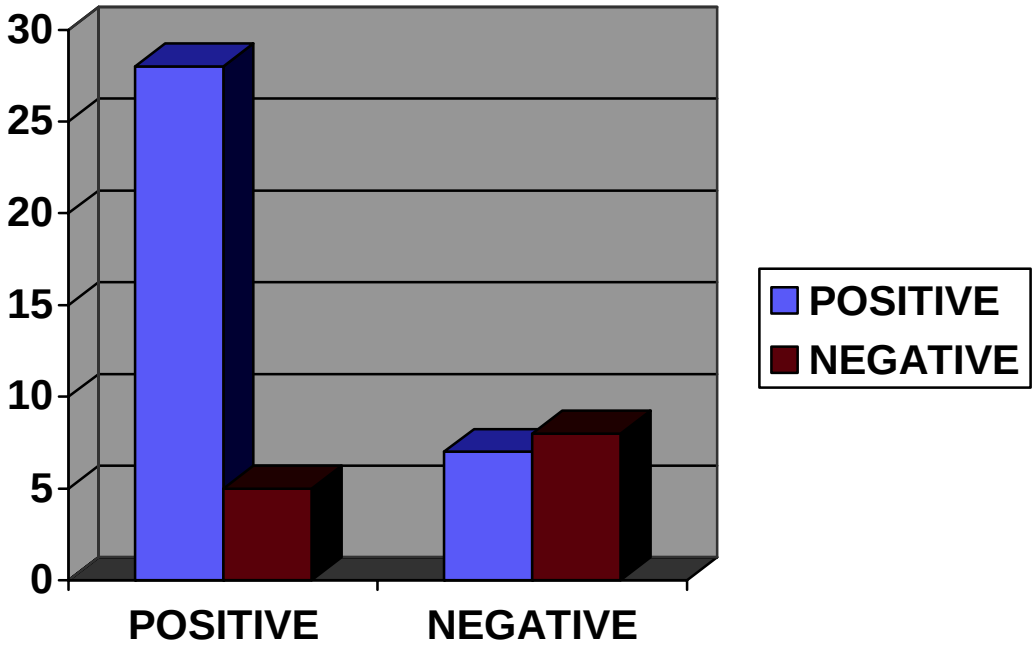
The HPV positivity is increased among the population who had undergone PS and are using IUCDs.Women who were using Barrier methods of contraception and OCPs show less positivity compared to others. Women who used OCPs also used barrier methods in between in my study group.

SMOKING AND HPV:

TABLE 16

HPV TEST

SMOKING	POSITIVE	NEGATIVE
PRESENT	28	7
ABSENT	5	8



84% 0f HPV positive women are smokers.This shows that there is a definite relation between smoking and HPV infection.

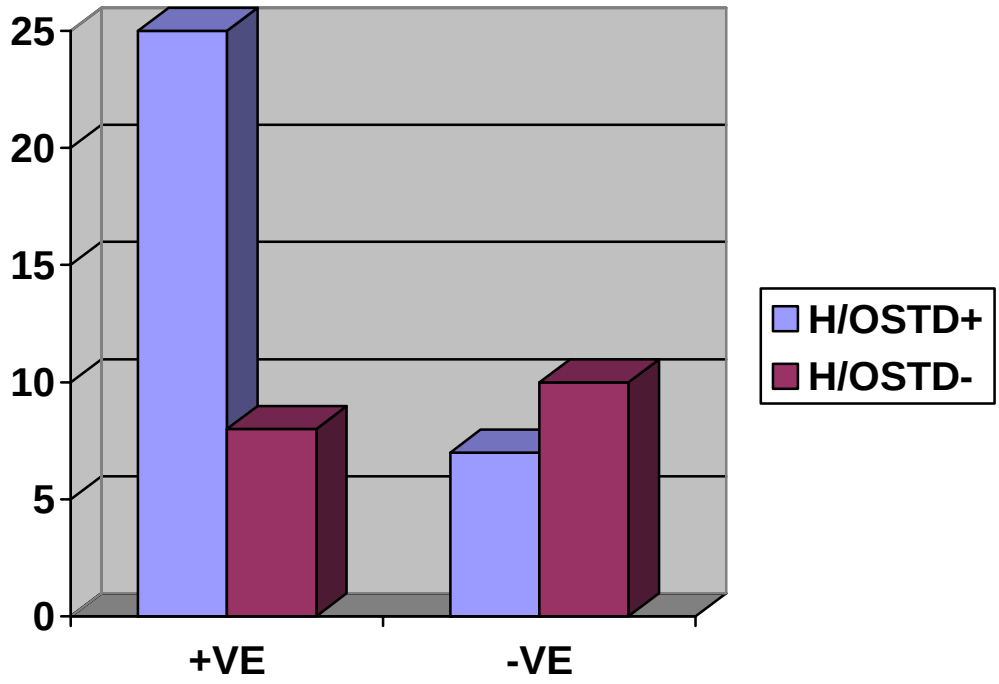
PREVIOUS HISTORY OF STD:

TABLE 17

HPV TEST

PAST H/O STD	POSITIVE	NEGATIVE
PRESENT	25	7
ABSENT	8	10

75% of women who test positive for HPV had a past h/o one or more episodes of Reproductive tract infection.



HPV IN HIGH AND LOW GRADE LESIONS.

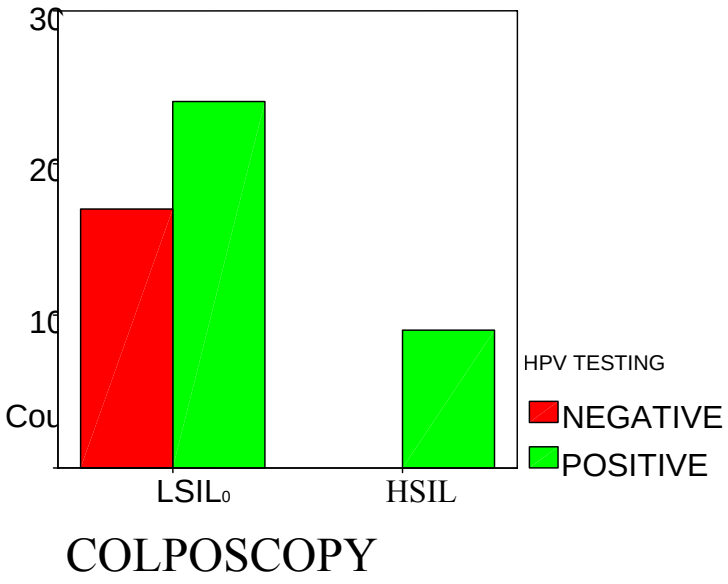


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TABLE 18

HPV TEST

COLPOSCOPY	NEGATIVE	POSITIVE
LSIL	17	24
HSIL	0	9
TOTAL	17	33

Almost 100% of HSIL lesions show positive for HPV infection and 60% of LSIL lesions show positive for HPV infection.

COMPOSITE TABLE SHOWING CO-FACTORS OF HPV LEADING ON TO PRECANCEROUS LESION OF CERVIX:

P Value showing significance

TABLE 19

HPV positivity	Colposcopic lesions	Past h/o STD	Smoking	Education	SES	Age at marriage	Age group

33	**0.017**	**0.049**	**<0.005**	**0.001**	**<0.001**	**<0.005**	**0.048**

This table shows the relative significance associated with HPV in causing pre cancerous lesions of cervix.This helps us in knowing the proportion of exposure and prevention.Thus Cancer cervix can be prevented by improving the SES ,Health education regarding early screening methods,appropriate treatment of SIL of cervix,avoiding tobacco and getting married >21 years.

DISCUSSION

HPV and Age group

This study is mainly conducted in reproductive age group between 25 and 35 years.The peak incidence of HPV infection is between 28 and 30 years.The peak age group in this study correlated well with the study conducted by Cherian Verghese in 2000.

The ICPO community based study on 2073 married women in Alipur Delhi showed a peak prevelance in the age group 25 to 29 years.

Grodione et al in his study of 2342 population documented an incidence of HPV in the age group 17 -25 years.Cherian Verghese of Kerala reported the peak age prevalence at 30years of age.

EDUCATION AND HPV

Education influences screening behavior through its effects on income and through its association with individual knowledge about cancer screening. The health-seeking behavior of many of the women in this setting is guided by traditional notions of ill health; many of them are not sure about the healing provided by modern medicine. Thus, communication problems that arose from differences among conceptions of health may also have contributed to the association between low levels of education and reduced participation. **Ref** Bradley J, Risi L, Denny L. Widening the cervical cancer screening net in a

South African township: who are the underserved? *Health Care Women Int* 2004; 25:227-41

Gwande et al studied abouy the HPV status in under developed areas and stated that it is a disease of poor educational status.

Verghese et al also concluded this in his study.

HPV AND SOCIO ECONOMIC STATUS

Low socio economic status is recognized as a risk factor for many problems including HPV infection.Women with low socio economic status have limited income ,restricted acsess to health care services,poor nutrition and a low level of issues about health care services and preventive behaviour.My study included women of low SES and hence icreased incidence.

Sanjos et al who conducted a study in Madhya Pradesh in 1997 found a60%Decrease of prevelance of HPV among upper SES.

Gwande et al in 1998 in his study detected that Poor genital hygiene prevalent among low SES is a risk factor for HPV.

Dutta et al 1990 and Verghese etal 1999 also found HPV prevelance to be increased among low SES.

PARITY AND HPV

HPV infection rate is high among women with increased parity. Pooled data from 8 case control studies from various continents show that as parity increases the HPV infection rate increases.The possibilities includes hormonal factors and trauma caused by delivery.

Cherian Verghese in 2000 in his study has comparatively showed increased incidence in higher parities compared to lower parities.

Fife et al in 1999 had shown increased prevelance of HPV among pregnant women , however only 20% of the infection continues to persist.

Anna R Guiliano et al in his study had shown there is increased incidence of HPV as the parity increases.

CONTRACEPTION

In June 2000, the National Institutes of Health (NIH), in collaboration with the Centers for Disease Control and Prevention (CDC), the Food and Drug Administration (FDA), and the United States Agency for International Development (USAID), convened a workshop to evaluate the published

evidence establishing the effectiveness of latex male condoms in preventing STDs, including HIV**.** A summary report from that workshop was completed in July 2001 which says that condom use prevents HPV infection to some extent.

Present study shows a comparatively less incidence of HPV infection among condom users.

Cherian Verghese in his study has documented a Very low incidence of HPV infection among barrier contraceptives.

Hippelianen et al in 1993 in his study had said that Population with non condom users show more incidence of HPV

AGE OF 1st SEXUAL INTERCOURSE AND HPV

Earlier is the age of marriage there is increased incidence of HPV infection because the transformation zone is exposed very much in the ecto cervix which has cells of increased multiplicity rate.

The National Health and Nutritional Examination Survey. NHANES was conducted from 1991-1994 by the National Center for Health Statistics and CDC using a complex, stratified, multistage probability cluster design in a sample population about the association between early age at first intercourse,and HPV infection.The study says that HPV infection rate is

increased among women of early age of 1st intercourse.The probable reasons given being it is a is a measure of lifetime rather than recent exposure.

Ref Journal of infectious diseases

11.15.02; Vol. 186; No. 10: P. 1396-1402; Katherine M. Stone; Kevin L. Karem; Maya R. Sternberg; Geraldine M. McQuillan; Alysia D. Poon; Elizabeth R. Unger; William C. Reeves

Present study shows a high incidence of HPV among women who marry less than 20 years , which is very much documented in other studies coducted by Elstrom et al in 1996 and Cherian Verghese in 2000.

INCIDENCE OF HPV IN HIGH AND LOW GRADE LESIONS:

HPV infection is sexually transmitted, affects the immature metaplastic cells of uterine cervix and, in an unknown proportion, results in squamous intraepithelial lesion (SIL) of differing severity was the report given by National Health Research Institutes, Taiwan, who conducted a A Multi-Center Survey of HPV in Cervical Intraepithelial Neoplasia (CIN) With Longitudinal

Follow-Up of LSIL Cases of cervix. An average estimation holds that about 60% of low grade SIL will regress, 30% will persist, 10% will progress to high grade lesions and less than 1% becomes invasive lesions.

Present study showed a high rate of incidence among HSIL (100%) and LSIL (60%) which is very much documented in many of the studies.

Verghese et al showed 100% incidence of HPV in HSIL and 33.3% in LSIL.

Van den brew etal in his stidy has demonstrated 100% incidence of HPV in squamous intra epithelial lesions.

Clavel et al had shown a 100% incidence of HPV in high grade cervical lesions.

Elfgren et al in his study of squamous intra epithelial lesions

Had shown84.8% of SIL showed HPV +vity and 28% of that lesions show persistence .

SMOKING AND HPV INFECTION.

A prospective study was conducted about the association between smoking and HPV by Aline Simen-Kapeu, Vesa Kataja, Merja Yliskoski, Kari Syrjänen, Joakim Dillner, Pentti Koskela, Jorma Paavonen *and* Matti Lehtinen in 2008 among 191 HPV infected samles which says that Smoking impairs the antibody response against HPV and hence their HPV positivity status is maintained for a long time.Ref **Scandinavian Journal of Infectious Diseases**2008, Vol. 40, No. 9, Pages 745-751

In the new study, Gunnell and colleagues underwent cervical screening of 146,104 women in a region of Sweden between 1969 and 1995. The study authors wrote that there appears to be a "synergistic" relationship between heavy smoking and high levels of a specific strain of HPV.

Current smokers who had signs of HPV infection at the time of their first Pap smear were more than 14 times more likely to show signs of precancerous lesions than current smokers who weren't infected.

And heavy smokers who had high levels of HPV when first tested were 27 times more likely to have precancerous lesions. Among nonsmokers, however, high HPV levels only raised their risk by six times.

Gunnell said that cigarette smoke affect the immune system. Both smoking and HPV affect molecules known as cytokines, which control tumor growth, he said.**Ref** Anthony Gunnell, M.A.Sc., researcher, Karolinska Institutet, Stockholm, Sweden; November 2006, *Cancer Epidemiology, Biomarkers & Prevention*

Sikstom et al1995 said that smoking is an important risk factor for HPV and the odds ratio associated was 1.4.

PREVIOUS H/O STD:

HPV infection rate is increased among women with a past h/o STD.

John W. Sellors, Tina L. Karwalajtys, Janusz Kaczorowski, James B. Mahony, Alice Lytwyn, Sylvia Chong, Joanna Sparrow, Attila Lorincz, and The Survey of HPV in Ontario Women (SHOW) Group studied about the Incidence, clearance and predictors of human papillomavirus infection in women.

Incident infection with carcinogenic HPV was highest in women aged 25-29 years, and risk factors were consistent with a h/o sexually transmitted infection in the past. Also a large proportion of the women who were HPV-positive appeared to have cleared the infection after one year.

Ref CMAJ. 2003 February 18; 168(4): 421–425

My study showed a 75% of HPV positive rate with a previous H/O STDS.

HPV INFECTION RATE AMONG VARIOUS RISK FACTORS IN PRESENT STUDY AND OTHER STUDY:

	PRESENT STUDY	CHERIAN VERGHESE 2000
AGE GROUP	28 TO 30 YEARS	30 YEARS
PARITY	MULTI PARITY	P2,P3 ↑INCIDENCE
EDUCATION	ILLITERATE AND LESS THAN PRIMARY SCHOOL	↑ IN ILLITERATES
SES	LOW SES	LOW SES
CONTRACEPTION	BARRIER CONTRACEPTIVES ARE SOME WHAT PROTECTIVE	↓INCIDENCE IN BARRIER USERS
SMOKING	INCREASED AMONG SMOKERS	↑ IN SMOKERS
PAST H/O STD	75% HAVE H/O STD	80% HAVE H/O STD
LSIL	60% SHOW POSITIVE	33.3% +VE
HSIL	100% SHOW POSITIVE	100% +VE

FOLLOW UP

All the HSIL and LSIL lesions are biopsied

		Biopsy	
	1	CIN3	LLETZ followed by Hysterectomy
HSIL9	5	Mod Dysplasia	LLETZ and colposcopy every 6 months
	3	Mild dysplasia	cryotherapy and Colposcopy every 6 months
	30	Inflammatory	Colposcopy every 6 months
LSIL41	7Cases	Mild dysplasia	cryotherapy colposcopy

4 Moderate dysplasia LLETZ Colposcopy every 6 months

These women of moderate dysplasia are in younger age group who want to preserve fertility.

SUMMARY

Although oncogenic HPV infection has been established as a causative factor of the precursors of cancer cervix as well as their progression to higher grade and eventually to malignancy, there are some other predisposing factors which play a substantive role in the causation and progression of these lesions. This study has tried to delineate these risk factors in cases of cervical dysplasia.

This study is a prospective study conducted in a sample of 50 women in the reproductive age group . Women with LSIL AND HSIL Lesions are selected ,cervical samples are taken from them and couriered to Renu Diagnostics lab.HPV infection is detected by HC 2 technique. It is an In vitro nucleic Acid Hybridization Microplate Assay with Amplification using Microplate chemiluminescene for the Qualitative Detection of 13 High-Risk types of human papillomavirus (HPV) DNA in cervical Specimen's..

The results are coded in RLU. HPV infection showed higher incidence among 28to30 years of age, among low SES and among illiterates. 100% of HSIL showed HPV infection and 60% of LSIL showed positive infection.

HPV infection shows higher incidence among low social status where the genital hygiene is poor and among illiterate women who have a very poor knowledge about sources of prevention.Women of low SES also get married soon and have decreased birth spacing which increases the incidence.

Following these women 73% of these lesions were inflammatory ,treated with antibiotics and followed up with colposcopy every 6 months.9 cases found to be moderate dysplasia and treated by LLETZ.10 cases found to be mild dysplasia ,Cryotherapy was done in these women. 1 lesion turned out to be CIN3 and proceeded with TAH.

CONCLUSION

Accumulated evidence based on etiologic associations and the differential world pattern points to cervical cancer being a preventable disease. Sexual hygiene and the use of barrier contraception (condom) may largely achieve this objective but there is a need for long-term education and acceptance.

Improvements in socio-economic standards would automatically reduce morbidity and mortality but this again is a long-term process.

Primary prevention, then, involves the education of a large segment of the population, especially the high risk groups, about sexual hygiene, barrier contraception and control of HPV infection.

Secondary prevention assumes vital importance in the context of the hurdles in implementing primary prevention methods. In a large country such India with a large, growing population and limited resources, population screening by Pap smear is neither pragmatic nor cost-effective. It is thus essential that we evolve our own strategies.

Women with an abnormal cervix should have additional studies including: Pap smear, biopsy, colposcopy and HPV DNA testing wherever possible.

ANNEXURE

PROFORMA

NAME `:

AGE :

S.NO :

ADDRESS :

OCCUPATION :

SE CLASS :

MENSTRUAL HISTORY

AGE AT MENARCHE

CYCLE / DAYS

LAST MENSTRUAL PERIOD

MARITAL HISTORY:

AGE OF MARRIAGE/FIRST INTERCOURSE Years

LIVING WITH HUSBAND

COITAL HISTORY

FREQUENCY OF COITUS

NO.OF SEXUAL PARTNERS

USE OF CONDOMS

OBSTETRIC HISTORY

PARTY

LAST CHILD BIRTH

STERLIZED

PERSONAL HISTORY

H/O OCP

H/O SMOKING

PAST HISTORY:

H/O STD's IN THE PAST

If Any Number Of Times

H/O CRYOTHERAPY DONE

H/O RADIOTHERAPY IN THE PAST

H/O CHEMOTHERAPY IN THE PAST

FAMILY HISTORY:

H/O MALIGNANICES IN THE FAMILY MEMBERS

CLINICAL EXAMINATION:

BUILD AND NOURISHMENT

LOCAL EXAMINATION:

SPECULUM EXAMINATION

VAGINA

CERVIX

ANY DISCHARGE

BIMANUAL PELVIC EXAMINATION:

CERVIX

:

UTERUS

FORNICES

COLPOSCOPIC FINDING:

CYTOLOGICAL STUDY OF CERVIX BRUSINGS

HUMAN PAPILLOMA VIRUS TESTING BY HC II:

OUTCOME : POSTIVE

NEGATIVE

ADVICE:

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SL.NO	age	age at menarche	age at 1 st esx. Ic	parity	lcb	contracep	colpos	hpv testing	ses group	edu group	smoking	h/Ostd
1	35	12	20	3	11	1	1	1	2	1	3	1
2	30	13	22	2	8	1	1	1	1	3	2	1
3	30	14	18	2	8	1	1	0	1	1	2	1
4	29	13	21	2	6	1	2	1	2	2	2	1
5	34	14	19	3	11	1	1	0	2	1	3	1
6	30	15	20	2	5	1	1	1	1	2	2	1
7	28	14	22	2	4	1	2	1	2	3	2	1
8	25	16	20	1	4	3	1	0	3	2	2	0
9	29	15	22	2	3	1	2	1	1	1	1	0
10	30	14	18	2	9	1	1	1	1	1	2	1
11	32	16	21	2	7	2	1	0	1	1	2	1
12	29	15	20	2	5	1	1	1	2	3	2	1
13	35	14	24	3	8	1	1	1	2	2	2	1
14	32	16	22	3	6	1	1	1	3	1	2	1
15	30	13	20	2	5	1	1	0	1	3	2	0
16	29	14	21	2	2	1	1	1	3	2	1	1
17	31	14	25	2	2	1	1	0	1	1	1	0
18	26	16	23	1	2	4	1	1	2	2	1	1
19	28	12	21	2	3	1	1	0	2	2	1	1
20	34	13	25	2	4	1	1	1	3	1	2	1
21	27	14	18	2	4	1	1	0	2	1	2	1
22	34	13	20	2	5	1	1	1	3	2	2	1
23	28	15	23	2	2	1	1	1	2	2	1	1
24	30	13	19	2	5	4	2	1	2	2	2	0
25	35	12	20	3	11	1	1	1	2	1	3	1
26	30	13	22	2	8	1	1	1	1	3	2	1
27	30	14	18	2	8	1	1	0	1	1	2	1
28	29	13	21	2	6	1	2	1	2	2	2	1
29	34	14	19	3	11	1	1	0	2	1	3	0
30	30	15	20	2	5	1	1	1	1	2	2	0
31	28	14	22	2	4	1	2	1	2	3	2	1
32	25	16	20	1	4	4	1	0	3	2	2	1
33	29	15	22	2	3	3	2	1	1	1	1	0
34	30	14	18	2	9	1	1	1	1	1	2	1
35	32	16	21	2	7	1	1	0	1	1	2	0
36	29	15	20	2	5	1	1	1	2	3	2	1
37	35	14	24	3	8	1	1	1	2	2	2	1
38	32	16	22	3	6	1	1	1	3	1	2	1
39	30	13	20	2	5	1	1	0	1	3	2	1
40	29	14	21	2	2	1	1	1	2	2	1	1
41	31	14	25	2	2	1	1	0	1	1	1	1
42	26	16	23	1	2	2	1	1	2	2	1	0
43	28	12	21	2	3	1	1	0	2	2	1	1
44	34	13	25	2	4	1	1	1	3	1	2	1
45	27	14	18	2	4	1	1	0	2	1	2	1
46	34	13	20	2	5	1	1	1	3	2	2	1
47	28	15	23	2	2	4	1	1	2	2	1	0
48	30	13	19	2	5	1	2	1	2	2	2	1
49	32	12	18	3	7	1	2	1	2	2	2	0
50	30	13	20	2	5	1	1	0	2	2	2	1

EDUCATION

3- 5^{th} to 10^{th} STD 2-<5^{th} STD 1-NIL

SES

1income ≥ Rs 3000 PM 2 income Rs2000PM 3 income Rs1500PM

SMOKING

1Tobacco chewers 2 Passive smoking

3 Active smoking

H/O STD

Present 1 Absent 0

HPV TESTING

Positive 1 Negative 0

COLPOSCOPY

LSIL 1 HSIL 2

CONTACEPTION

1 PS

2 BARRIER

3 OCP

4 IUCD

SL.NO	age	age at menarche	age at 1 st esx. lc	parity	lcb	contracep	colpos	hpv testing	ses group	edu group	smoking	h/Ostd
1	35	12	20	3	11	1	1	1	2	1	3	1
2	30	13	22	2	8	1	1	1	1	3	2	1
3	30	14	18	2	8	1	1	0	1	1	2	1
4	29	13	21	2	6	1	2	1	2	2	2	1
5	34	14	19	3	11	1	1	0	2	1	3	1
6	30	15	20	2	5	1	1	1	1	2	2	1
7	28	14	22	2	4	1	2	1	2	3	2	1
8	25	16	20	1	4	3	1	0	3	2	2	0
9	29	15	22	2	3	1	2	1	1	1	1	0
10	30	14	18	2	9	1	1	1	1	1	2	1
11	32	16	21	2	7	2	1	0	1	1	2	1
12	29	15	20	2	5	1	1	1	2	3	2	1
13	35	14	24	3	8	1	1	1	2	2	2	1
14	32	16	22	3	6	1	1	1	3	1	2	1
15	30	13	20	2	5	1	1	0	1	3	2	0
16	29	14	21	2	2	1	1	1	3	2	1	1
17	31	14	25	2	2	1	1	0	1	1	1	0
18	26	16	23	1	2	4	1	1	2	2	1	1
19	28	12	21	2	3	1	1	0	2	2	1	1
20	34	13	25	2	4	1	1	1	3	1	2	1
21	27	14	18	2	4	1	1	0	2	1	2	1
22	34	13	20	2	5	1	1	1	3	2	2	1
23	28	15	23	2	2	1	1	1	2	2	1	1
24	30	13	19	2	5	4	2	1	2	2	2	0
25	35	12	20	3	11	1	1	1	2	1	3	1
26	30	13	22	2	8	1	1	1	1	3	2	1
27	30	14	18	2	8	1	1	0	1	1	2	1
28	29	13	21	2	6	1	2	1	2	2	2	1
29	34	14	19	3	11	1	1	0	2	1	3	0
30	30	15	20	2	5	1	1	1	1	2	2	0
31	28	14	22	2	4	1	2	1	2	3	2	1

32	25	16	20	1	4	4	1	0	3	2	2	1
33	29	15	22	2	3	3	2	1	1	1	1	0
34	30	14	18	2	9	1	1	1	1	1	2	1
35	32	16	21	2	7	1	1	0	1	1	2	0
36	29	15	20	2	5	1	1	1	2	3	2	1
37	35	14	24	3	8	1	1	1	2	2	2	1
38	32	16	22	3	6	1	1	1	3	1	2	1
39	30	13	20	2	5	1	1	0	1	3	2	1
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41	31	14	25	2	2	1	1	0	1	1	1	1
42	26	16	23	1	2	2	1	1	2	2	1	0
43	28	12	21	2	3	1	1	0	2	2	1	1
44	34	13	25	2	4	1	1	1	3	1	2	1
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46	34	13	20	2	5	1	1	1	3	2	2	1
47	28	15	23	2	2	4	1	1	2	2	1	0
48	30	13	19	2	5	1	2	1	2	2	2	1
49	32	12	18	3	7	1	2	1	2	2	2	0
50	30	13	20	2	5	1	1	0	2	2	2	1

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