CYTOLOGY, VISUAL INSPECTION AIDED BY ACETIC ACID (VIA) AND TOLUIDINE BLUE TEST FOR THE EARLY DETECTION OF CERVICAL NEOPLASIA-A COMPARATIVE STUDY

By

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ABBREVIATIONS

ASCUS	-	Atypical Squamous cells of undetermined significance
CIN	-	Cervical intraepithelial lesion
CIS	-	Carcinoma in situ
DAWA	-	Dense aceto white area
HAWA	-	Hazy aceto white area
HIV	-	Human Immunodeficiency Virus
HPV	-	Human papilloma virus
HSIL	-	High grade squamous intraepithelial lesion
IMB	-	Inter menstrual bleeding
INF	-	Inflammation
LBC	-	Liquid based cytology
LSIL	-	Low grade squamous intraepithelial lesion
NVP	-	Negative predictive value
PCB	-	Post-coital Bleeding
PMB	-	Post menopausal bleeding
PMOL	-	Pre malignant oral lesions
PPV	-	Positive Predictive Value
RCI	-	Reid colposcopy index
SCC	-	Squamous Cell Carcinoma
SES	-	Socio-economic Status
VIA	-	Visual inspection with acetic acid
VILI	-	Visual inspection with Lugol’s iodine
TZ	-	Transformation Zone
WDPV	-	White Discharge per Vagina

TABLE OF CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF FIGURES

INTRODUCTION

Worldwide, cancer of the cervix uteri is the second most common cancer in women, accounting for 15% of all malignancies and continues to be a cause of great concern to women's health, being associated with agonizing morbidity and high mortality with an incidence of 5,28,000 new cases per year (2,66,000 deaths in 2012). Almost 70% of the global burden falls in areas with lower levels of development, and more than one fifth of all new cases are diagnosed in India.[1] In developed countries the incidence is as low as 1-8 per 1,00,000 women Cancer cervix is a serious health problem in India[2].Annual number of cervical cancer cases in India is 122,844 and the annual number of deaths due to cervical cancer is 67,477[3]. India's cervical cancer age standardized incidence rate (30.7 per1, 00,000) and age standardized mortality rate (17.4 per 1, 00,000) are the highest inSouth Central Asia[4].

Simultaneously, there is also evidence that India is on the verge of a large HIV epidemic. The Indian National AIDS Control Organization estimates that the number of people living with HIV is approximately 5.1 million (38% of whom were women). This is of great concern when given the strong association between HIV and HPV infections and evidence of more rapid progression of HPV infections to cervical neoplasia in HIV infected women.

Invasive cancer of cervix has been considered a preventable cancer because ithas a long pre-invasive state, cervical cytology screening programs are available andthe treatment of pre-invasive lesions is effective[4a]. The unique accessibility of thecervix to direct visualization and the possibility of cellular and tissue sampling haspermitted extensive investigations on lesions of cervix. There is an excellent

evidence that invasive cancer of the cervix develops from carcinoma in situ or dysplasia. Furthermore the development of HPV vaccine goes long way in preventing cancer cervix.

Therefore screening of the cervix by cytology, visual aided inspection (VIA) screening methods and colposcopy can significantlyreduce the rate of invasive cancers. Downstaging of cervical cancer is the detection of the disease at an earlierstage when it is still curable. Detection is done by nurses and other paramedical healthworkers using a simple speculum for visual inspection of cervix. In places whereprevalence of cancer is high and cytological screening is not available, down-staging screening is useful. It can certainly minimize the cancer death through earlydetection[5].

Pap smear has become a routine method of cervical cancer screening. Itsclinical utilization is rapidly expanding due to the simplicity of the technique, costeffectiveness and less time taken to obtain the diagnosis[6]. Cytology has got certainlimitations like low sensitivity of 51% and a false negative rate of 49%.

Visual inspection with acetic acid where in the cervix is inspected after applying dilute acetic acidand then inspected by naked eyes for evidence of disease, has potential advantages over traditional screening techniques in poorly resourced locations. It does not require asecond person for interpretation of results or a repeated visit by the patient to collect thereport as there is immediate feedback of test results to the patient and importantly,treatment can be provided immediately after the test.

The colposcope is a low power, binocular microscope for study of surfaceepithelium and underlying connective tissue stroma along with vascular pattern[7]. It isan optical method of visualizing lower female genital tract under bright illuminationusing stereoscopic vision. Colposcopy is complementary as well as superior tocytology. It is a simple non-invasive OPD procedure.

It helps in determiningindications for cervical biopsy, locating sites and the extent of biopsy. It helps inavoiding traumatic diagnostic methods like cervical conization for minor lesions andat the same time significant lesions are not overlooked.Colposcopic directed biopsy of suspicious areas provides the final diagnosis inmost situations and is taken as the gold standard in diagnosis of neoplastic lesions.

NEED FOR STUDY

Global evidence demonstrates that the key to reducing cervical cancer morbidityand mortality is early detection coupled with timely treatment of cervical precancerouslesions. Cervical cytology, often referred to as Pap smear, is perhaps the most known ofavailable screening methods.

Although performing a Pap test may seem relatively simple,from both a clinical and programmatic perspective, a large number of steps are requiredto take adequate smear, process and analyse the specimen and inform patients of the results. If any of these steps are unreliable or logistically burdensome, the entirescreening program could breakdown and with it, the potential for any public healthbenefit. Unfortunately, many, if not all, of these steps can be problematic in manydeveloping countries viza, lack of political will, poor organizational backup, financialconstraints and priority given to other health issues like infectious disorders control etc. Ignoranceof the masses about the disease and consequences are other important reasons for setback.

WHO states that cancer cervix is a preventable disease. In India, there lies a stark difference between the fact and reality as the methods employed towards preventing cancer cervix is still lacking in leaps and bounds. Given this reality and fact that screening byPap smear (cytological screening) does have pitfalls and burden of the disease ishighest in low resource countries. If screening & subsequent treatment is to have ameasureable effect on the burden of disease borne by women and the health care system,it is apparent that cervical cancer screening based on an approach other than just papsmears is needed.

Hence other solutions have to be found like-

1. Visual Inspection of cervix- VIA (visual inspection by acetic acid, VILI (visual inspection by Lugol's iodine) or Toluidine blue dye test
2. Use of magnoscope instead of Colposcopy
3. Single visit approach
4. Treatment with cryosurgery for VIA positive women
5. Education and counselling
6. Increasing coverage by camp approach
7. Low cost HPV tests
8. HPV vaccines

Toluidine blue dye test has been used in screening & diagnosis of oral mucosal epithelial abnormalities & vulvalneoplasia. VIA, VILI have been recommended by WHO as an alternative to cytology to pick up a patient at risk for cancer cervix. The present study was undertaken to assess the sensitivity, specificity, positive predictive values and accuracy of VIA, Toluidine blue dye test used instead of lugol's iodine (VILI) and Cervical cytology in picking up pre invasive or invasive cancer of cervix, thereby assessing its efficacy in screening the same for an early detection and prompt treatment of cervical neoplasia.

OBJECTIVE OF THE STUDY

1. To correlate the findings of cytology, VIA and Toluidine blue test for the early detection of cervical neoplasia.
2. To find the relative efficacy of VIA, Toluidine blue test and Cytology in detecting the pre-malignant and malignant lesions of the cervix.

REVIEW OF LITERATURE

In a retrospective study conducted by Joura EA et al. which included 24 women with vulvar epithelial neoplasia (VIN) &74 women with nonneoplastic epithelial disorders, among those who attended a vulvar clinic at a University Hospital during a two year period underwent Vulvoscopy, staining of vulvar epithelium with 1% toluidine blue and punch biopsy. Vulvar epithelium demonstrated toluidine blue staining in 100% of the patients with VIN 3, in 83% of women with VIN 1-2, in 50% of the women with squamous cell hyperplasia and in 10% of the women with lichen sclerosus. The differences in staining between the groups were statistically significant ($P < .001$). The sensitivity of toluidine blue staining for the detection of VIN was 92%; the negative predictive value 96%; specificity for strong staining was 88%[8].

In a study conducted by Arbyn M et al. among women in sub-Saharan Africa, India and other parts of the developing world, five screening methods viza, naked eye visual inspection of the cervix after application of diluted acetic acid (VIA), or Lugol's iodine (VILI) or with a magnifying device (VIAM), the Pap smear and human papilloma virus testing with the Hybrid Capture-2 assay (HC2), were evaluated in 11 studies. More than 58,000 women, aged 25–64 years, were tested with 2–5screening tests and outcome verification was done on all women independent of the screen test results. The outcome was presence or absence of cervical intraepithelial neoplasia (CIN) of different grades or invasive cervical cancer. Verification was based on colposcopy and histological interpretation of colposcopy-directed biopsies. Negative colposcopy was accepted as a truly negative outcome. VIA showed a sensitivity of 79% (95%, CI 73–85%) and 83% (95%, CI 77–89%), and a specificity of 85% (95%, CI 81–89%) and 84% (95%, CI 80–88%) for the

outcomes CIN2 or CIN3, respectively. VILI was on average 10% more sensitive and equally specific. VIAM showed similar results as VIA. The Pap smear showed lowest sensitivity, even at the lowest cut off of atypical squamous cells of undetermined significance (57%; 95% ,CI 38–76%) for CIN2 but the specificity was rather high (93%; 95%, CI 89–97%). The HC2-assay showed sensitivity for CIN2 of 62% (95%, CI 56–68%) and a specificity of 94% (95%, CI 92–95%)[9].

In a study conducted by R. Sankarnarayan et al. Among the rural women, four types of visual detection approaches for cervical neoplasia were investigated in India: a) naked eye inspection without acetic acid application, widely known as 'downstaging'; b) naked eye inspection after application of 3-5% acetic acid (VIA); c) VIA using magnification devices (VIAM); d) visual inspection after the application of Lugol's iodine (VILI). Downstaging has been shown to be poorly sensitive and specific to detect cervical neoplasia and is no longer considered as a suitable screening test for cervical cancer. VIA, VIAM and VILI are currently being investigated in multicenter cross-sectional studies (without verification bias), in which cytology and HPV testing are also simultaneously evaluated. These studies will provide valuable information on the average, comparative test performances in detecting high-grade cervical cancer precursors and cancer. Results from pooled analysis of data from two completed studies indicated an approximate sensitivity of 93.4% and specificity of 85.1% for VIA to detect CIN 2 or worse lesions; the corresponding figures for cytology were 72.1% and 91%. VIAM and VILI are currently being investigated in multicentre cross-sectional studies (without verification bias), in which c ytology and HPV testing are also simultaneously evaluated. These studies will provide valuable information on the average,

comparative test performances in detecting high-grade cervical cancer precursors and cancer.One of these studies is a 4-arm trial addressing the comparative efficacy of VIA, cytology and primary screening with HPV DNA testing. This trial will provide valuable information on comparative detection rates of CIN 2-3 lesions[10].

A study was conducted byRana T et al. at gynaecological clinic in Lady Willingdon Hospital. Out of 100 subjects, 85 were negative with both screening techniques. 24 were positive with VIA while Pap smear was positive in 12 subjects. Histological diagnosis of CIN/cancer was made in 16 out of the total 26 patients who underwent biopsy. The sensitivity of VIA was 93% and of Pap smear was 83%. Corresponding specificities were 90% and 97%. VIA was more sensitive than Pap smear which was statistically significant (P value < .05). The positive predictive value (PPV) of VIA was 62.5% versus 83% for Pap smear which is statistically significant (P value < 0.001) the negative predictive value (NPV) of VIA was 98% versus 97% for cytology. There was no significant difference between the NPV of both tests (P value equals 1). Overall, VIA demonstrated an accuracy of 90% as compared to 96% for cytology[11].

In a prospective study done by Neelam Pradhan, Pap smear was done in 200 women, 100 with healthy and 100 with unhealthy cervix attending gynaecology OPD at T. U. Teaching hospital Kathmandu. Cervical dysplasia was seen in 8 women with Unhealthy cervix, 7 were mild and 1 was severe dysplasia. In women with healthy cervix, 5 had mild, 1 had moderate dysplasia. 82% women with healthy cervix and 83% with unhealthy cervix had nonspecific inflammation. Women who were married before 20yrs and higher parity were more likely to have unhealthy cervix[12].

In a study conducted by S SShastri et al. cytology, HPV testing VIA, VIAM and VILI were carried out concurrently on 4039 women aged 30-65yrs. All women were investigated with colposcopy and biopsies were taken from 939 women who had colposcopic abnormalities. The sensitivities of cytology were 57.4%, HPV testing 62%, VIA 59.7%, VIAM 64.9% and VILI 75.4%. Specificities were 98.6%, 93.5%, 88.4%, 86.3% and 84.3% respectively[13].

Belinson JL et. al.in 2013,conducted a study in which visual inspection of cervix with 5% acetic acid was performed on women aged 35- 45 years in rural china. Women with doubtful lesions, had colposcopy and cervical biopsy. The sensitivity of visual inspection equalled or exceeded reported rates for conventional cervical cytology. Visual inspection and colposcopy have similar profiles. The benefit of an inexpensive mode of diagnosis and treatment algorithm will be a powerful incentive to persue visual inspection for cervical cancer screening in developing countries[14].

Denny Lynette et al. in the year 2004,conducted a study, in which women were screenedby direct visual inspection with acetic acid and other methods. If an abnormality was identified, these women were referred for colposcopy and biopsy. This two stage screening for cervical cancer provides an alternative to conventional method for low resource settings[15].

Van Linda LE et al. in 1936,conducted a study and later summarised it. They state in their summary that the study conducted on the patients attending family planning clinics for regular gynaecological examination had acetic acid applied to cervix followed by gross visualization without magnification. Patient with suspicious acetowhite area and normal papanicolaou tests were referred for colposcopic evaluation (9 patients had CIN-I and 4 CIN-III). 22% had koilocytosis, 19% had

benign histological findings.51 patients had suspicious lesions also at colposcopy for which biopsies were performed and 34% had normal colposcopic findings. They concluded, visual inspection of cervix after acetic acid increased the detection rate of cervical dysplasia otherwise missed by papanicolaoutest[16].

Debra Eisenberger et al.conducted a study in 1978,in which she stated,the three types of error leading to false-negative results of Pap smears like (1) sampling error (2) Screening error (3) Interpretative errors, which can be improved by using the Ayres spatula first followed by the endocervical brush. Fewer smears will be obscured by blood which could result in more squamous intraepithelial lesions being detected. Screening errors can be prevented by correct timing of screening and adequate patient preparation. Ideally Pap smear is taken in the middle of menstrual cycle or Immediate pre and post-menstrual period. Interpretation errors can be avoided by following standard grading system of diagnosis cervical cytology and proper training of cytopathologist[17].

Paul T Wertlakeet. al.in 1979 found out that speculoscopy, when combined with the Papanicolaou smear as a screening test, yields a higher percentage of women with biopsy-confirmed cervical pathology than the use of the Papanicolaou smear as a sole screening method[18].

Ericmegevand MD et al. in 1961, conducted a study in which, 76 womenhad positive reactions to acetic acid. Among the 2350 women with negative reactions, 254 had positive cervical smears; only 11 of these had histologic high-grade SIL. In contrast, 20 of the 61 women with positive cytology and positive acetic acid test had high-grade SIL on histology. Therefore, the acetic acid reaction enabled the observer to detect 20 of the 31 women (64%) who exhibited a high-grade SIL both on cytology

and histology. In conclusion, locations where access to cytopathology is limited, naked-eye visualization of the cervix after the application of diluted acetic acid warrants consideration as an alternative in the detection of cervical premalignant lesions[19].

Monica Jonsson RNM et. al. 1997 stated that aceto whitening of the cervix and cytology has low sensitivity as a predictor of HIV infections as determined by PCR. Howe DT et. al. 1991. In their study on 100 women with suspected cervical intraepithelial neoplasia (CIN) attending the colposcopy clinic with histological diagnosis made from excision biopsies taken by large loop excision of the transformation zone (LLETZ) were compared with those from colpscopically directed punch biopsies taken concurrently. One unsuspected micro invasive tumour was found and the diagnosis made by LLETZ was significantly worse than that made by punch biopsy in 24 cases (24%: 95% CI 15.6 to 32.4%). LLETZ improves the accuracy of diagnosis of CIN[20].

Divya Hegde et al. conducted a study in 2011, in which 225 women in reproductive age group attending the Gynaecology department at K.S Hegde Charitable Hospital were enrolled in the study. A Papanicolaou smear and visual inspection of the cervix with acetic acid was done. All women then underwent colposcopy using the video colposcope. All patients who tested positive on screening then underwent colposcopy guided biopsy. Pap smear of LSIL and above was taken as abnormal. Out of 225 patients, VIA was positive in 27(12%) patients and pap smear was abnormal in 26(11.7%). There were 15 LSIL, 6 HSIL and 5 were squamous cell carcinoma. On biopsy, there were 15 mild dysplasia, 2 moderate dysplasia, 4 severe dysplasia and 3 squamous cancers. Pap smear had a sensitivity of 83%, specificity of

98%, positive predictive value of 80 % and negative predictive value of 97.9%. VIA had a sensitivity of 70.8%, specificity of 95%, and positive predictive value of 62.9 % and negative predictive value of 96.5%. They concluded that diagnostic values of VIA is comparable to pap smear, and it performs well in detecting high grade lesion and that VIA can be used as a screening modality for cervical cancer in low resource[21.]

Samira Khan et al. 2007 in their study out of 300 women, aged 25 - 65 years, the positive predictive value (PPV) of low or high threshold VIA, VILI and cytology were 22.0%, 72.7%, 57.7% and 45.5%; such that the compounding the negative predictive value (NPV) were 80.0%, 80.0%, 88.9% 77.5%. Overall accuracy of high threshold VIA (76 %) was comparable to VILI 75.8%; cytology having 66% and low threshold VIA with 33 %. High threshold VIA and VILI have higher accuracy for detection of precancerous lesions of cervix than Pap smear indicating that these test to be implicated for cancer screening which is more cost effective[22].

Elite L et al.in2004,conducted a study on 2009 sexually active women who were 30 years or older and they had never been screened before, underwent cervical cytology and VIA in the Aimags Central Hospital. Women with abnormal test results and 5% of women with normal results were recommended to have colposcopy with or without biopsy. They found that among the patients taken for study, Visual inspection with acetic acid was abnormal in 254 (12.6%); Pap smear showed atypical squamous cells of undetermined significance or worse in 3%. Using cervical intraepithelial neoplasia 2 or higher disease on biopsy as the end point, the test parameters for VIA are sensitivity of 82.9% (95% CI = 81.3%-84.5%), specificity of 88.6% (95% CI = 87.2%-90.0%), positive predictive value of 12.2% (95% CI = 10.8%-13.6%), and negative predicative value of 99.7% (95% CI = 99.5%-99.9%). The test parameters

for Pap smear are sensitivity of 88.6% (95% CI = 87.2%-90.0%), specificity of 98.5% (95% CI = 98.0%-99.0%), positive predictive value of 51.7% (95% CI = 49.5%-53.9%), and negative predicative value of 99.8% (95% CI = 99.6%-100%).

Khodakarami N et. al. 2011, In tier study,comprising of total 100 women with the mean age 36.0 years, the sensitivity, specificity, PPV, NPV, and accuracy of the Pap test, the VIA, and the colposcopywere 23.5, 100, 100, 86.5, and 87%; 62.5, 98.8, 90.9, 93.2, and 92.9%; and 46.7, 97.6, 77.8, 91, and 89.8%, respectively, for cervical neoplasia. The Pap test had low sensitivity but high specificity, whereas VIA had a high sensitivity in addition to being easy and low-cost. Adjuvant methods of screening such as VIA can be a valuable alternative to the Pap test for cervical cancer screening in low-resource settings[24].

In a prospective study, 400 women were screened using the Pap smear, VIA and colposcopy. The sensitivity of VIA (96.7%) was much higher than that of Pap smear (50%) and almost as high as that of colposcopy (100%). The specificity of VIA (36.4%) was much lower than that of Pap smear (92.4%) and colposcopy (96.9%) resulting in high false positive rate for VIA. The advantages of VIA method are its low cost, ease of use, high sensitivity and immediate results. Its main limitation is high rate of false positive results which may lead to over treatment if see and treat policy is applied[25].

In an analytical cross sectional study, VIA and cytological smears were carried out on non-pregnant women aged 30-60 years. Women with positive VIA and positive cytology and one in ten negative women (control) were biopsied. 5010 women were enrolled, 4813 (96.1%) were screened. 574 (11.9%) had colposcopy. 1743 biopsies were obtained of which 528 were controls. The sensitivity of VIA was

70.4% versus 47.7% for cytology. VIA specificity was 77.6% versus 94.2% for 13cytology. They concluded that VIA has acceptable test qualities and can be used in low resource settings as a large scale screening method[26].

In a prospective study, in 1921, asymptomatic women underwent a complete clinical evaluation including Pap smear and VIA. Participants with any positive test were referred for colposcopy and biopsy. More women were tested positive by VIA than on the Pap smear. The positive predictive value for detection of CIN 2 was 8.5% for VIA and 6.3% for Pap smear. It was also observed that 2.3% VIA positive patients failed to return for follow up as compared to 26.3% Pap smear positive patients, which is statistically significant. VIA is useful for detection of precursor lesions of cervical cancer not only in low resource settings but also in well-equipped health and cancer centers[27].

A total of 11,834 healthy women were subjected to VIA, VILI, conventional Pap smear and Hybrid Capture II (HCII) assay. Women who had a positive result from any of these tests were subjected to colposcopy and biopsies. VIA was positive in 61.8% of the women with CIN 1, 57.0% of those with CIN 2, 35.0% of women with CIN 3 and in 21 of 28 (75%) of women with cancer. Approximately 10% of women with no detectable disease had an abnormal VIA. Regarding VILI, 83.3% of women diagnosed with CIN 1 and 62.5% of those with CIN 3 had an abnormal test. VILI failed to detect one of three cases of cancer. Both the sensitivity, specificity and positive predictive value of VIA and VILI in detecting CIN 2 or CIN 3 could be significantly improved depending on the combination with Pap smear or HCII[28].

The Toluidine blue dye test used in our study has so far been used in the detection of vulvar and oral neoplasia. There is no documentation about the dye being used on the cervix for cervical neoplasia detection. We are conducting this new study and hence the references quoted below are those that of Toluidine blue dye used in the detection of pre malignant oral lesions.

Juhi Upadhyay et al. Used Toluidine blue in their study and attempted to evaluate the efficacy of toluidine blue vital dye for detection of pre malignant oral lesions (PMOL's). The study included 47 biopsies Toluidine blue positive (TBP) = 35 and Toluidine blue negative (TBN) = 12, of which 23 cases were confirmed as dysplastic(TBP=17 and TBN=6), 7 as hyperkeratosis (TBP=4 and TBN=3), 8 as epithelial hyperplasia(TBP=6 and TBN=3) and 5 as other benign lesions(TBP=4 and TBN=1). The validity test revealed a sensitivity of 73.9% and specificity of 30%. The positive predictive value was 54.8% and negative predictive value of 50%. The study intends to highlight the false negative result (26.1%) which was mainly attributed to mild dysplasia and the false positive (32.6%) which included hyperkeratosis, hyperplasia, lichen planus and traumatic ulcer[29].

Navneet Sharma et al. Compared the results of exfoliative cytology with Toluidine blue in diagnosing dysplasia in leukoplakia, Toluidine blue dye test showed 50% sensitivity and 83.3 % specificity. In comparison to chemiluminescent light examination, cytology showed 42.9% sensitivity and 79.3% specificity. Chemiluminescent light examination showed 60% sensitivity and 70% specificity compared to Toluidine blue. Overall accuracy of exfoliative cytology was less than toluidine blue, whereas latter showed superior but comparable results to chemiluminescent illumination in detecting dysplasia[30].

MORPHOLOGY OF UTERINE CERVIX

Cervix is the narrowed, caudal portion of the uterus. It is conical in shape with a truncated apex directed downwards and backwards. It measures 2.5 cm and is continuous above with the body of the uterus and below it protrudes into the vagina forming fornices.

The four fornices are anterior, posterior and two laterals. The posterior fornix is deeper than the anterior. The junction between cervix and corpus is called isthmus. The cervix is divided into two portions: The portiovaginalis which is that part protruding into the vagina and the portiosupravaginalis, which lies above the vagina and below the corpus. The portiovaginalis is covered by nonkeratinizing squamous epithelium. Its canal is lined by a columnar mucus-secreting epithelium which is thrown into a series of V-shaped folds that appear like the leaves of a palm and are therefore called plicae palmitae.

The upper border of the cervical canal is marked by the internal os, where the narrow cervical os widens out into the endometrial cavity. The lower border of the canal, the external os, contains the transition from squamous epithelium of the portiovaginalis to the columnar epithelium of the endocervical canal. This occurs at a variable level relative to the os and changes with hormonal variations that occur during a woman's life. It is in this active area of cellular transition that the cervix is most susceptible to malignant transformation[31].

Transformation zone:

The cervix is composed of columnar epithelium, which lines the endocervical canal and squamous epithelium, which covers the ectocervix. The point at which they meet is called the squamocolumnar junction (SCJ). It is a dynamic point that changes in response to puberty, pregnancy, menopause and hormonal stimulation. In neonates, the SCJ is located at the ectocervix. At menarche, the production of oestrogen causes the vaginal epithelium to fill with glycogen. Lactobacilli act on the glycogen to lower the pH, stimulating the subcolumnar reserve cells to undergo metaplasia. Metaplasia advances from the original SCJ 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 SCJ to the physiologically active SCJ. As the metaplastic epithelium in the transformation zone matures, it begins to produce glycogen and eventually resembles the original squamous epithelium, colposcopically and histologically[32].

The original squamous epithelium of the vagina and ectocervix has four layers[32]

1. **Basal layer (Stratum Germinatum):** It rests on the basement membrane.

It consists of a single row of cuboidal or columnar cells with scanty. Basophilic cytoplasm and centrally placed round to oval large nucleus.

2. **Para basal or Prickle cell layer:** It is above the basal layer, 4-10 cells in. Thickness consisting of large polyhedral cells with basophilic cytoplasm and centrally placed nucleus, arranged in an irregular mosaic pattern.

3. **Intermediate cell layer:** It forms the bulk of the epithelium. It is also called clear cell layer. The cells are large oval to polygonal with irregular vesicular nuclei and give a characteristic basket weave pattern. The cytoplasm is rich in glycogen.

4. **Superficial layer or Stratum corneum:** It is made up of flattened, elongated or polygonal cells with acidophilic cytoplasm and small pyknotic nuclei. The cells detach from the surface (exfoliation).

AETIOPATHOGENESIS OF CERVICAL CANCER:

1) **Age -** It occurs at about 2 peaks of age, at 30-35 years and 50-55years of age. Preinvasive lesions occur 10 years before.

2) **Sexual activity, marriage and childbearing –**Sexually active woman is two to four times more likely to develop cancer than in sexually inactive woman. Young age at first intercourse, multiple sexual partners and high parity have been implicated as the risk factors for CIN and cervical cancer.

3) **Race –** The women of certain races, notably orthodox Jews are almost immune to cervical cancer. Carcinoma cervix is unusually common in Africans.

4) **Social and economic factors –** The disease is more prevalent among women belonging to low socio economic status.

5) **Coitus –** The practice of coitus is now established as being a prime cause of cervical malignant disease. It is almost unknown in groups of nuns and virgins. Early age of 1st intercourse and multiple partners are associated with higher risk of developing cervical cancer.

6) **Infection with HPV (Human Papilloma Virus)–**HPV infection has been detected in up to 99% of women with squamous cervical carcinoma.

Specific HPV types are associated with cervical cancer. Low risk includes types 6,11,42,44 and high risk types include 16,18,31,33,35,39,45,51,52,56,58. HPV subtypes 16 and 18 are found in 62% of cervical carcinomas. The mechanism by which HPV affects cellular growth and differentiation is through the interaction of viral E6 and E7 proteins with tumor suppressor genes p53 and Rb respectively. Inhibition of p53 prevents cell cycle arrest and cellular apoptosis, which normally occurs when damaged DNA is present, whereas inhibition of Rb disrupts transcription factor E2F, resulting in unregulated cellularproliferation[33].

7) **Risk associated with smoking**-It seems consistent that the risk of cervical cancer is invariably associated with human papillomavirus (HPV) infection partly via sexual transmission. Active smoking may also be related to cervical cancer because tobacco smoke constituents/metabolites or mutagens/carcinogens will be conveyed to the cervical mucus and act as independent initiators/promoters in the carcinogenesis or interact with the oncogenes of HPV.

8) **HIV and cervical neoplasia**- strong association between HIV and HPV infections and evidence of more rapid progression of HPV infections to cervical neoplasia in HIV infected women.

9) **Use of OC Pills**- OC Pills predispose to cervical neoplasia although women on OC pills are adivced yearly pap smear for monitoring of changes in cervical epithelium. There are trials undergoing regarding the same but as of now there is no proven study.

NATURAL HISTORY OF DISEASE

A clear understanding of the natural history of cervical cancer is key to planning and implementing a rational, cost-effective cervical cancer prevention program[34]**.** Accepted models of natural history of cervical cancer have changed in recent years The first cervical cancer prevention programmes were based on the

premise that the disease developed from precursor lesion (broadly known as dysplasia), progressing steadily from mild to moderate to severe dysplasia to carcinoma in situ (CIS), and then to cancer. In fact, it now appears that the direct precursor to cervical cancer in high-grade dysplasia, which can progress to cervical cancer over a period of up to 10 years. Most lower-grade dysplasia regresses or does not progress

CERVICAL INTRAEPITHELIAL NEOPLASIA

In 1961, International Committee on Histological Terminology defined dysplasia as "all other disturbances of differentiation of the squamous epithelium of lesser degree than carcinoma in situ. WHO defines, "dysplasia is a lesion in which part of the thickness of the epithelium is replaced by cells showing varying degrees of atypia".

The International Committee on Histological Terminology defined carcinoma in situ as "lesion of the epithelium on which, throughout its thickness, no differentiation takes places". This was also the view taken by Govan et. al. who insisted upon complete loss of stratification and of cellular differentiation as defining criteria. The WHO definition of a carcinoma in situ is "a lesion in which all or most of the epithelium shows the cellular features of carcinoma".

SQUAMOUS METAPLASIA

An understanding of squamous metaplasia is the key to understanding the development of premalignant disease of the cervix[35]. Squamous metaplasia occurs in every woman and is that process whereby columnar epithelium is changed to

squamous epithelium; the area which has been transformed from columnar epithelium is called the Transformation Zone (TZ).

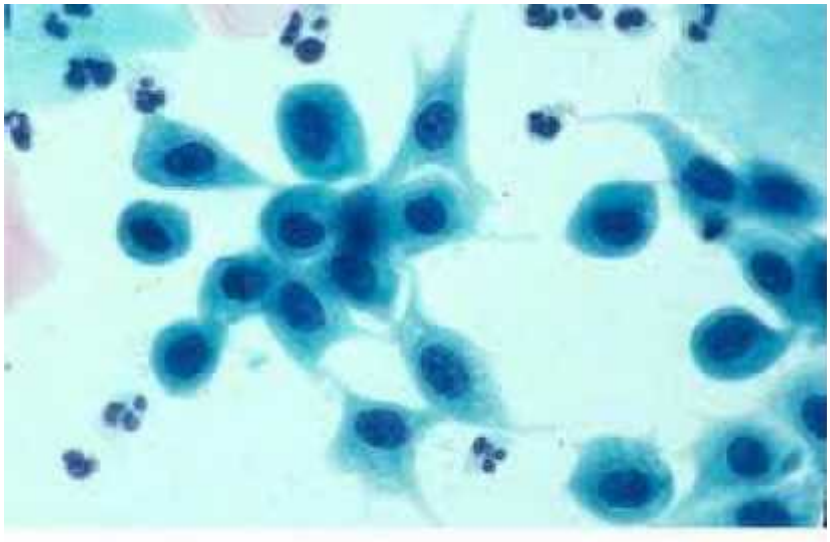


Figure 1- Squamous Metaplasia

DIFFERENTIATION

Some differentiation is expected in dysplasia, although this may be difficult to detect in some of the most severe forms. The definition of carcinoma in situ requires that the epithelium shows no differentiation as the surface is reached, so that the immature and undifferentiated cells occupy the whole of the thickness of the epithelium. The presence of surface differentiation is the only way of distinguishing between carcinoma in situ and dysplasia but, with adoption of the CIN terminology this distinction becomes meaningless, so that deliberations over the presence or absence of surface differentiation are pointless. The proportion of the epithelium which shows differentiation is a more useful indicator of the degree of CIN, although it is not, as some authors suggest, the only criterion to be taken into account[36].

Furthermore, it must be remembered that CIN develops by a metaplastic process and that a metaplastic epithelium may show no differentiation, even when there is no nuclear atypia. It is thus possible to make a diagnosis of a minor degree of CIN in the complete absence of differentiation, if the nuclear changes are sufficiently mild.

NUCLEAR ABNORMALITIES

Nuclear enlargement, hyperchromasia, chromatin clumping and irregularities in size and shape are features which characterize the nuclei in CIN. These are appearances which may reflect the aneuploidy DNA content of the cells and they are very important criteria for the diagnosis of CIN and for determining its grade.

MITOTIC ACTIVITY

The normal squamous epithelium of the cervix has only a little mitotic activity, with the mitotic figures being confined to the parabasal layers. In CIN, the number of mitotic figures is increased and they may be present at any level in the epithelium. The commonest of these abnormal configurations is the “three-group metaphase” in which the main mass of the chromatin is lined up along the equatorial plate, but small groups of chromosomes are apparently separated and lying on either side. Two-group metaphases may also sometimes be seen.

Taking all these features into account, the grades of CIN may be characterized in the following way.

CIN I- The essential feature of CIN I is that while the cells the lower one third of the epithelium show nuclear abnormalities, the cells in the upper and middle thirds of the epithelium undergo cytoplasmic differentiation. The normal maturation process and differentiation from basal and parabasal layers to the intermediate and superficial

layers are maintained. In the upper layers koilocytosis are characterised by perinuclear halos, well defined cell borders, nuclear hyperchromasia, irregularity and enlargement. Mitotic features are present in lower one third layer.

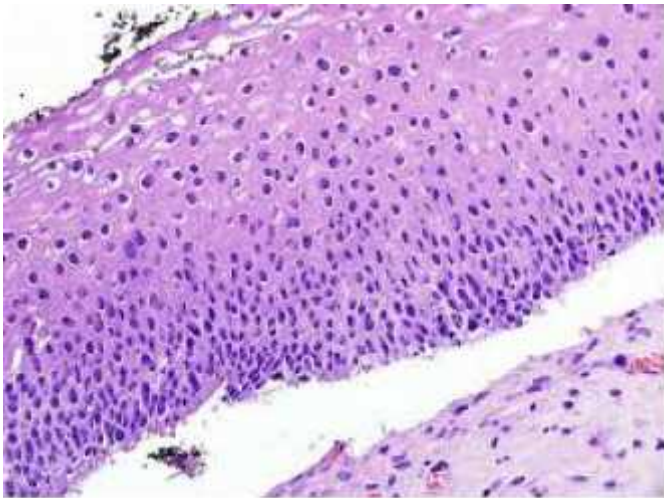


Figure 2– CIN I

CIN II- The histological features of CIN II are similar to those of CIN I except that undifferentiated non-stratified cells with pleomorphic nucleoli and a high nucleocytoplamic ratio extend beyond the lower third of the epithelium but not into upper third. The cells in the upper third of the epithelium undergo a variable degree of stratification and of cytoplasmic differentiation. Mitotic figures are present in the lower two-thirds of the epithelium and are not uncommonly of abnormal form.

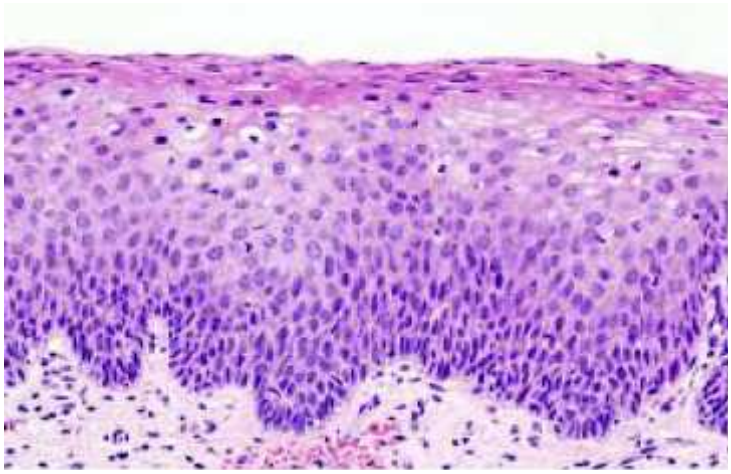


Figure 3: CIN II

CIN III- In this condition undifferentiated, non-stratified, basaloid cells with nuclear crowding, indistinct boundaries and a high nucleocytoplasmic ratio occupy more than thirds, or the full thickness, of the epithelium. The degree of nuclear pleomorphismis often greater than that seen in CIN I or II.

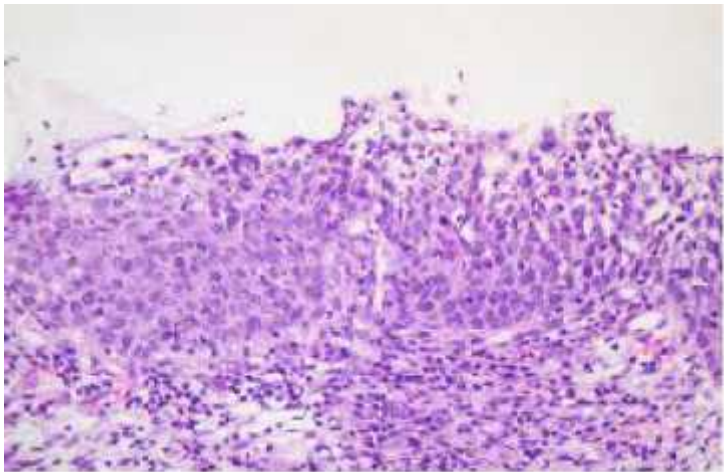


Figure4 : CIN III

CURRENT VIEWS OF THE NOMECLATURE OF CIN

The system of nomenclature for cervical epithelium abnormalities that is used now is a two-tier system which recognizes the unity of the disease process and is in keepingwith current therapeutic approaches.

A working party was convened jointly by the National Health Service CervicalScreening programs, National coordination Network and the British Society forColposcopy and Cervical Pathology in December 1990[37]. The working party consideredthe three alternative possibilities of a) using the term CIN without division into grades, b)division of CIN into two grades and c) division of CIN into three grades. Theyrecommended the continuation of epithelial abnormality to encompass those lesions inwhich a diagnosis of CIN cannot be certainly made (basal abnormalities of uncertain significance).

Basal abnormalities of uncertain significance

The histological features of this entity are:

1. A minimal degree of nuclear pleomorphism limited to the basal layers in the absence of severe inflammation.
2. The features of CIN I in the presence of severe inflammation.
3. A thin epithelium in which the features of CIN (grade not specified) would have been inappropriate, but in the presence of severe inflammation.

In all these instances, the changes may be associated with the features of HPV infection. In any of the above circumstances the finding of abnormal mitotic figures should result in a diagnosis of CIN. A diagnosis of basal abnormalities of uncertain significance in a colposcopic biopsy is an indication for subsequent colposcopic review.

PREVENTION & SCREENING OF CERVICAL CANCER

Primary Prevention

It aims at reducing the incidence of cervical cancer by identifying the causes and risk factors and eliminating or preventing those from exerting their effect. The different strategies are,

- Use of condom
- Raising the age of marriage and of first birth
- Limiting the number of sexual partners
- Maintenance of local hygiene
- Avoiding or quitting smoking and minimizing exposure to environmental tobacco smoke
- Diet rich in fresh vegetables and fruits
- Effective therapy of STD
- Cancer education

Removal of cervix during hysterectomy as a routine for benign lesion is a definite step in prevention of stump carcinoma.

Secondary Prevention

It involves identifying and treating the diseases in earlier stages .This is done by screening procedures.

Screening

Cancer screening is the main weapon for early detection of cervical cancer at a pre invasive or premalignant stage. Cancer screening may be defined as the "Search for unrecognized malignancy by means of rapidly applied tests"[38].

Disease

The disease to be screened should fulfil the following criteria before it is considered suitable for screening-

1. The condition sought should be an important health problem (in general, the prevalence should be high)
2. There should be a recognizable latent or early asymptomatic stage
3. The natural history of the condition, including development from latent to declared disease should be adequately understood (so that we can know at what stage the process ceases to be reversible)
4. There is a test that can detect the disease prior to the onset of sign and symptoms.
5. Facilities should be available for confirmation of the diagnosis
6. There is an effective treatment
7. There should be an agreed on policy concerning whom to treat as patients

8. There is a good evidence that early detection and treatment reduces morbidity and mortality.
9. The expected benefits (e.g. The number of lives saved) of early detection exceeds the risks and costs.

Cervical neoplasia is an important disease that meets most of these criteria. It has been conceived a preventable cancer.

- The uterine cervix is easily accessible for clinical evaluation.
- Pre cancer phases of cervix usually progress in a gradual well documented fashionand can be easily detected.
- The treatment of cervical neoplasia in its pre invasive state can be undertakenwith low complication rate and a good expectation of success.
- The detection of cervical cancer precursors and their eradication leads to decreased incidence of invasive cervical cancer.
- Reductions in incidence and mortality seem to be proportional to the intensity ofscreening programs.

In highly screened populations, the cervical cancer incidence rate has dropped by 70-90% whereas the unscreened population continues to experience high incidence of the cancer.

Screening Test

For a screening test to be effective it must fulfill certain basic requirements

a) The test must detect the disease in a stage where early treatment will provide a superior prognosis to treatment in a later stage
b) The test must have sufficient sensitivity to detect the disease in an early stage

c) The test must be sufficiently specific to distinguish nonspecific changes from the disease.
d) The test must be cost effective
e) The test must be sufficiently simple, easy, safe and rapid to administer
f) The screening procedure must be acceptable to those undergoing screening

A screening test is not intended to be a diagnostic test. It is only an initial examination. Those who are found to have positive test results are referred for further diagnostic workup and treatment.

Types of Screening

a) Mass Screening- screening of a whole population irrespective of the particular risk individual may run of contracting the disease in question
b) High risk/ selective Screening- is conducted on a population identified to have high risk factors
c) Opportunistic Screening- is the screening of anyone who seeks health care
d) Multiphasic Screening- application of two or more screening tests on combination to a large number of people at one time then to carry out separate screening test for single diseases

Methods of Cervical Cancer Screening:

1. Conventional exfoliative cytology
2. Liquid based cytology
3. Automated cervical screening techniques
4. Visual inspection with acetic acid (VIA)

5. Visual inspection with Lugol's iodine (VILI)/ Toluidine blue dye test

6. Speculoscopy

7. Cervicography

8. HPV DNA testing

9. Colposcopy

10. Fluorescence spectroscopy

11. Polar probe

RECOMMENDATIONS FOR CERVICAL SCREENING

As infection by HPV is considered to be the most important causative factor or risk factor for aetiopathogenesis of cervical cancer, screening before the onset of sexual activity will not be very productive.

Acute infection with HPV is common after initiation of sexual activity and very often these infections are transient, clearing within 2 years. However these infections can cause cytological abnormalities which are of low grade nature but may necessitate further diagnostic procedures. The transient nature of most common HPV infections, the long pre-invasive phase of dysplasia and the potential harm that can result from diagnostic and therapeutic procedures are factors that should be taken into consideration before introduction of the screening programs at an early age.

Data suggests that there is little risk of missing an important cervical lesion until 3-5 years after initial exposure to Human papilloma virus (HPV). Screening before the 3 year period may result in over diagnosis of cervical lesions that would regress spontaneously. An upper age limit is necessary for health care professionals

who do not askpatient about their sexual history and for adolescents who are unable or unwilling to disclose prior consensual or nonconsensual intercourse.

Cost effectiveness is a major issue in cancer cervix screening programs. The absolute risk of developing invasive squamous cell cancer as a result of negative test in small (fewer than 5 women/100,000/ year).

Table 1- A comparison of ACS guidelines with USPSTF guidelines on screening for cervical Cancer

Criteria	**USPSTF guidelines**	**ACS 2002 guidelines**	**ACOG**
Age to intitiate screening	No direct evidence to determine optimum age to start screening. Within 3 years of onset of the onset of sexual activity or by age 21 years	Three years after the onset of sexual activity; no later than age 21	Recommendations by ACOG and ACS are the same
Screening frequency	At least every 3 years (High grade dysplasia or rapidly progressive cervical disease is unlikely to develop or be over looked with	Annually with Conventional cytology or every 2 years with liquid based cytology until age 30, then, women with 3 consecutive normal tests may be screened every 2-3 years. However, if	Unlike ACS, ACOG does not base its recommendations on the screening method used. It suggests annual screening for a period of 2-3 years, If 3 consecutive negative

	3 years after a normal examination in an immune competent woman who has had multiple normal smears)	there is H/O in-utero DES exposure, HIV or immune suppression, yearly or more frequent screening should continue	Pap results are obtained at age 30 or older. More frequent screening should be performed in women with HIV, in-utero DES exposure or immunosuppression
Screening after hysterectomy	No cytological testing after total hysterectomy for benign condition	No cytological testing after total hysterectomy for benign condition	-
Discontinuation	After age 65 if there has been adequate recent screening with normal pap findings. Screening should continue if the patient has never been screened or if information about previous screening is not available	After 70 years of age, women with intact cervix who have 3 consecutive normal cervical cytology tests and no abnormal tests within the previous 10 years. If information about previous screening is not available, women with H/O of cervical cancer or in-utero DES exposure, HIV, immunosuppression should continue	ACOG recommendations endorse those of ACS and USPSTF

		cervical screening as long as they are in good health	
Routine inspection for HPV infection	Insufficient evidence	Not yet FDA approved if approved, conventional or liquid based cytology combined with test for DNA from high risk HPV subtypes should be performed not more than every 3 years	

USPSTF- US Preventive Services Task Force

ACS- American Cancer Society

ACOG- American College of Obstetrics and Gynaecology.

PAP SMEAR

History

The introduction of the use of evaluation of cellular material from the cervix and vagina for the diagnosis of cervical carcinoma is generally attributed to Dr. George Papanicolaou, an anatomist, who in 1928 published his report “New Cancer Diagnostics” which was supported by Babes in the same year. Dr.Papanicolaou and Dr. Herbert Trautrefined the vaginal pool cellular collection technique in 1941. In 1943, they published their findings and conclusions in the monograph, “Diagnosis of Uterine Cancer by the Vaginal Smear”. This procedure was named the Pap test. Dr. J. Ernest Ayre in 1947 introduced the wooden spatula to scrape the cervix and harvest the cells directly from the transformation zone. This spatula was referred to as the Ayre’s Spatula. In 1962 Dr. Hugh Davis introduced the Davis pipette that was used to collect the cells from the cervix and vagina by irrigating with saline.

Timing of Pap smear

Ideally Pap smear is taken in the middle of menstrual cycle. Immediate pre and post-menstrual periods are avoided.

Patient Preparation

1. Not to use any vaginal drugs for one week before taking sample
2. Not to douche vagina for 48 hours before Pap test
3. Abstinence from coitus for 24 hours prior to test
4.Patient should not be bleeding or not to have vaginitis

Sampling Sites

1) **Vaginal cytology-** Specimen is collected from the posterior vaginal fornix in a pipette by suction, smeared on to a slide, fixed and stained by pap staining. Advantage of this method is that it can be done by even the patient herself. However the quality of specimen is very poor 50% false negative rate
2) **Cervical Scrape-** It consists of the superficial cells from the external os and lower endocervix taken by means of special wooden or plastic spatula. For many years only type cervical spatula was the Ayre's spatula. The extended tip Aylesburry spatula with a longer endocervical limb has a higher rate of satisfactory smear.

Collecting the specimen

1) Patient in lithotomy position. Introduce speculum into vagina to expose cervix
2) Place the small end of Ayre's spatula high into the canal
3) Rotate the spatula to 360 degrees thoroughly scraping the squamo columnar junction and obtain a good endocervical component.
4) Spread the material onto a clean glass slide and fix it

Specimen Adequacy

A fully satisfactory smear as one containing both squamous cells and endocervical metaplastic cells. In an adequate smear 8000-12000 well preserved and well visualized squamous epithelial cells should be present covering more than 10% of slide surface. For liquid based cytology number is 5000. Adequate endocervical component should at a minimum consist of two clusters of well-preserved endocervical and squamous metaplastic cells with each cluster containing 5 cells.

NORMAL CYTOLOGY

Two types of epithelia are present within the uterine cervix

1) Non keratinising squamous epithelium lining ectocervix
2) Columnar epithelium lining endocervix. Both these epithelia are under hormonal control.

Cervical epithelium consists of following cells:

1) Superficial squamous cells: mature, usually polygonal squamous epithelial cells. Cytoplasm is cyanophilic or eosinophilic and nucleus is pyknotic.
2) Intermediate squamous cells: Mature, polygonal squamous epithelial cells. The cells are of same size as superficial cells. Their cytoplasm is cyanophilic and nuclei vesicular.
3) Parabasal cells: The cells are oval or round immature squamous epithelial cells. The cytoplasm is basophilic with smooth cytoplasmic borders.
4) Basal cells: If basal cells are present in the smear, it can be assumed that a pathologic process has damaged the upper layers of squamous epithelium.

The cytoplasm is basophilic and scanty. The nuclei are of the same size as parabasal cells[39 40].

Advantages of cytology are ideal for mass screening, high specificity, easy to perform, less time taken to obtain the diagnosis and detection of lesion in endocervical canal.

Disadvantages of cytology are low sensitivity, need for laboratory with high Human expertise, not possible to locate the lesions and high cost[41]. The first documented incident of deficiencies in gynecologic cytology laboratories was reported by Hutchinson et al showed that commonly used devices for the performance of the Pap

smear collected between 600,000 and 1.2 million cervical epithelial cells but that fewer than 20% of these collected cells were transferred onto the glass slide[42].

Limitations of Pap smear[43]

1) Inadequate samples constitute about 8% of the specimens received.
2) False negative results as high as 20-30% have been reported, which occurred due to clumping of cells when the cells are not uniformly spread on the glass slide.
3) Sometimes other contents of the cervical specimen such as blood, bacteria and yeasts contaminate the sample and prevent the detection of abnormal cells. If exposed to air for too long before being fixed on the slide, cervical cells can become distorted.
4) Human error is probably the primary threat to accurate interpretation. An average Pap smear slide contains 50,000-300,000 cells that must be examined and if the sample contains only a few abnormal cells within a crowded background of healthy cells, the abnormal cells may be missed.

GRADING SYSTEM FOR REPORTING OF CERVICALCYTOLOGY

In 1998, the first National Cancer Institute workshop held in Bethesda, Maryland resulted in development Bethesda system for cytological reporting. Recently, the terminology was refined in Bethesda III system (2001)-

• Atypical squamous cells (ASC)

• Low grade squamous intraepithelial lesion (LSIL)

• High grade squamous intraepithelial lesion (HSIL)

Table 2- Grading System for Reporting of Cervical Cytology

SL No	Bethesda system	Dysplasia / CIN system (WHO)	Papanicolaousytem
1.	Within normal limits	Normal	I
2.	Infection (organism should be specified)	Inflammatory atypia	II
3.	Reactive &reparative changes		
4.	Squamous cell abnormalities - Atypical squamous cells 1. Of undetermined significance (ASC-US) 2. Exclude high grade lesions	Squamous atypia, HPV atypia, exclude LSIL Exclude HSIL	
	Low grade squamous intraepithelial lesion (LSIL)	HPV atypia (koilocytic) Mild dysplasia – CIN1	III
	High grade squamous intraepithelial lesion (HSIL)	Moderate dysplasia – CIN2 Severe dysplasia Carcinoma in situ –CIN 3	IV
5.	Squamous cell carcinoma	Squamous cell carcinoma	V

Pap tests with cellular abnormalities that are more marked than those attributable to reactive changes but that quantitatively or qualitatively fall short of definitive diagnosis of ‘squamous intraepithelial lesion’ are placed in a category called ‘atypical squamous cells’. These specimens are further categorized as of undetermined significance (ASCUS) or cannot exclude high grade (ASC-H).

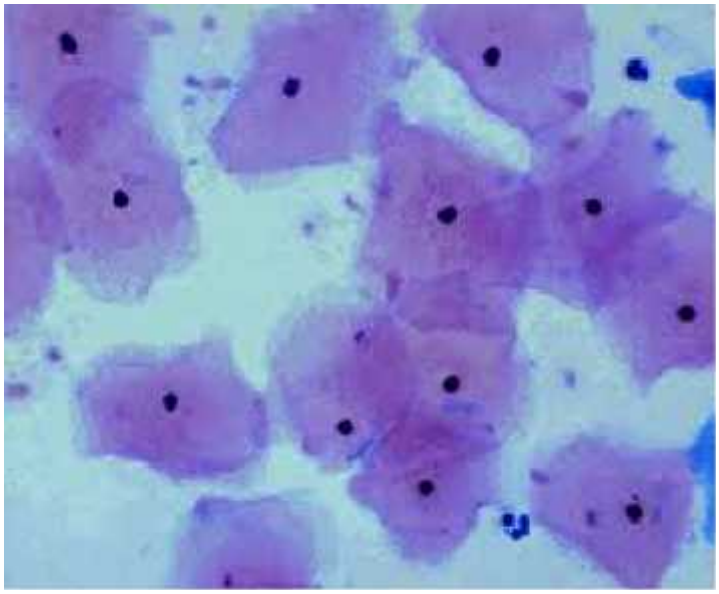


Figure 5- Normal Cytology

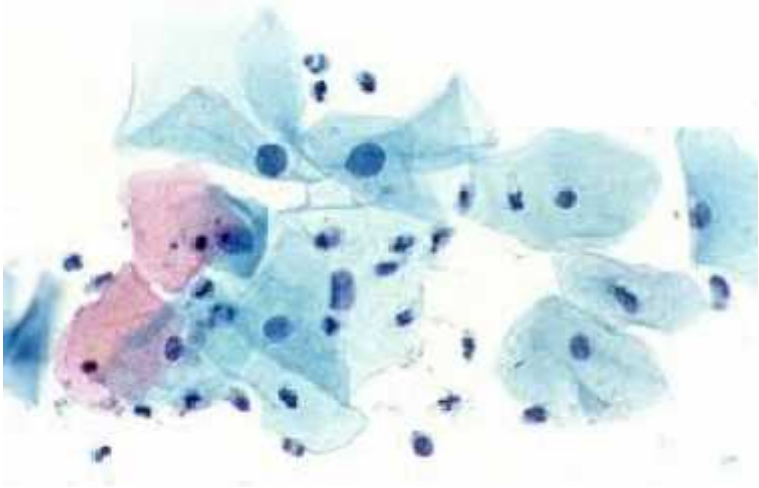


Figure 6- ASCUS

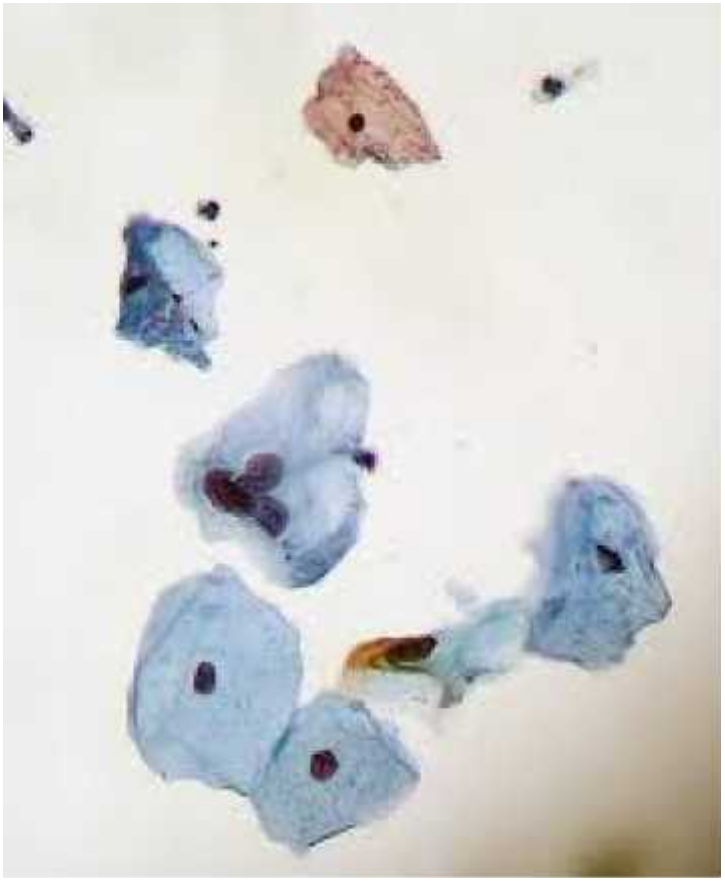


Figure 7- LSIL

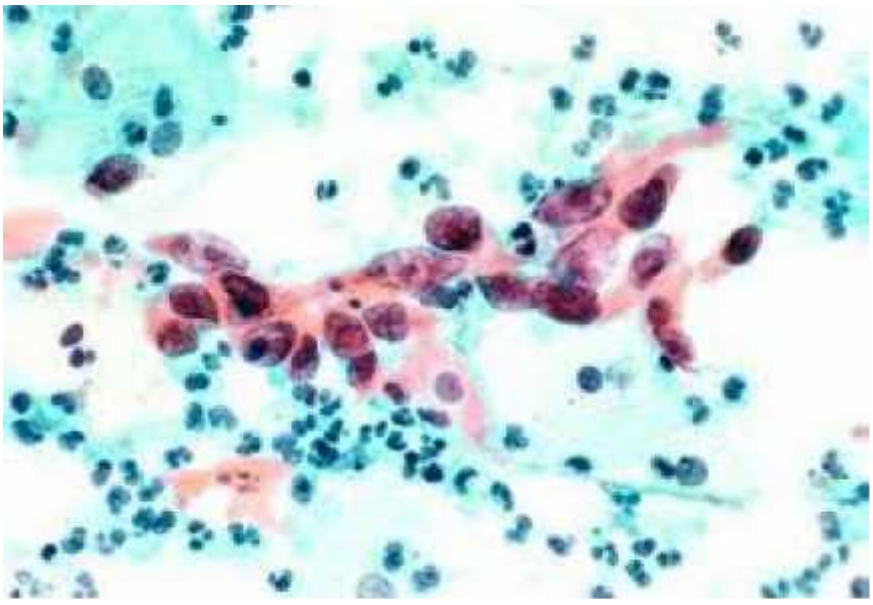
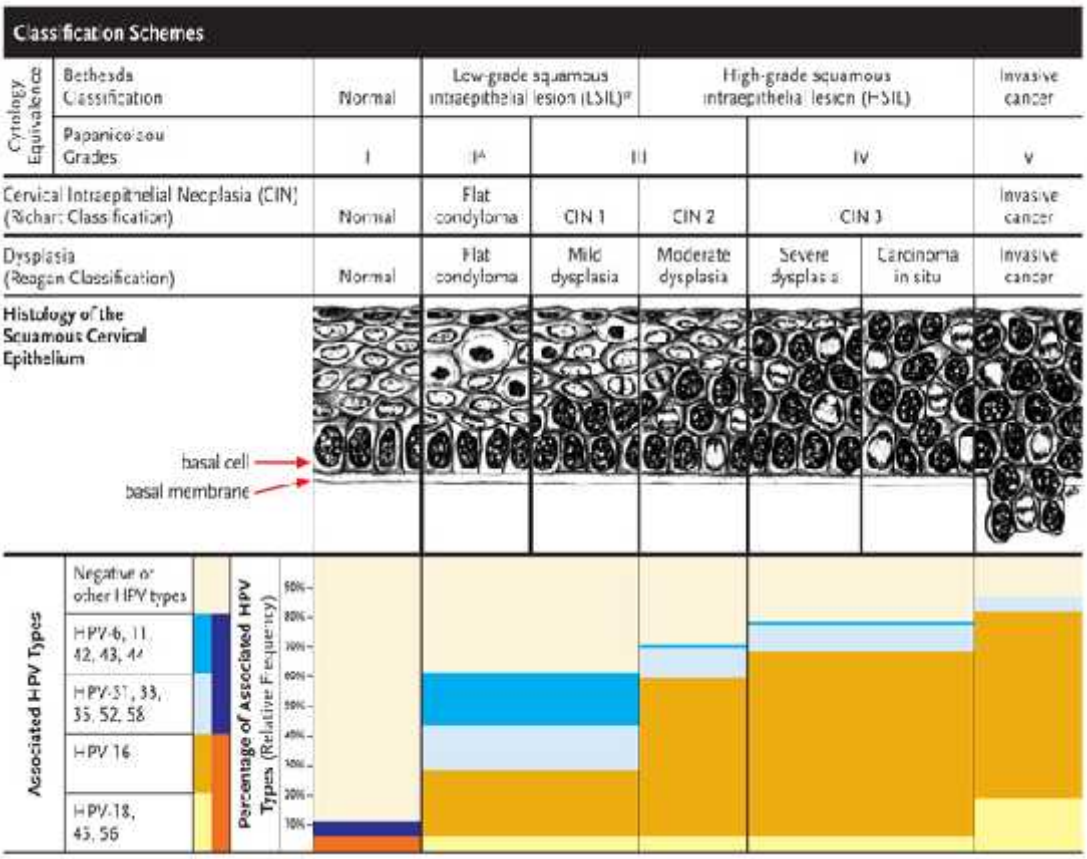


Figure 8 -HSIL

The various grading systems for cervical cytology have been discussed above. Given below is Table 3, in which all the classification and grading systems have been put together.

Table3 : Classification schemes



VISUALIZATION TECHNIQUES

History of research on visual inspection

Historically before the advent of programmatic screening by Pap smears health care providers relied on looking at the cervix to detect abnormalities. Schillers test has been used for many years in differentiating mature normal from immature abnormal epithelium. Walter Schiller in 1929 recommended iodine staining for selecting the areas for biopsy. An improvement over Schillers test was Toluidine blue test described by Ralph M. Richart of New York in 1963.Then in 1982, Ottaviano and La Torre published an important study involving 2,400 women who were examined visually and colposcopically after a cervical wash with acetic acid[44]. Over the last several decades, visualization techniques based on identifying cervical lesions using light of various wavelengths have been developed. These techniques could potentially be used for cervical screening, either as an adjunct to cervical cytology or as replacement for cytology.

There are two general categories of visualization techniques-

First general category of visualization techniques includes approaches that use broad band light (i.e., the entire spectrum of light composed of both wavelengths that are visible and nonvisible to the naked eye). Like-

1) Visual inspection by acetic acid (VIA)
2) Visual inspection by Lugol's iodine (VILI)
3) Toluidine blue test
4) Visual inspection with acetic acid and magnification aided visual inspection (VIAM)
5) Colposcopy
6) Cervicography
7) Speculoscopy

Second General Category of visualization techniques includes the methods that use specific wavelengths of light produced by lasers or specialized light sources and electro optical sensors to detect cervical disease. These devices are being evaluated for 4 possible clinical indications.

a) As adjuncts to cervical cytology where the device will be used in addition to cervical cytology for primary cervical cancer screening
b) For triage of women with an abnormal cervical cytology, much as colposcopy or HPV DNA is used
c) As a method to localize sites for cervical biopsies
d) As a primary screening device that could provide an alternative or replacement to cervical cytology.

Eg- Polar probe

First generation category-

Visual Inspection with Acetic acid (VIA)/DVI- Direct Visual Inspection, is the inspection of the cervix after application of a dilute solution of 3-5% acetic acid. Visual inspection/ Acetic acid test/ Visual Inspection with Acetic Acid (VIA) is the examination of cervix after application of 3-5% acetic acid. Thecervix is inspected after one minute. Lesions which stain acetowhite are regarded as positive for VIA.

Principle of VIA: Acetic acid dissolves mucus. It effectively removes the thin mucous covering ofthe epithelial surface. It further penetrates into the surface cell causing coagulation ofintracellular proteins and dehydration of the intracellular compartment. These actionsessentially make the cells more refractory toward light because the space between nucleiand their surrounding proteins for light energy to pass through is decreased. As a resultmore light is reflected back changing the

perceived color of the area in view. Areas where light is more heavily reflected have an obvious whitish coloration and are described as acetowhite epithelium.

Visual Inspection with Lugol's iodine (VILI)/ Schiller's Iodine test- In which cervix is inspected with naked eye after the application ofLugol's iodine. Areas of healthy tissue will stain mahogany brown while areas of abnormal cells would turn white or yellow. In cases of immature squamous metaplasia, there will be partial iodine uptake. The application of acetic acid or iodine highlights the areas with abnormalities and enables the clinician to take biopsies in the affected area of the cervical epithelium.

The normal squamous epithelium is rich in glycogen and takes up a dark brown stain with iodine. Rapidly proliferating cells utilize all the glycogen and hence are deficient in glycogen. They remain unstained. Hence iodine negative areas are considered abnormal[45].

Toluidine blue dye test[29]**-** Toluidine blue, discovered during 1960s, is a basic metachromatic dye of thiazine group that shows affinity for the perinuclearcristernae of DNA and RNA. The use of tolonium chloride (Toluidine blue) for in-vivo staining of suspicious lesions of oral cavity that stains tumor cells, normal mucosa andleukoplakia differentially.

Reichart (1963) first reported the use of 1% tolonium chloride stain in delineation of neoplastic epithelium of the cervix[46]. Its use in- vivo is based on the fact that dysplastic and anaplastic cells contain quantatively more nucleic acids and increased mitoses than normal surrounding epithelium and stains nucleus . Another mechanism appears to be greater penetration and temporary retention of the dye in the

intercellular spaces of rapidly dividing cells in-vivo RNA. However, the mechanism by which the dye differentially stains malignant and dysplastic tissues remains unclear.

Epstein et al. in 2003 showed that use of toluidine blue in oral cavity is more sensitive than clinical examination alone, and compared to iodine staining (sensitivity of 73%), toluidine blue (sensitivity 91.2%) has yielded better results. For pre malignant oral lesions (PMOL) the sensitivity is about 72-100% and the specificity being 45-93%.Recent reports have concluded toluidine blue retention in high risk PMOL's and high-risk molecular clones, even in lesions with minimal or no dysplasia. However, the detection of low-grade oral dysplasia has been less consistent. Further there has not been any agreement on the intensity of the stain uptake, though suggested dark royal blue staining to be the true positive outcome of test with different histological pattern of uptake. Inspite of claims from several authors for use of toluidine blue vital dye for the detection of PMOL's needs serious re-considerations. The present study was conducted to evaluate the efficacy of toluidine blue vital dye as a screening method to adjunct the current in use screening methods for the early detection of cervical neoplasia. Also to further discuss its efficacy and desirability of its use in cervical neoplasia screening.

Visual Inspection with acetic acid and Magnification Aided Visual Inspection (VIAM)-Inspection of the cervix after the application of 3% solution of acetic acid usinga low (4x-6x) magnification and a built in light source. The patient is referred to a higher centre for further evaluation if aceto white areas are found. The technique

can be taught to semiskilled personnel and may be an attractive option for primary screening inunderdeveloped countries.

Speculoscopy- Lonky brothers Stewart and Neal, and Marin in 1984 conceived theidea to use chemical light for anatomic illumination. Speculoscopy is an in-vivo test performed by using the Speculative ,a special “blue-white” chemiluminescent disposable light along with acetic acid and low level magnification to visually examine the cervix and vaginal wall.

Normal cervical tissue absorbs the very precise spectrum of light and appears dark blue or purple. Diseased and abnormal cells with increased keratinization and nuclear cytoplasmic ratio reflect the light and appear bright white. Speculoscopic diagnoses are considered positive if there is a distinct acetowhite area, and suspicious if either the acetowhite lesion disappeared within 30-60 seconds of being sighted. If the acetowhitelesion is equivocal to define, or if some glare is seen in the endocervical canal then endocervical neoplasm is suspected. The sensitivity of speculoscopy ranged from 84.2% to 94.7% and the specificity from 84.2% to 100%. When speculoscopy is compared to cerviscopy which uses a regular light source and no magnification, acetowhite areas can be better identified. Many clinical studies have demonstrated that speculoscopy combined with Pap smear is a more complete cervical cancer screening method than Pap smear alone. PapSure, a combination of Pap smear and speculoscopy is the only direct visual cervical cancer screening method to receive clearance by the USFDA.

Colposcopy[47]-Colposcopy means to look into the vagina (i.e.colpo means vagina, scope means to look). It was first described by Hans Hinselman of Germany in 1925. It is performed using a colposcope, an optical instrument that suppliesmagnification (typically 5 - 25x) and often records photographs. Magnification provided by colposcope is 6-40 times. Blue/ green filter is used for visualization of vascular pattern, as they appear dark and visibly contrasted against the surrounding epithelium.It allows accurate delineation of suspicious areas for tissue biopsy. Colposcopy is required in abnormal Pap smear cytology, to locate abnormal areas, to obtain directed biopsy, for conservative therapy under colposcopic guidance and for follow up of cases treated conservatively

Indications for colposcopy include-

1. Pap smear consistent with HPV infection, dysplasia, or cancer (LSIL or HSIL)
2. Pap smear with ASCUS favor dysplasia or repeated ASCUS
3. Pap smear with repeated unexplained inflammation
4. Abnormal appearing cervix
5. Infection with oncogenic HPV
6. Aceto positivity in visual inspection with acetic acid (VIA) and visual inspection with acetic under magnification (VIAM)
7. Iodine negative areas on visual inspection with Lugol's iodine (VILI)
8. Leukoplakia of cervix

Lesions that are more likely to be missed or under-read by colposcopic examination include endocervical lesions, extensive lesions that are difficult to sample and necrotic lesions.

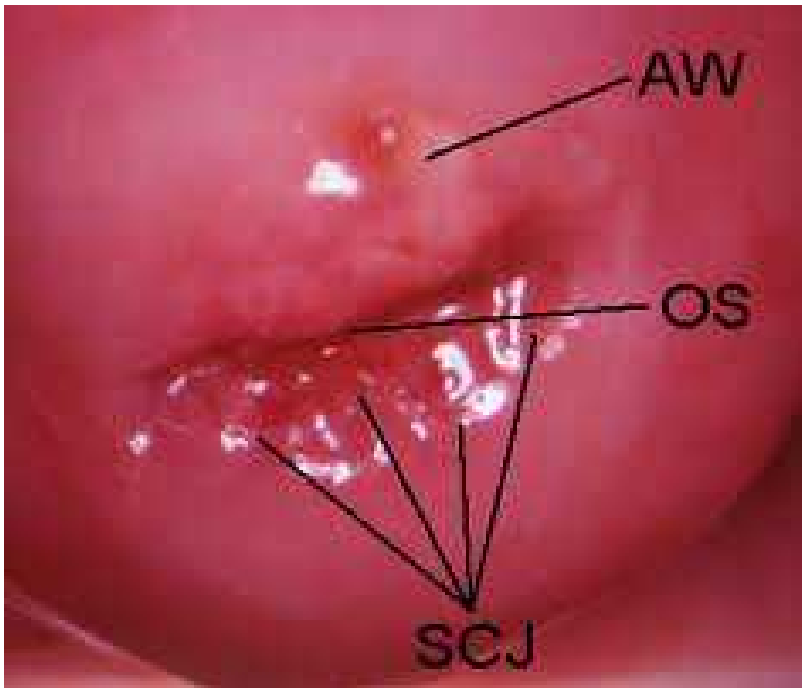


Figure 9- Normal Colposcopic Image

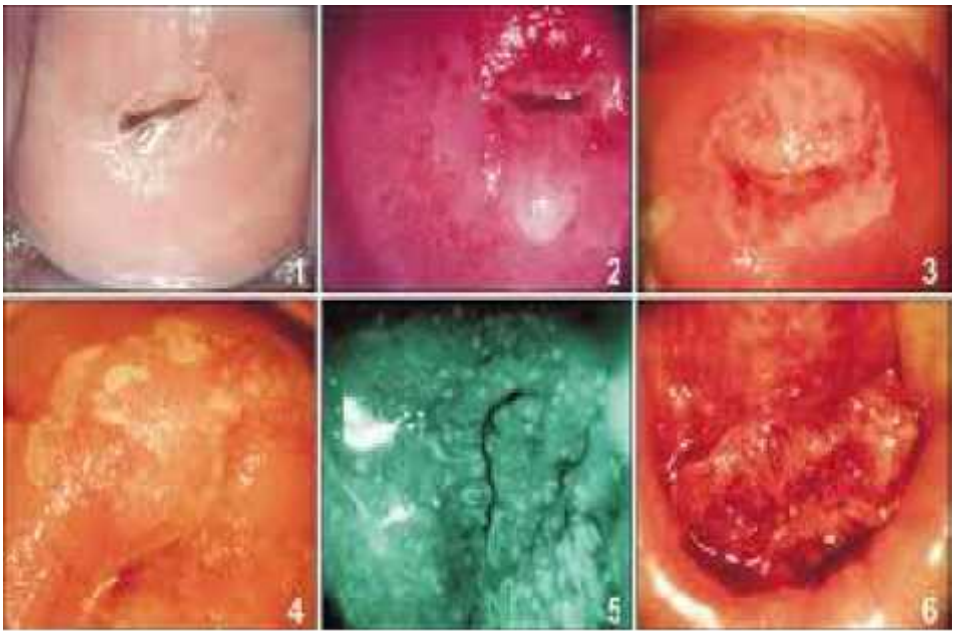


Figure 10- Abnormal Colposcopic Images

Contraindications*:* No absolute contraindications exist.

The patient's ability to tolerate a standard speculum examination is the only true limiting factor. Active cervicitis and vulvovaginitis should be treated before under the examination because inflamed tissues can alter the ability to obtain an accurate assessment.

Coppleson's Grading System

Grade I (Insignificant)-Shiny or semitransparentacetowhite epithelium. Borders are not sharp. No punctuations are mosaic. Short inter-capillary distance.Absent atypical vessels.

Grade II (Significant)-Dense acetowhite epithelium or grey opaque epithelium. Borders sharp. Dilated irregular, coiled vessels. Coarse punctuations, mosaic. Atypical vessels.Irregular surface color.

Role of Colposcopy in screening

Colposcopy greatly enhances diagnostic accuracy. When used complimentary to Cytology, sensitivity of colposcopy is between 87-99%. But its specificity is lower and varies between 23-87%.

Problems encountered in colposcopy may arise due to

1. **Inadequate expertise**: An inexperienced colposcopist may find difficulty in assessment of various lesions. Recognition of squamocolumnar junction is crucial to identify the upper limit of the lesion. A novice colposcopistmay give more importance to minor grades of mosaic or punctations than major grades of acetowhite epithelium leading to biopsy from a wrong area.

2. **Interpretive problems and limitations**:

There are various conditions which create confusion in colposcopic differentiation. Immature or active metaplastic epithelium may be difficult to differentiate from early grades of CIN. Vascular pattern may lead to confusing picture. Colposcopy maybe unsatisfactory at times.

3. **Failure to follow standard diagnostic protocol**:

Deviation from an established protocol increases the probability of inaccurate diagnosis resulting in inappropriate treatment which may prove disastrous[48].

4.**Post menopausal women** :

In post menopausal women due to atrophic changes the squamo columnar junction gets flushed in to the vagina which makes it difficult for visualisation and examination to repost the findings.

Colposcopic Directed Biopsy:

Biopsy should be taken under colposcopic guidance from a point within the most abnormal area. Histopathology provides the final confirmation of diagnosis in most situations, even though certain conditions cannot be pinpointed such as inflammatory conditions. It is of paramount importance in deciding the modality of treatment and type of surgery. Thus it is imperative to have active interaction between the colposcopist, cytologist and histo pathologist to correlate their findings for achieving optimum results in the management of female lower genital tract lesions[49].

Cervicography[50]-

This method was first described by Adolf Stafl in 1981. A photograph of the cervix is taken after the application of 3-5% solution of acetic acid. Cerviscope is a special camera equipped with an extension tube and ring flash. After development, the

film is projected on 2 x 2 m screen and a colposcopy expert reads the picture from a distance of 150 cm. The cervigram is interpreted as negative, atypical or positive. The cervigrams are treated as

1. Negative
2. Atypical – recommending repeat cervigram and Pap test in 6 to 12 weeks
3. Positive – with colposcopy recommended, if the cervigram indicated.

In summary, cervicography alone has an inferior sensitivity compared to cytology, and therefore is not recommended in settings where adequate cytology services are available. As an adjunct to Pap smear screening, cervicography may increase the sensitivity for detecting cervical abnormalities but will decrease the specificity, potentially resulting in increased referrals for colposcopy. It is useful when a colposcopistis not available for spot evaluation.

Downstaging screening of cancer cervix-

According to WHO it is defined as the detection of disease in an earlier stage (when still curable) by nurses and other paramedical workers by using a simple speculum for visual inspection of the cervix. It is an experimental approach suggested by WHO as alternative to regular cytological screening.

In the developing countries, where effective mass screening cannot be extended and the majority of cases of carcinoma cervix are diagnosed at an advanced stage, down staging screening offers at least an early detection of disease. The strategy is however, not expected to lower the incidence of cancer cervix but it can certainly minimize the cancer death through early detection.

Objective of the visual examination is solely, to be able recognize clinically normal from abnormal cervix and refer abnormal looking cases for further evaluation and diagnosis. All findings should be carefully recorded in the provided printed forms. The gross appearance of the cervix is classified into Normal, Abnormal or Suspicious of Malignancy.

Normal Cervix-

A normal cervix appears smooth, round, pink lubricated with clear mucoid secretion and has a central hole the “external os”. The shape of the external osvaries with parity, being round in a nulliparous woman and slit like or cruciate in a multipara. Cervix in postmenopausal women appears atrophic.

Abnormal Cervix- This category will include all benign looking lesions, like

1. Hypertrophy
2. Redness or Congestion
3. Irregular surface/ Distortion
4. Simple erosions (that do not bleed on touch)
5. Cervical polyps (with smooth surface)
6. Abnormal discharge (foul smelling, dirty/ greenish, white/ cheesy, blood stained)
7. Nabothian follicles
8. Prolapsed uterus

Suspicious of Malignancy- Malignancy should be suspected when there is erosion that bleeds on touch or a growth with an irregular surface. Both of these lesions may be friable and bleed on touch or may be accompanied with an offensive discharge.

Second generation category-

Polar Probe- Real time electronic device is used for detection of cervical neoplasia. It detects the existence of cervical cancer and pre cancer by measuring two sets of physical parameters, voltage decay and scattering of various wavelengths of light by different tissues. When applied directly to cervix the device instantly recognizes six types of tissues from normal to abnormal tissue. It may be used in primary screening or as an adjunct to cytology. Excellent discrimination of polar probe at high-grade end of spectrum and its ability to give instantaneous results makes it especially appearing ashigh risk, poorly compliant population in rural areas and in developing countries where return visit colposcopy are especially difficult. The sensitivity is similar to that ofcytology and specificity better than cytology in some settings.

NEW SCREENING TECHNOLOGIES UNDER INVESTIGATION AIMED AT IMPROVING PAP TEST ACCURACY

New technologies for cytology screening are intended to reduce the false negative rate, improve sensitivity and specificity of screening, improve the adequacy of the pap smear and potentially improve laboratory productivity.

Liquid- Based Cytology

a) Thin Layer preparation/ Thin Prep FDA approved-

Interpretation of Pap smear slides maybe hindered by poor sampling, uneven cell distribution or improper fixation of the slide. Important findings may be obscured by uneven sample distribution, cellular clumping and debris.The Liquid based/ thin layer preparation system is designed to correct this problem. It improves the sensitivity of the Pap test to the stated goal of 80%. The cell sample is obtained with a cytobrush, and Ayre spatula or a cervical broom device. The sample is not smeared

directly on a glass slide. Instead the sample is rinsed in a vial containing liquid preservative. This technique transfers 80 – 90% of the cells to the liquid media, as compared with only 10 – 20% transferred to the glass slide with conventional cytology testing. The technique removes most mucus protein and fresh red blood cells from preparation, distributes the cells uniformly, improves fixation and the preservation of the cellular architecture, maintains diagnostic clusters and ensures uniform sampling of the material removed from the cervix. It eliminates air dying.

Furthermore, this method provides representative residual material in collection media that can be used for additional / adjunctive testing (ex-HPV testing). The cells are retrieved from the vial by passing the liquid through a filter, which traps the larger epithelial cells, separating them from the small blood and inflammatory cells. A predetermined number of cells are drawn onto a filter membrane, which is then applied toa glass slide in a monolayer. This leads to a thin layer of diagnostic cells properly preserved and more easily interpreted by cytologist. This technique reduces the rate of unsatisfactory smears encountered with conventional cytological testing by 70 to 90 %.

There are some disadvantages with this technique. Interpretation of monolayers is different from conventional studies, requiring retraining of cytotechnologists and cytopathologists. Cells may be overlooked because they may resemble benign metaplastic cells. Also glandular abnormalities are more difficult to assess.

b) Cytorich/ Surepath – Awaiting FDA approval

It uses collection technique similar to that employed by Thin Prep. Unlike thin prep, however Cytorich is approved for use only with broom like device. Mixing the solution separates clumps of cells. Centrifuging against a density gradient separates debris and inflammatory cells. Cells are suspended and allowed to settle onto a slide by gravity. The slide is then stained and evaluated by a cyto pathologist. This process reduces the number of unsatisfactory slides and results in increased sensitivity.

Automated cytological screening-

Interpretation of cervical cytology is considered to be very difficult (Richart,1995). The training programs for cyto technologists are long, require an educated student and require a high degree of discipline and pattern recognition skills. Even after completing an adequate training program, cytotechnologists require several years of practical experience before they can make consistent diagnosis on the normality of the smears. Hence it is not surprising that false negative rates are high. Manual re-screening is possible to reduce this rate, but this comes at the expense of increased false positives, longer screening time and diminished productivity. Methods of computer assisted screening currently available

Auto Pap screening system-has been approved by the U.S F.D.A for primary screening and rescreening of samples initially read as normal. This technique uses an automated microscope coupled to a special digital camera.The system scans the slides and uses computer imaging techniques to analyze each field of view on the slide. Computer algorithms are then used to rank each slide on the basis of the probability that the sample may contain abnormality. It selects 10-20% of slides labeled as formal following routine screening by cytotechnologist with the highest probability of having

abnormal cells. The algorithm includes a variety of visual characteristics, such as shape and optical density of the cells. The selected slides are then reviewed by a cytotechnologist or cytopathologist which of the cells are likely to be abnormal. This technique reduces false negative rate by 32%. It is not in widespread use. It has been shown to be superior to conventional pap test screening in identifying ASCUS, LSIL and HSIL

Pap Net-uses neural network computing technology to improve accuracy in Pap smear screening. It uses the computer to spot suspicious patterns not obvious to the naked eye and displays the abnormal cells on a high resolution color video screen for interpretation and diagnosis by a skilled laboratory technician. Received FDA approval for the automated rescreening of Pap smears that have been read as normal.

Auto Cyte- Uses slides that are prepared by the thin layer cytology systems (CytoRich Method) and can screen up to 300 slides within 24 hours. For each abnormal slide, the most significant abnormal cellular features and the interpretation of each are captured, stored and processed by a series of algorithms. These images are presented to a human reviewer who then determines whether manual review is required. After the human reviewer has entered an opinion, the device reveals its determination based on a ranking as to whether manual review is warranted. When human reviewer and computer agree that no review is needed, a diagnosis of 'within normal limits' is given. Manual review is required for any case if designated by either the cytologist or the computer ranking.

HPV DNA detection and typing

Cervical cancer and its precursor are cause principally, of not exclusively, by HPV infection (Schiffman 1993).The prevalence of HPV varies dramatically, depending on the age group and techniques used to detect the virus. It is much more common in the younger patients than the older .There is also recent evidence that, in many women HPV infection is transient and is cleared within the first 12 – 24 months. Therefore, in younger women, HPV infection is more a marker of sexual activity than of cervical cancer risk, whereas the persistence of HPV in older (less sexually active) is an indication of increased cervical cancer risk. Primary screening for HPV DNA in younger women may probably detect many women with cervical HPV, few of whom have significant disease. This will lead to many unnecessary colposcopic evaluations. However, in older women, in whom the prevalence of disease is lower and its significance is greater, HPV testing may offer a sensitive and specific way of detecting women at risk of developing squamous intraepithelial lesion.

The test for HPV include

1. PCR
2. Hybrid Capture Tube test (HCT)
3. Hybrid Capture II test (HC II)
4. HPV test (Called the DNA with Pap)
5. Pre Tect HPV-Proofer

HPV detection by PCR- PCR and southern blotting are highly sensitive and highly specific tests, can detect even a single molecule of HPV DNA. During the past 10 years, PCR has been the “gold standard” technique in HPV diagnosis. DNA is extracted by the high-salt method. HPV DNA is detected by PCR with primer GP05+

and GP06+. Confirmation of the specificities of the PCR products is done by hybridization with digoxigenin-labeled oligonucleotide probes specific for HR HPV types. Afterhybridization, the positive spots on the film are graded according to the signal intensities are weak, moderate or strong. Approximately 20 copies of HPV, i.e., 20 SiHa cells mixed with 200ng of human fibroblast DNA, give a weakly positive signal. The recognized disadvantages of PCR are its extremely high analytical sensitivity and potential for contamination, leading to false-positive results.

HPV detection by DNA test- The hybrid capture technique is based on the formation of RNA-DNA hybrids between HPV DNA that may be present in clinical specimens and complementary unlabeled HPV RNA probes. The RNA-DNA hybrids are captured and immobilized by anti-hybrid antibodies. Immobilized hybrids are reacted with a monoclonal antibody reagent that is conjugated to alkaline phosphatase, and the complexes are detected via a chemiluminescent substrate reaction. In HCT, a tube luminometer is employed, whereas in HC II, a microplateluminometer reads the light output and displays the assay results as relative light units (RLU). HPV positivity or negativity is based on comparison to a standard positive reference (RLU of a clinical specimen divided by the mean RLU of three positive calibrator references).

The HCT Probe B cocktail detects a limited number of high-risk HPV types, including HPVs 16, 18, 31, 33, 35, 45, 51, 52, and 56 and has been reported to have a diagnostic sensitivity similar to that of the Pap smear. HC II detects HPV types at an increased sensitivity compared to that of HCT. The advantage of this test is that it may provide a semi quantitative measure of viral load. It has been suggested that viral load may lend prognostic and diagnostic value

HPV detection by mRNA test-

Persistent expression of E6/ E7 oncogenes could serve as an indicator of progression to cervical intraepithelialneoplasia and invasive cancer. E6/ E7 mRNA transcripts are detected by mRNA based molecular techniques and may therefore be of higher prognostic value and improve the specificity and PPV compared to HPV DNA testing in screening.Generally malignant transformation of the cervical cells is indicated by expression of 2 to 1000 copies of HPV E6, E7 mRNA per cell. Total mRNA is extracted using the RNeasyMiniprotocol . Individual identification of E6/ E7 mRNA full-length transcripts from HPV 16, 18, 31, 33 and 45 is performed with the PreTect HPV Proofer assay .Several investigators have found that among older women, the combination of a Pap smear and HPV DNA screening detects more than 95% of patients with high grade lesions, 100% with invasive cancers and 70% with low grade lesions.

One of the most promising roles of HPV testing is to determine which women with single smear showing ASCUS require colposcopic evaluation.

Advantages

- High positive predictive value (PPV)
- The ease with which the specimens can be collected even by unsophisticated medical personnel
- Does not require the extensive training that conventional cytological screening personnel most undergo.
- More sensitive than unaided visual inspection of cervix
- Potential for applying it to large populations at low cost.

The use of HPV DNA testing in addition to cytology improves the sensitivity compared to HPV testing alone. According to the consensus recommendations, HPV DNA testing can be added to cytology for screening of women over age 30 years. If results are negative for both HPV DNA and cytology, there is no need to screen again for 3 years. If the cytology is negative but the DNA test is positive, repeat screening with both cytology and HPV DNA testing is indicated in 6 – 12 months, as the likelihood of developing high grade neoplasiais low. If repeated results are positive for either cytology or HPV DNA, the clinician should proceed to colposcopy.

Pap Sure

It is a in-office, non-invasive method for performing direct visual screening of examination of cervix. It has been approved by the FDA for use in all women undergoing Pap smears.

Treatment-

The treatment of cervical cancer depends upon the age of the patient, desirability of the woman to retain her reproductive function , the type of lesion present and the stage of the disease. Below Table 4 gives a brief and concise overlook on the options available for treatment of cervical cancer.

Table 4: Standard Treatment Options for Cervical Cancer [56]

In situ carcinoma of the cervix (this stage is not recognized by FIGO)	Conization
	Hysterectomy for postreproductive patients
	Internal radiation therapy for medically inoperable patients
Stage IA cervical cancer	Conization
	Total hysterectomy
	Modified radical hysterectomy with lymphadenectomy
	Radical trachelectomy
	Intracavitary radiation therapy
Stages IB, IIA cervical cancer	Radiation therapy with concomitant chemotherapy
	Radical hysterectomy and bilateral pelvic lymphadenectomy with or without total pelvic radiation therapy plus chemotherapy
	Radical trachelectomy
	Neoadjuvant chemotherapy
	Radiation therapy alone
	Intensity Modulated Radiation Therapy (IMRT)
Stages IIB, III, and IVA cervical cancer	Radiation therapy with concomitant chemotherapy
	Interstitial brachytherapy
	Neoadjuvant chemotherapy
Stage IVB cervical cancer	Palliative radiation therapy
	Palliative chemotherapy
Recurrent cervical cancer	Radiation therapy and chemotherapy
	Palliative chemotherapy
	Pelvic exenteration

METHODOLOGY

Source of Data: Women attending Gynaecology OPD at B.L.D.E. Uniersity's SHRI B.M.Patil Medical College and Research Hospital, Bijapur.

Methods of collection of Data:

A. Study Design: Prospective study

B. Study Period: Two years (2012-2014)

C. Sample Size: 100 cases who fulfilled selection criteria

Inclusion criteria:

1. Women aged between 30-65yrs
2. Parous women.

Exclusion criteria:

1. Pregnant women
2. Clinically visible growth on cervix
3. Prior hysterectomy or procedure on the cervix
4. Women who are not sexually active

Procedure:

Written and informed consent were obtained from all the participants after brief explanation of the procedure.

History:

Menstrual and obstetric history was taken in relation to the presenting complaints. History of any previous surgery on cervix was noted.

Examination:

The patient was placed in dorsal position, the labia separated and the Cusco's self retaining speculum gently inserted without the use of lubricant or jelly. The cervix

was exposed and visualized for any gross pathological features under adequate light and findings were recorded.

Pap smear:

After preliminary inspection of the cervix, a Pap smear was taken using Ayre's spatula. The squamocolumnar junction was scraped with the Ayre's spatula by rotating full 360 degree. The scrapings were evenly spread on a glass slide and immediately fixed by dipping in the jar containing equal parts of 95% ethyl alcohol and ether and transported to the cytopathological laboratory. Smears were analyzed by senior pathologist. Revised Bethesda System was used for describing Pap smearresults.

Visual inspection with acetic acid (VIA):

After taking Pap smear, 3-5% acetic acid was applied on the cervix and cervix was inspected after one minute. Repeat application of 3-5% acetic acid was done if required. The following were noted:

1. The presence or absence of aceto white patch
2. Location of aceto white in relation to squamocolumnar junction
3. Intensity of aceto white patch
4. Margin of aceto white patch

Information Agency for Research on Cancer (IARC) guidelines for reporting VIA test results[51].

Positive VIA test:

a. Sharp, distinct, well defined, dense aceto white areas with or without raised margins, abutting the squamocolumnar junction

b. Strikingly dense aceto white areas in the columnar epithelium

c. Condyloma and leukoplakia occurring closer to the squamocolumnarjunction turning intensely white after application of acetic acid

Negative VIA test:

a. No aceto white lesion observed on the cervix

b. Polyps protruding from the cervix with bluish-white areas

c. Nabothian cysts appearing as button like areas or as whitish acne or pimples

d. Faint line-like or ill-defined aceto whitening at the squamocolumnarjunction

e. Dotted areas in the endocervix

f. Shiny, pinkish, cloudy, bluish-white lesions, faint patchy lesions or doubtful lesions with ill-defined, indefinite margins blending with the rest of the cervix.

g. Angular, irregular, digitalizing aceto white lesions resembling geographical regions far away from the transformation zone (satellite lesions)

h. Streak-like aceto whitening in the columnar epithelium

i. When red spots are observed in the cervix against a pinkish-white hue

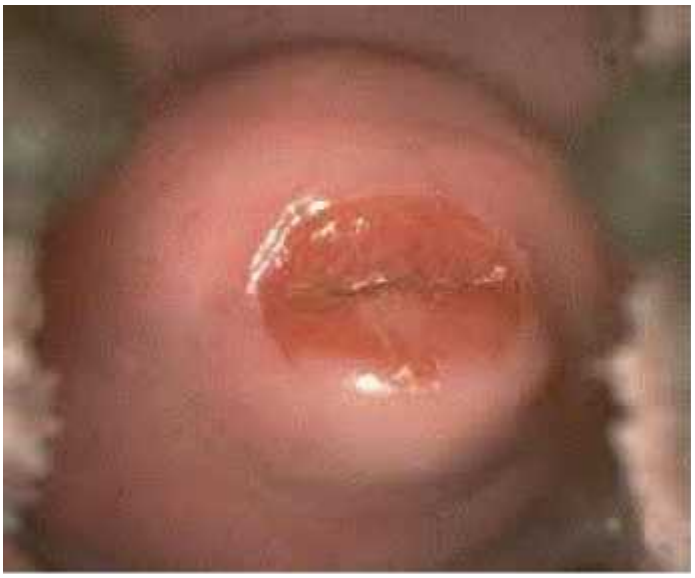


Figure 11- Normal- Negative VIA

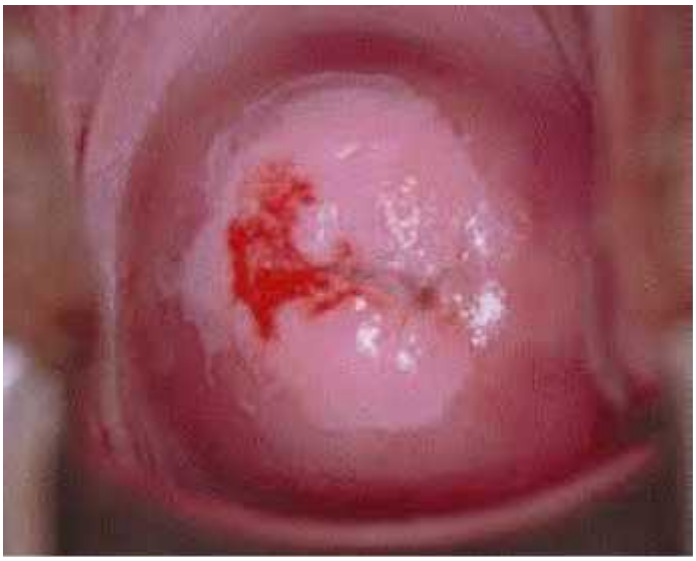


Figure 12- Positive VIA

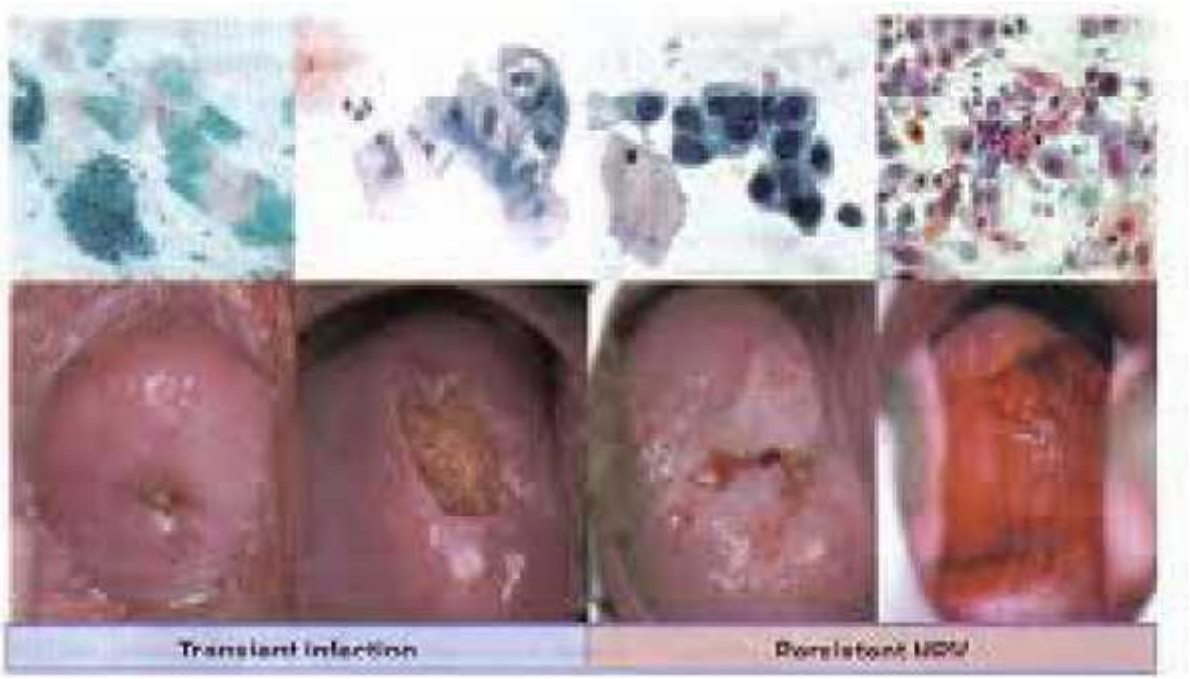


Figure 13 - VIA Images in HPV infection

Toluidine blue test:

Procedure: the squamo-columnar junction of the cervix is pre-washed with 1% acetic acid followed by application of a swab soaked in 1% aqueous solution of Toluidine blue dye. After 1 minute re-washing with 1% acetic acid is performed to remove excess dye. Abnormal areas are stained royal blue.

The patients with positive test results with any or all the tests of screening and 10% of negative cases in all groups selected randomly will be subjected to colposcopy and directed biopsy.

Colposcopy:

In all women colposcopy was done. Colposcopy was performed using normal saline, green filter and acetic acid. Findings were recorded and colposcopy diagnosis was made based on Modified Reid Colposcopic Index (RCI)[52].Ried et al (1983) defined three objective categories based on colposcopicindex using four colposcopic signs i.e. colour, margin (including surface contour), vascular pattern and iodine response. Each category is offered scores of 0 to 2.

Summation of scores is done.

Scores of 0-2: Predictive of minor lesion (CIN1 or HPV)

3-5: Middle grade lesion (CIN1 – II)

6-8: Significant lesion (CIN II – III)

Table 5: COMBINED COLPOSCOPIC INDEX[53]

Colposcopic sign	0(zero)	1(one)	2(two)
Margin	Condylomatous or micropapillary contour, indistinct aceto whitening, flocculated or feathered margins. Angular jagged lesions. Satellite lesions and aceto whitening beyond the transformation zone.	Regular lesions with straight outlines	Rolled peeling edges. Internal demarcation between areas of differing appearance
colour	Shiny, snow-white color , indistinct aceto whitening	Intermediate shade (shiny gray)	Dull oyster white
Vessels	Fine- caliber vessels, poorly formed patterns	No abnormal vessel	Definite punctations and mosaicism
Iodine	Positive iodine uptake	Partial iodine	Negative staining of significant lesion

Figure 14: Colposcope **Figure 15: Ayre's spatula**

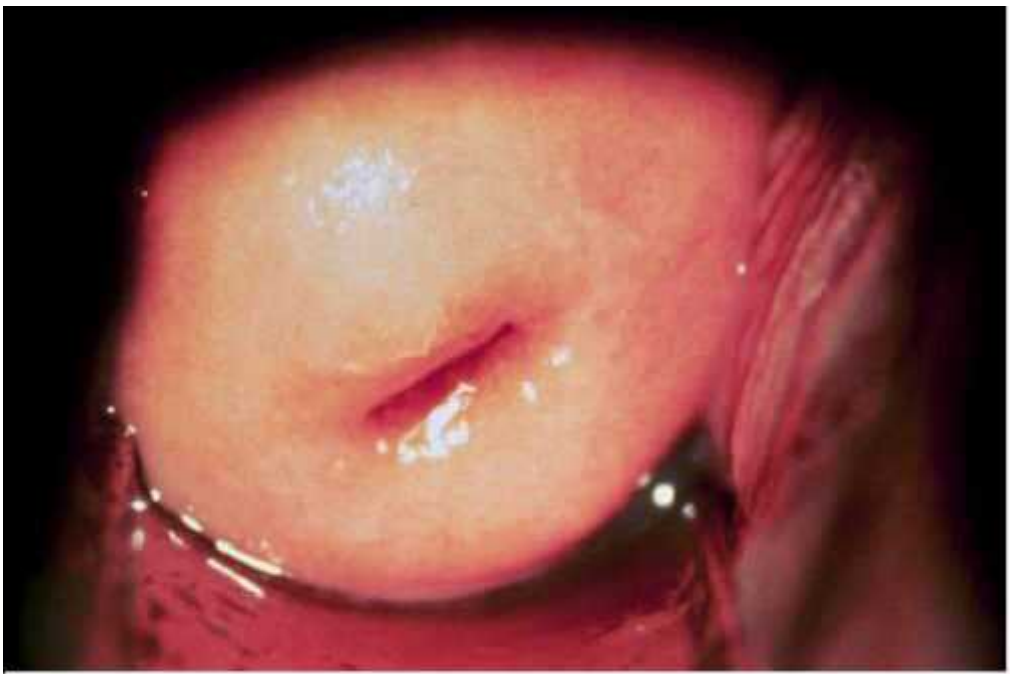


Figure 16: Normal cervix

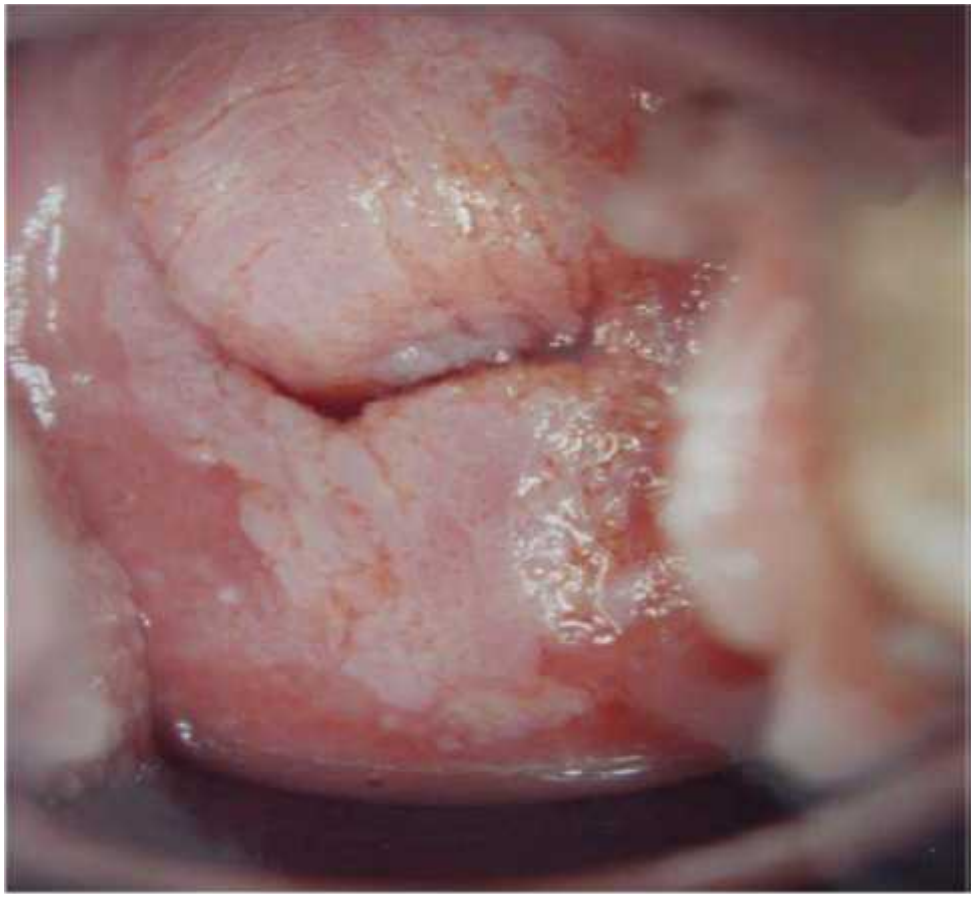


Figure 17: Sharp, distinct, well-defined, dense acetowhite areas with or without raised margins abutting the squamocolumnar junction (HSIL)

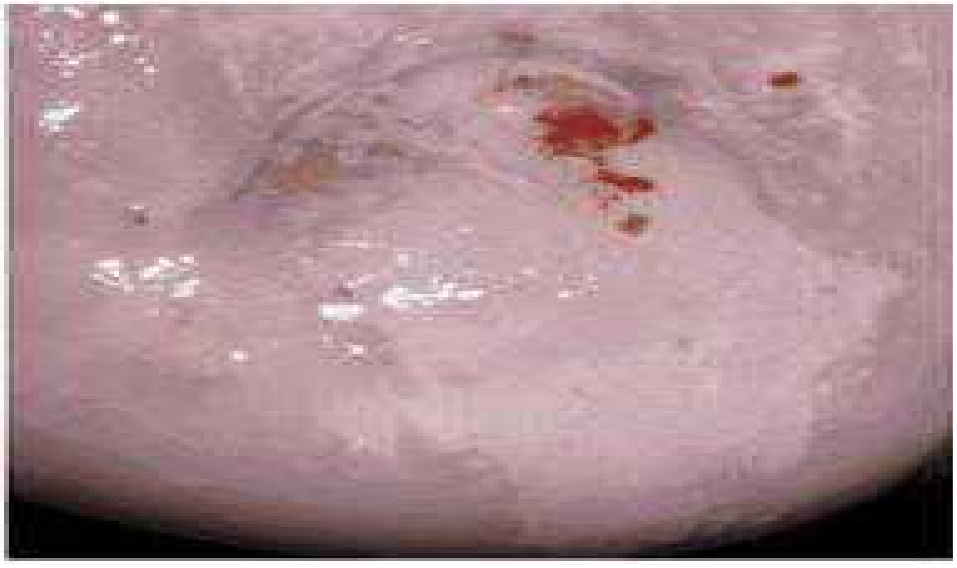


Figure 18: Strikingly dense acetowhite areas (LSIL)

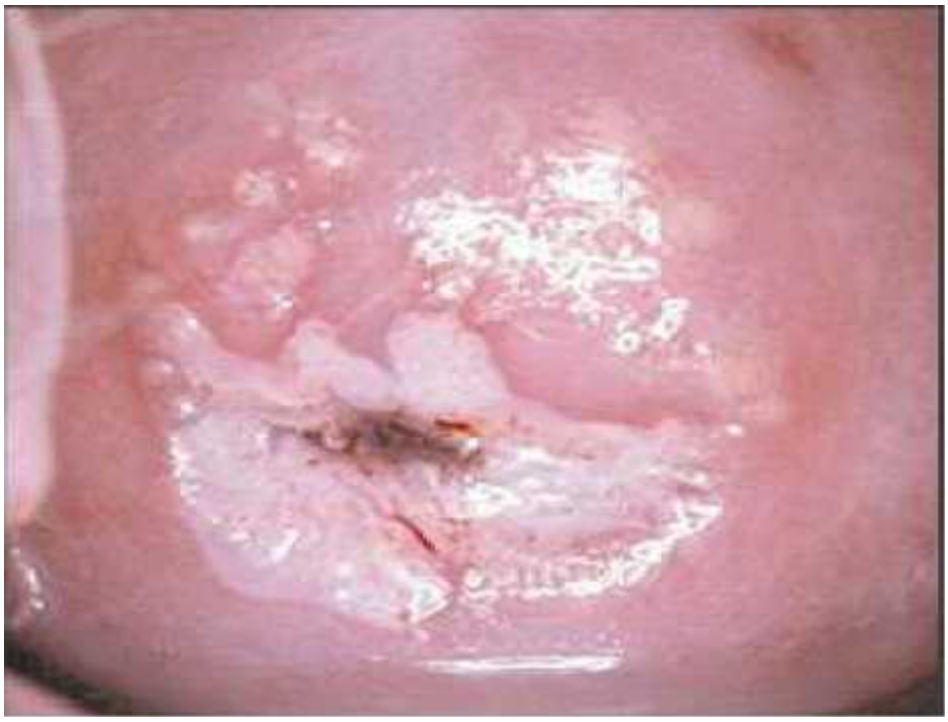


Figure 19 : Strikingly dense acetowhite areas in the columnar epithelium

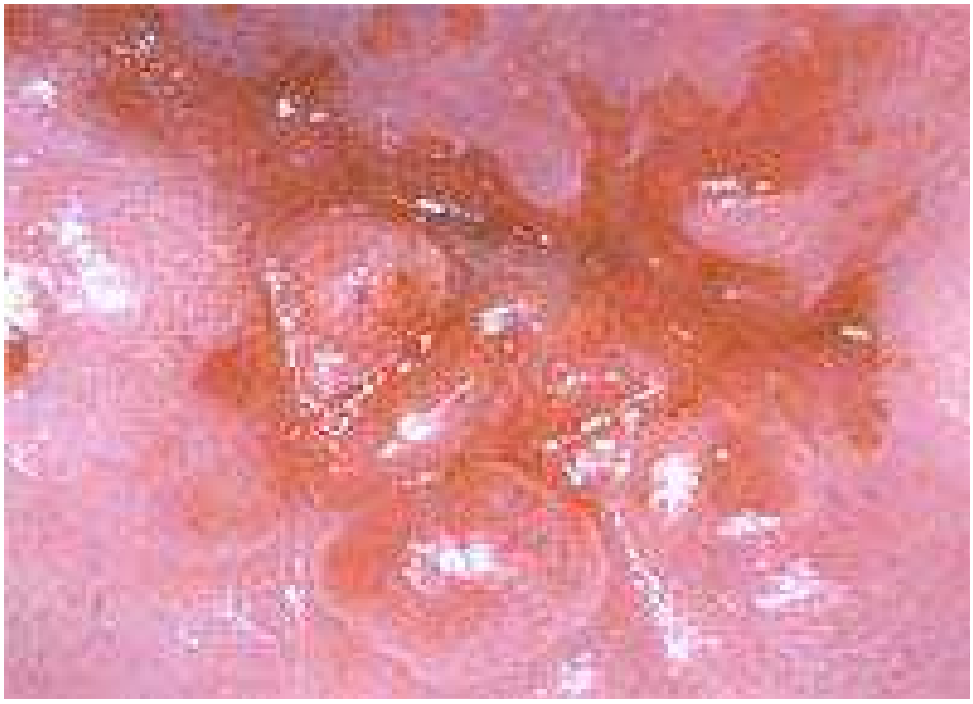


Figure 20: Strikingly dense acetowhite areas with atypical vascular pattern (HSIL)

Colposcopic guided biopsy:

Biopsy was taken from abnormal area under colposcopy guidance using cervical punch biopsy forceps. Four quadrant biopsy was taken from ectocervix at the squamocolumnar junction if no abnormality was detected on colposcopy. The specimen was sent for histopathological examination in formalin solution. Slides were analyzed by senior consultant pathologist. Biopsy results were categorized as

1. Cervicitis/ metaplasia
2. CIN-1 (mild dysplasia/ correlating with LSIL)
3. CIN-2/3 (moderate to severe dysplasia/ correlating with HSIL)
4. Squamous cell carcinoma

STATISTICAL ANALYSIS

The statistical analysis was done by calculating diagnostic efficacy of each test. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), false positive rate, false negative rate and accuracy were calculated for Pap smear, VIA, Toluidine blue dye test and colposcopy with colposcopy directed biopsy results as the gold standard.

RESULTS

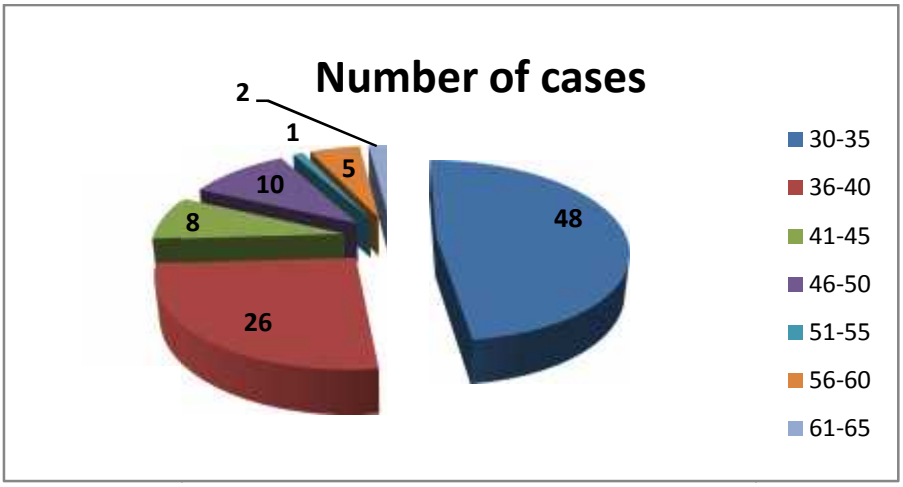
The study was performed on 100 women who attended the Department of Obstetrics and Gynaecology at Sri B.M. Patil Medical College & Research Hospital, Bijapur. The objectives of the study was to correlate the findings in women with unhealthy cervix by cytology, visual inspection aided by acetic acid test (VIA), Toluidine blue dye test, colposcopy and colposcopic directed biopsies in detecting the premalignant and malignant lesions of the cervix and to find the efficacy of individual tests. The detailed analysis of the study conducted and the results are computed together after all the tests were employed to arrive at a conclusion.

The 100 women who were in the study group belonged to the age group of 30-65 years. The Table 6 and the Graph 1 shows age wise distribution of cases.

Table 6: Distribution of cases according to age:

Age group	Number of cases (N= 100)
30-35 years	48
36-40 years	26
41-45 years	08
46-50 years	10
51-55years	01
56-60 years	05
61-65 years	02

Graph 1: Distribution of cases according to age:



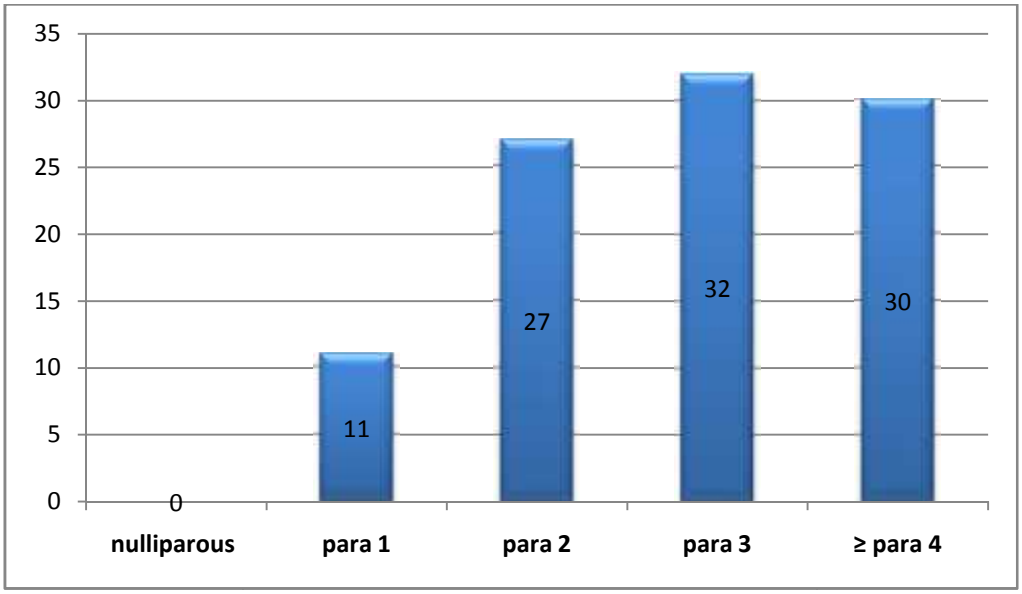
Maximum no of cases were in the age group of 30-35 years ie 48 cases (48%). The mean age was 32.35 years.

The women in the study group were primipara to grand multipara. The Table 7 and Graph 2 shows the distribution of cases based on parity.

Table 7: Distribution of cases based on parity:

Parity	Number of cases (N=100)
Nullipara	0
Para one	12
Para two	27
Para three	31
≥Para four	30

Graph 2: Distribution of cases based on parity:



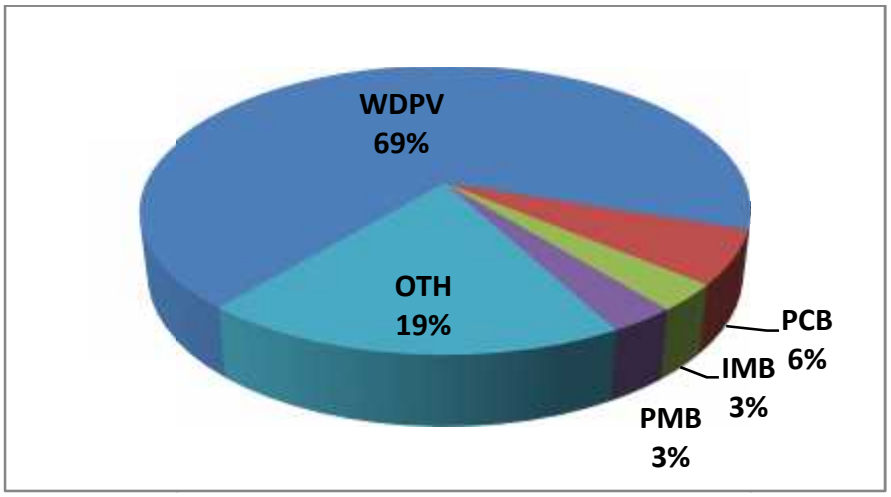
Majority of study group were in para 3 (31%) and ≥ para 4 (30%) group.

The women in the study group presented with symptoms of white discharge per vagina, post coital bleeding, inter menstrual bleeding, post menopausal bleeding, pain abdomen and backache. The Table 8 and Graph 3 shows the various symptoms with which the patients presented and the distribution of cases with each of the symptoms

Table 8: Distribution of cases based on symptoms:

Symptoms	Number of cases (n=100)
White discharge per vagina	69
Post coital bleeding	6
Inter menstrual bleeding	3
Post menopausal bleeding	3
Others (pain abdomen, backache)	19

Graph 3: Distribution of cases based on symptoms:



WDPV- White discharge per vagina

PCB- Post coital bleeding

IMB- Inter menstrual bleeding

PMB- Post menopausal bleeding

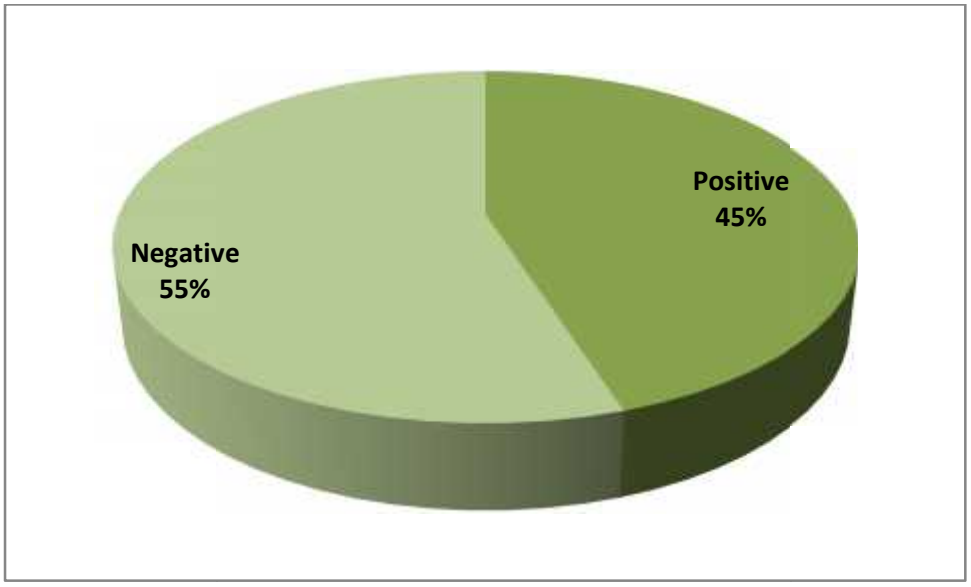
The commonest symptom was white discharge per vagina.

The women recruited for the study were subjected to visual inspection aided by acetic acid (VIA), where in 3-5% of dilute acetic acid was applied to the cervix and observed by naked eyes. Test was considered positive result by the presence of aceto white area and the others negative. The Table 9 and Graph 4 show the number of positive and negative cases on VIA.

Table 9: VIA RESULTS

Total	**Number of cases (n=100)**
Positive	45
Negative	55

Graph 4: VIA RESULTS



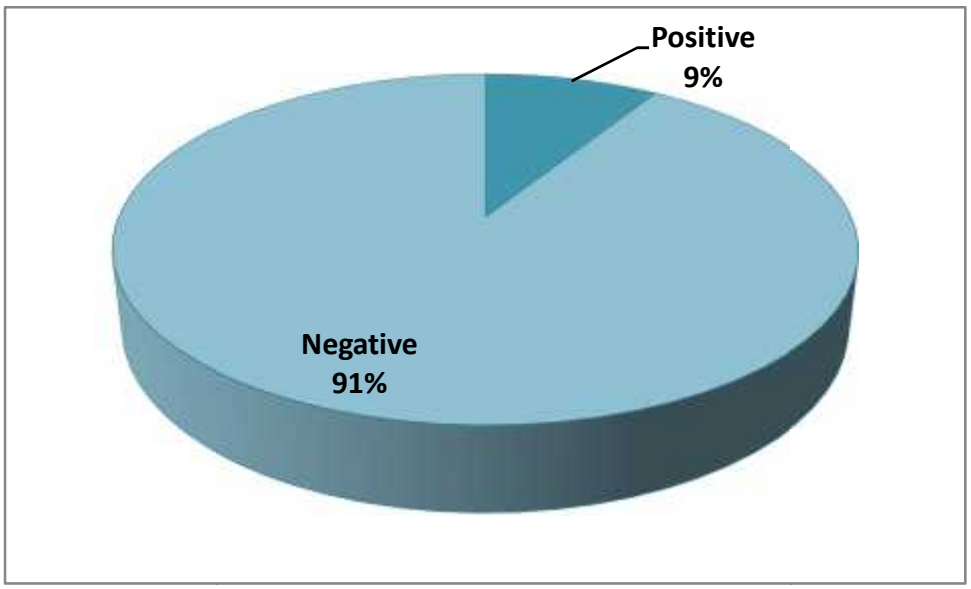
45 women out of 100 had a positive result and 55 out of 100 had a negative result on VIA.

The Toluidine blue dye test, where1% Toluidine blue dye was applied to the cervix. If the area on cervix stained royal blue test was considered to be positive. If no stain is taken up then the test result was considered negative. Table 10 and Graph 5 show the results of Toluidine blue dye test.

Table 10: TOLUIDINE BLUE DYE TEST RESULTS

Total	**Number of cases (n=100)**
Positive	9
Negative	91

Graph 5: TOLUIDINE BLUE DYE TEST RESULTS



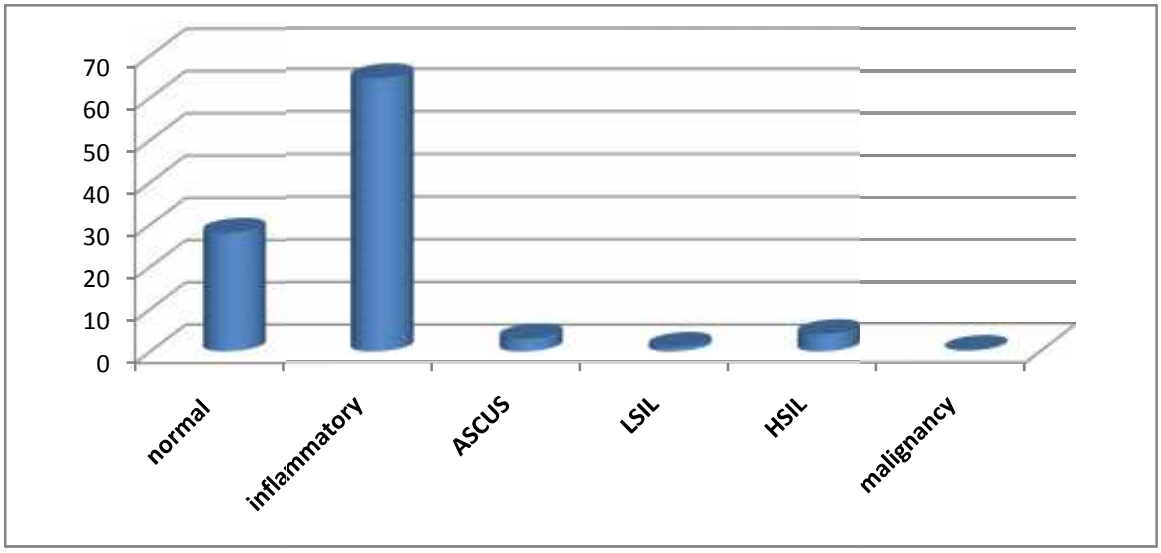
9 out of 100 women were positive to the Toluidine blue dye test and 91 had a negative result.

All the 100 women underwent cytological examination. The findings on cytology have been computed below in the following Table 11 and Graph 6.

Table 11: PAP Smear results

Outcome	Number of cases (n=100)
Normal	28
Inflammatory	64
ASCUS	3
LSIL	1
HSIL	4
Malignancy	0

Graph 6: Pap smear results



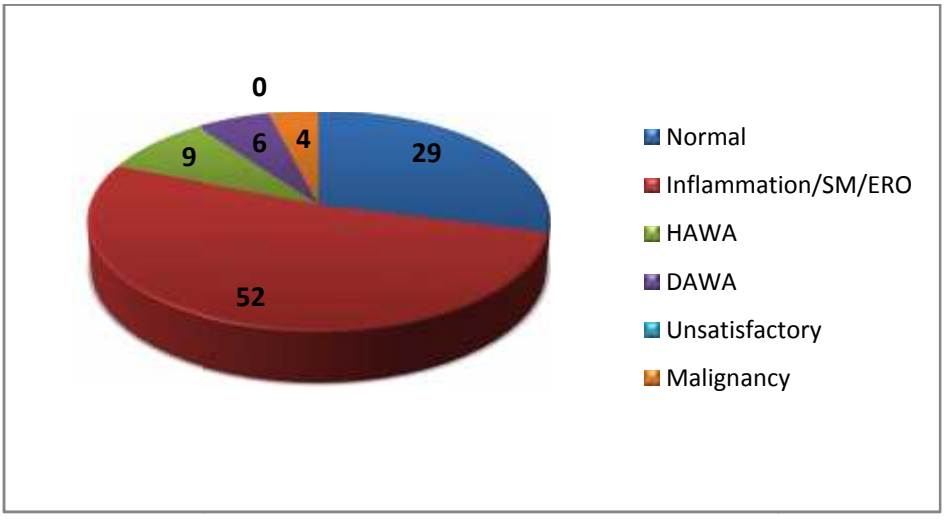
Pap smear revealed that 64% had an inflammatory smear and 5% had a positive Pap smear. The result of Pap smear was considered positive if it revealed LSIL, HSIL, carcinoma in situ or invasive cancer. Among 5 positive cases, there were 1 LSIL, 4 HSIL and no case of malignancy.

Examination of cervix by Colposcopy is the gold standard screening method used in the study. All the cases were subjected to colposcopy examination. Table 12 and Graph7 depicts various colposcopic findings and the distribution of cases with different colposcopic findings.

Table 12: Colposcopy results

Outcome	Number of cases (100)
Normal	29
Inflammation/squamous metaplasia/erosion	52
Hazy/faint aceto white areas. Fine punctations or Mosaicism	9
Dense aceto white areas. Coarse punctations or Mosaicism	6
Unsatisfactory	0
Malignancy (intense aceto white lesion, coarse irregular punctations, cork screw vessels)	4

Graph 7: Colposcopy results



HAWA- Hazy aceto white area

DAWA- Dense aceto white area

SM- Squamous metaplasia

ERO- Erosion

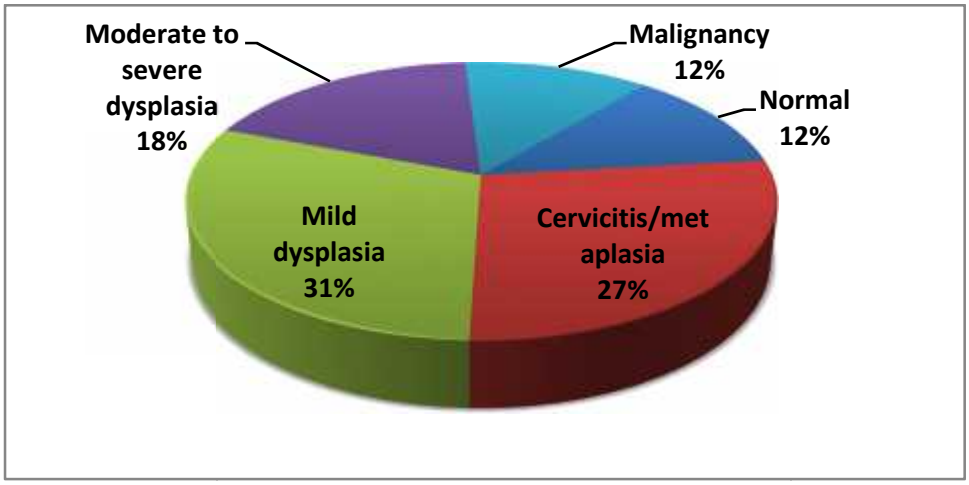
On Colposcopy 19 women were found to have a positive result (19%) and the remaining were normal. Colposcopy was considered positive if it revealed lesions of hazy aceto white areas/fine punctations/mosaicism and above. Among the 19 women with abnormal colposcopies there were 9 cases of hazy aceto white areas/fine punctations/mosaicism, 6 cases of dense aceto white areas/coarse punctations/mosaicism and 4 cases suspicious of malignancy.

The cases with positive findings by VIA or Toluidine blue dye test or Cytology and Colposcopy and also those who were suspicious were subjected to biopsy and the findings were confirmed. Following Table 13 and Graph 8 shows the confirmatory biopsy findings done on all the positive cases (n=33).

Table 13: Biopsy results

Outcome	Number of cases (n=33)
Normal	4
Cervicitis/metaplasia	9
Mild dysplasia	10
Moderate to severe dysplasia	6
Malignancy (squamous cell carcinoma)	4

Graph 8: Biopsy results



The positive biopsy includes 20 cases out of 100. Biopsy was considered positive if it revealed mild dysplasia and above. It includes 10 mild dysplasia (LSIL), 6 moderate to severe dysplasia (HSIL) and 04malignancies. Out of 04malignancies none had visible growth on per speculum examination.

Comparision between the Pap smear and colposcopy findings were made to find the total positive cases thereby formulating the diagnostic value of Pap smear, its sensitivity, specificity, PPV, NPV and accuracy. The Table 14 contains the information regarding the same.

TABLE 14: COMPARISION BETWEEN PAP SMEAR AND COLPOSCOPY

PAP SMEAR		COLPOSCOPY					
		Normal	**INF/ Metaplasia/ Erosion**	**HAWA/ Fine punctuation / Mosaicism**	**DAWA/ Coarse puncatations/ Mosaicism**	**Unsatisfactory**	**Malignancy**
NORMAL	28	22	6	0	0	0	0
INFLAMMATION	64	7	46	9	2	0	0
ASCUS	3	0	0	0	1	0	2
LSIL	1	0	0	0	1	0	0
HSIL	4	0	0	0	2	0	2
MALIGNANCY	0	0	0	0	0	0	0
TOTAL	100	29	52	9	6	0	4

INF-inflammation

HAWA- hazy aceto white area

DAWA- dense aceto white area

5 cases out of 100 women were positive on Pap smear. 19 out of 100 women were positive on colposcopy. All the 5 positive cases of Pap smear were also positive out of 19 of colposcopy proven positive cases. 9 cases of hazy aceto white areas/ fine punctuation/ mosaicism were under reported as inflammatory and 2 under reported cases of inflammation were actually cases of dense aceto white areas/coarse punctations/mosaicism. 2 cases of malignancy and 1 case of dense aceto white area/coarse punctuations/mosaicism was under reported as ASCUS on pap smear.

All the 5 positive cases of pap smear were subjected to confirmatory biopsy. The Table 15 depicts the biopsy findings in each of the positive cases.

Table 15: Biopsy of pap smear positive cases

PAP SMEAR	BIOPSY				
	Normal	**Cervicitis/metaplasia**	**Mild dysplasia**	**Moderate dysplasia**	**Malignancy**
POSITIVE (5 cases)	-	-	-	2	3

Table 16: Diagnostic efficacy of pap smear

PAP SMEAR	COLPOSCOPY		TOTAL
	Positive	**Negative**	
Positive	5	0	5
Negative	14	81	95
TOTAL	19	81	100

Tests	%
Sensitivity	26.3
Specificity	100.0
Positive predictive value	100.0
Negative predictive value	85.3
Accuracy	86.0

Comparision between VIA and colposcopy was done and the results are given in Table 17.

Table 17: Comparison Between VIA And Colposcopy

VIA		COLPOSCOPY					
		Normal	**INF/ Metaplasia/ Erosion**	**HAWA/ Fine punctuation/ Mosaicism**	**DAWA/ Coarse puncatations/ Mosaicism**	**Unsatisfactory**	**Malignancy**
Positive	45	0	26	9	6	0	4
Negative	55	29	26	0	0	0	0
TOTAL	100	29	52	9	6	0	4

45 cases out of 100 women were positive on VIA. 19 out of 100 women were positive on colposcopy. 19 casesofVIA positive cases were also the 19 colposcopy proven positive cases. 26 cases of VIA were false positive which were cases of inflammation/erosion/metaplasia. No false negative cases.

Among 45 positive cases of VIA 30 cases were subjected to confirmatory biopsy added with their cytology and colposcopy finding. The Table 18 depicts the distribution of VIA positive cases according to their biopsy findings.

Table18 : Biopsy VIA positive cases

VIA	BIOPSY				
	Normal	**Cervicitis/metaplasia**	**Mild dysplasia**	**Moderate dysplasia**	**Malignancy**
POSITIVE (45 cases)	16 (of which biopsy was not done on 15 cases and 1 case was normal)	9	10	6	4

Table19 : Diagnostic efficacy of VIA

VIA	COLPOSCOPY		TOTAL
	Positive	**Negative**	
Positive	19	26	45
Negative	0	55	55
TOTAL	19	81	100

Tests	**%**
Sensitivity	100.0
Specificity	67.9
Positive predictive value	42.2
Negative predictive value	100.0
Accuracy	74.0

Comparision between the Toluidine blue dye test with the colposcopy findings were made and the result is given in Table 20.

TABLE 20: comparison between Toluidine blue test and colposcopy

Toluidine Blue Test		**COLPOSCOPY**					
		Normal	**INF/ Metaplasia/ Erosion**	**HAWA/ Fine punctuation/ Mosaicism**	**DAWA/ Coarse puncatations/ Mosaicism**	**Unsatisfactory**	**Malignancy**
Positive	9	0	1	0	4	0	4
Negative	91	29	51	9	2	0	0
TOTAL	100	29	52	9	6	0	4

9 cases out of 100 women were positive on Toluidine blue dye test. 19 out of 100 women were positive on colposcopy. 8 positive casesof Toluidine blue dye test were positive out 19 of coploscopy proven positive cases. 1case was false positive which is a case of inflammation/metaplasia/erosion. 9 cases of hazy aceto white areas/ fine punctations/ mosaicism and 2 cases of dense acetowhite areas/ mosaicism/ coarse punctations were falsely negative with Toluidine blue dye test.

All the 9 positive cases on toluidine blue dye test were subjected to biopsy. The Table 21 gives information regarding the biopsy findings in each of the Toluidine blue positive cases.

Table 21:Biopsy Toluidine blue Dye Test positive cases.

Toluidine Blue Dye Test	BIOPSY				
	Normal	**Cervicitis/metaplasia**	**Mild dysplasia**	**Moderate dysplasia**	**Malignancy**
POSITIVE (9 cases)	-	1	-	4	4

Table 22: Diagnostic efficacy of toluidine blue test

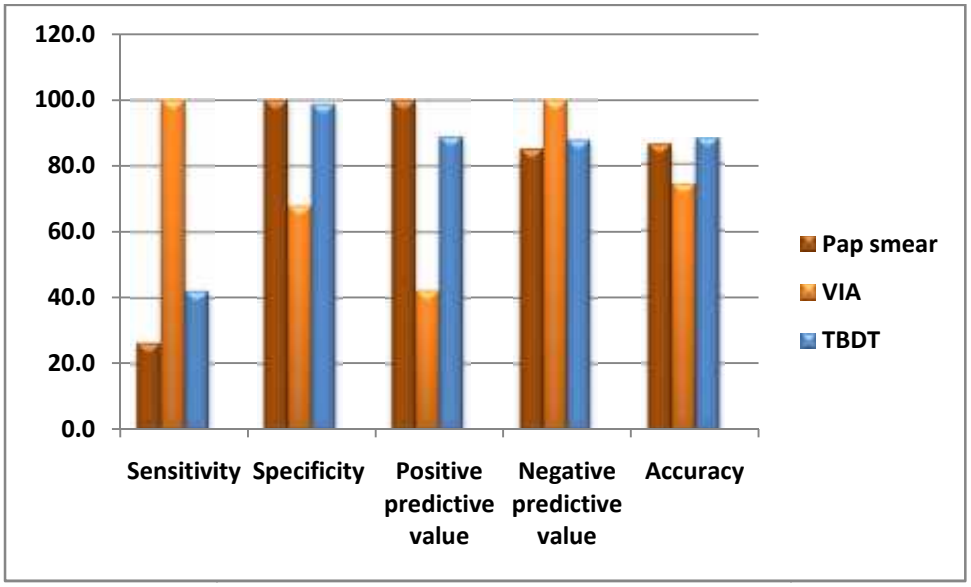
Toluidine Blue Dye test	**COLPOSCOPY**		**TOTAL**
	Positive	**Negative**	
Positive	8	1	9
Negative	11	80	91
TOTAL	19	81	100

Tests	**%**
Sensitivity	42.1
Specificity	98.8
Positive predictive value	88.9
Negative predictive value	87.9
Accuracy	88.0

Table 23:Comparision of diagnostic efficacy of various tests

Diagnostic efficacy	Pap smear	VIA	Toluidine test
Sensitivity	26.3	100.0	42.1
Specificity	100.0	67.9	98.8
Positive predictive value	100.0	42.2	88.9
Negative predictive value	85.3	100.0	87.9
Accuracy	86.0	74.0	88.0

Graph 9: Comparision of diagnostic efficacy of various tests



VIA has the highest sensitivity of 100% , followed by Toluidine blue dye test, which has slightly better sensitivity of 42.1% than Pap smear which has the least sensitivityof 26.3%.

Specificity of Pap smear was the highest at 100% followed by toluidine blue dye test which is 98.8% specific, least specific is VIA at 67.9%.

The positive predictive value of Pap smear was 100 %. The PPV of toluidine blue dye test and VIA is 88.9% and 42.2% respectively.

The NPV of VIA is the highest at 100%, followed by toluidine blue dye test at 87.9% and the least is that of pap smear at 85.3 % .

Accuracy of Toluidine blue dye test was high at 88% and Pap smear at 86%. Least accurate is VIA 74.0%.

Table 24: False Negative rates of test.

Test	False negative rates(%)
Pap smear	73.7
VIA	0.0
Toluidine test	57.9

Table 25 : False Positive rates of test.

Test	False positive rates(%)
Pap smear	0.0
VIA	32.1
Toluidine test	1.2

DISCUSSION

The incidence of cervical cancer can be reduced by as much as 80% if the quality, coverage and follow- up of screening methods are of high standard[53]. Frequently repeated cytology screening programs have led to a large decline in cervical cancer incidence and mortality in developed countries.

Cytology based screening programs have achieved very limited success in developing countries like India due to lack of trained personnel, laboratory facilities, equipments, high cost of services and poor follow-up. It has become necessary to find out alternative screening procedure to cytology which has high sensitivity and specificity[53].

The present study was carried out in the Department of Obstetrics and Gynaecology at Sri B M Patil Medical College & Research Hospital, Bijapur from 2012-2014. One hundred cases who fulfilled the selection criteria were recruited for the study.

Maximum number of cases was found to be in the age group 30-35 years (48%). Mean age was 32.35 years. Majority of the study group were Para three (31%) and Para four and above (30%). The commonest symptom with which the patients presented with, was recurrent white discharge per vagina (69%).

In our study, 45 out of 100 women showed a positive result and 55 women showed negative result on VIA (visual inspection aided by acetic acid). VIA positivity rate depends upon type of criteria used and population screened (high risk or general

population). Sensitivity of VIA was the highest ie 100% and was similar to study conducted by Bharaniet. al. in their study the sensitivity of VIA was found to be 100% too. The specificity and PPV of VIA was low at 67.9% and 42.2% respectively but the NPV was highest at 100%. Accuracy of VIA is 74.0%. Of the 45 VIA positive cases and 19 cases were coploscopically proved to be positive aswell , in which 9 cases of hazy acteo white areas or fine puncatation or mosiacism, 6 cases of dense aceto white areas or coarse punctations or mosaicism and 4 cases of malignancy were found. The malignancy cases had no visible growth on cervix on per speculum examination. Further, among the 45 positive cases of VIA 30 cases were subjected to confirmatory biopsy of which 10cases of mild dysplasia, 6 cases of moderate to severe dysplasia and 4 cases of malignancy were found.

26 cases were falsely positive on VIA. Therefore the cases were selected for biopsy by taking even cytology and colposcopic findings into consideration. Of the 26 cases, biopsy was not done on 15 cases, 1 case was normal on biopsy, 1 case of moderate dysplasia which was not reported by pap smear and the colposcopic findings were suspicious. The rest 9 cases belonged to cervicitis or metaplasia group

Another screening method employed was the toluidine blue dye test used instead of the lugol's iodine in order to find out its efficacy and use as an adjunct to the available screening methods. Of the 100 cases 9 were positive and 91 were negative on toluidine blue dye test. It has the less sensitivity at 42.1%, but specificity is high at 98.8%, the PPV is 88.9% and the NPV 87.9%. Accuracy is high at 88%. Of the 9 positive cases 8 cases 4 cases of dense aceto white area or coarse punctations or

mosaicism and 4 cases of malignancy were found. 1 case was false positive , a case of inflammation or metaplasia or erosion.

All the 9 positive cases were subjected to biopsy, of which 4 cases moderate to severe dysplasia and 4 cases of malignancy were found. 1 case was of cervicitis or metaplasia.

In our study, sensitivity of Pap smear was very low ie 26.3%. This is because 9 cases of hazyaceto white areas/ fine punctations/ mosaicism, 3 cases of dense aceto white areas /coarse punctations/ mosaicism, 2 cases of malignancy were under reported as Inflammatory/ ASCUS. As a screening test, the Pap smear has been found to have a low sensitivity and the low sensitivity rate has been attributed to the presence of infection and inflammation in high number in the developing countires. The specificity of Pap smear is high ie 100%, the PPV was 100% and the NPV was 85.3%. Accuracy of Pap smear was 86%.

In a multicentric study by Sankaranarayanan et. al. showed sensitivity of Pap smear ranging from 36.6% to 72.3% and specificity ranging from 87.2% to 98.6%[10] [54]. In a study conducted by Goel et al the sensitivity of Pap smear was found to be 50% and specificity was 97%[41].

In a comparative study done by Tejaswini. B. H. the Sensitivity of VIA was 95%, Specificity was 55%, PPV of 61%. Out of 210 biopsy positive patients, 100 showed cervical cytology suggestive of precancerous and cancerous lesion. The sensitivity of Pap smear was 43% , Specificity was 97%, PPV of Pap smear was 90%. The overall Accuracy of VIA was 72% and Cervical cytology was 74%.

Positive cases were subjected to biopsy and majority were reported as either normal or chronic cervicitis.

In another study by Divya Hegde, out of 225 patients, VIA was positive in 27(12%) patients and Pap smear was abnormal in 26(11.7%). Pap smear had a sensitivity of 83%, specificity of 98%, PPV of 80 % and NPV of 97.9%. VIA had a sensitivity of 70.8%, specificity of 95%, PPV of 62.9 % and NPV of 96.5%.

In a study done by Afshan, the Sensitivity of Pap smear was found to be 43.2%, high Specificity of 95.2% and the PPV was 84.2%[58]. The results of various studies have been put together for comparison with our study results in the below table .

Table 26 : **Comparison of various study results**

Studies conducted	Sensitivity		Specificity		PPV		Accuracy	
	Pap smear	VIA	Pap smear	VIA	Pap smear	VIA	Pap smear	VIA
Sankaranarayanan et. al[10 55]	36.6% to 72.3%	-	87.2%to 98.6%	-	-	-	-	-
Goel et al[25]	50%	-	97%	-	-	-	-	-
Tejaswini. B. H.[57]	43%	95	97%	55%	90%	61%	74%	72%
Divya Hegde[21]	83%	70.8%	98%	95%	80%	62.9%	-	-
Afshan[58]	43.2%	-	95.2%	-	84.2%	-	-	-
Rana T et.al[11]	83%	93%	97%	90%	83%	62.5%	96%	90%
In our study	26.3%	100%	100%	67.9%	100%	42.2%	86%	74%

The results in various study were found to be comparable with our study. All the 5 positive cases of pap smear were subjected to biopsy on which 2 cases of moderate to severe dysplasia and 3 malignancy cases were found. The high number of malignancy cases in our study is attributed to the judicious method of case selection which has made it imperative in getting high incidence of malignancy cases.

The women with cervicitis/metaplasia in our study were treated with antibiotics. Women with cervical ectopy underwent cryocautery. The cases with malignancies were given option of surgery at our hospital or reference to higher centre for further management. It is evident that VIA had higher sensitivity of 100% as compared to toluidine blue dye test at 42.1% and cytology at 26.3%, the specificity and positive predictive value of Pap smear was high at 100% while the specificity of Toluidine blue dye test was 98.8% and its PPV at 88.9% where as the specificity of VIA was much lower at 67.9% and PPV at 42.2% Hence VIA, Toluidine blue dye test, cytology and Colposcopy and confirmed by biopsy gives reliable results by which, patients of cervical lesions have a relatively higher chance of detection of pre malignant and squamous intraepithelial lesions/malignancy as compared to any procedure when performed alone.

CONCLUSION

Following conclusions were drawn from the present study:

1. The majority of women were in the age group 30-35 years.

2. The commonest symptom was recurrent white discharge per vagina.

3. VIA has high sensitivity but low specificity.

4. Toluidine blue dye test has high specificity and low sensitivity.

5. Pap smear has high specificity but low sensitivity.

6. Colposcopy has high sensitivity and specificity. Hence it is used as gold standard test in the study in detecting premalignant and malignant lesions of the cervix.

5. The specificity and positive predictive value of Pap smear was high and that of toluidine blue dye test was slightly lower but the lowest was of VIA.

6. Accuracy of Toluidine blue dye test was the highest followed by Pap smear compared to VIA which has lower accuracy than the other 2 tests.

7. VIA, toluidine blue dye test and cytology added with gold standard colposcopy and colposcopy guided biopsy, when used together in patients of cervical lesions have a relatively higher chance of detection of squamous intraepithelial lesions/malignancy as compared to either procedure when performed alone.Multiple tests performed yields reliable result in which chance of missing a positive case is minimal hence it is recommended than performing a single test.

SUMMARY

The study was performed on 100 women in the Department of Obstetrics and Gynaecology at Sri B M Patil Medical College & Research Hospital, Bijapur. The objectives of the study was to correlate the findings in women with unhealthy cervix by cytology, visual aided by acetic acid test (VIA), Toluidine blue dye test and to assess the efficacy of all the tests in early detection of the premalignant and malignant lesions of the cervix.

The sensitivity, specificity, positive predictive value, negative predictive value, false negative rate, false positive rate and accuracy of Pap smear, VIA, Toluidine blue test were compared with colposcopy as the gold standard.

- Majority of the women included in the study were 30-35 years of age (48%).
- The commonest symptom was recurrent white discharge per vagina (69%).

VIA was positive in 45 cases and negative in 55 cases out of 100 cases. 19 cases were colposcopy proven positive cases of which 9 cases of hazy acteo white areas or fine puncatation or mosiacism, 6 cases of dense aceto white areas or coarse punctations or mosaicism and 4 cases of malignancy were found. Among the 45 positive cases of VIA 30 cases were subjected to confirmatory biopsy of which 10 cases of mild dysplasia, 6 cases of moderate to severe dysplasia and 4 cases of malignancy were found. Sensitivity was high at 100% and specificity low at 67.9%. PPV was 42.2% and NPV was 100%. Accuracy was 74.0%.

• Pap smear revealed 5 positive cases, 64 had inflammatory smears and 28 cases smears were within normal limits. The sensitivity and specificity of Pap smear was 26.3% and 100% respectively. Accuracy was 86%. Sensitivity was low because 9 cases of hazyaceto white areas/ fine punctations/ mosaicism, 3 cases of dense aceto white areas /coarse punctations/ mosaicism, 2 cases of malignancy were under reported as Inflammatory/ ASCUS. All the 5 positive cases of pap smear were subjected to biopsy on which 2 cases of moderate to severe dysplasia and 3 malignancy cases were found.

Correlation of VIA, Toludine blue dye test, Pap smear revealed that VIA had higher sensitivity at 100% as compared to Toluidine blue dye test and cytology at 42.1% and 26.3% respectively. Cytology had high specificity and positive predictive value at 100% and that of Toluidine blue was 98.8% and 88.9% respectively. VIA specificity was lower at 67.9% and PPV was 42.2%.

Accuracy of Toluidine blue dye test was highest at 88%, cytology at 86% and that of VIA was 74% in our study.

From our study it can be concluded that the chance of missing a premalignant or malignant case while performing a single test can be reduced by applying multiple tests like VIA, Toluidine blue dye test and cytology, which yields reliable results when performed together along with colposcopy, thereby results in early detection of premalignant and malignant lesions of the cervix. Hence multiple tests are recommended wherever the facility is possible.

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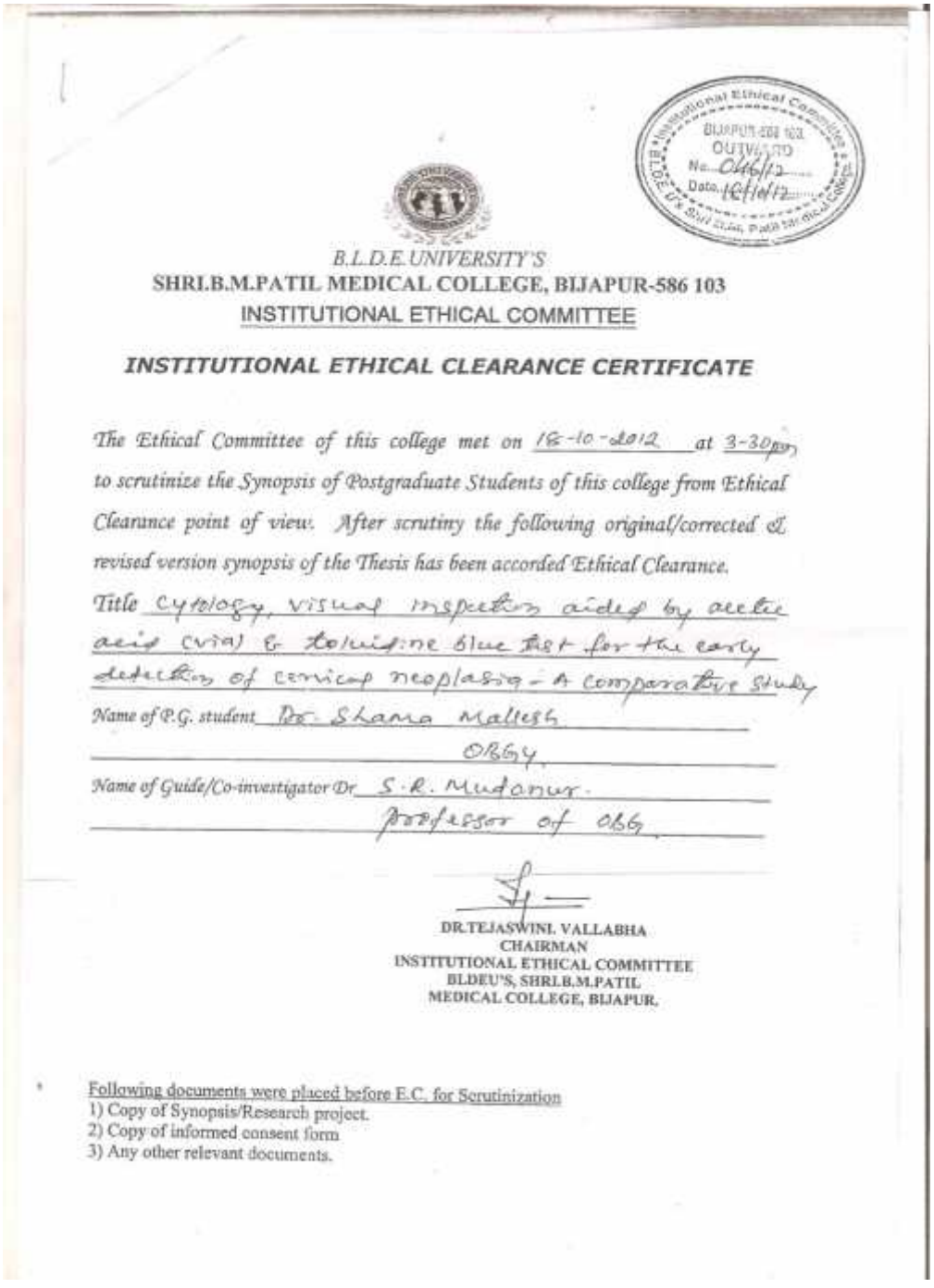
ANNEXURE



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SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 16-10-2012 at 3-30pm to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.

Title Cytology, visual inspection aided by acetic acid (via) & toluidine blue test for the early detection of cervical neoplasia - A comparative study

Name of P.G. student Dr. Shama Mallesh
OBGy.

Name of Guide/Co-investigator Dr S.R. Mudanur.
Professor of OBG.

DR.TEJASWINI. VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

RESEARCH INFORMED CONSENT FORM

TITLE OF THE PROJECT:

"CYTOLOGY, VISUAL INSPECTION AIDED BY ACETIC ACID (VIA) AND TOLUIDENE BLUE TEST FOR THE EARLY DETECTION OF CERVICAL NEOPLASIA" – A COMPARATIVE STUDY.

PG GUIDE'S NAME: DR. (prof) S R MUDANUR

Purpose of Research:

I have been explained about the reason for doing the study and selecting me as a subject of the study. This study is to find the efficacy of Cytology, VIA, Toluidine blue dye Test in detecting the premalignant and malignant lesions of cervix.

Procedure:

I understand that I will be a part of this study. My history and physical findings will be taken from the case paper and will be evaluated in a systematic way.

Risks and Benefits:

Minimal side effects like pain, bleeding and discomfort can occur. I understand that this procedure is not expected to aggravate any side effect or cause detrimental effect to me. By subjecting patient to all the three methods of detection, cervical cancer can be detected in pre cancer stage and can be cured.

Alternatives:

Even if I decline the participation in the study, I will get the routine line of management.

Privacy and Confidentiality:

The only people to know that I, am a research subject are members of the research team. No information about me or information provided by me during the research will be disclosed to other without my written permission except:

1. In emergency to protect my rights and welfare.
2. If required by law

I understand that the medical information produced by this study will become a part of hospital records and will be subject to the confidentiality and privacy regulation of BLDE University's Shri .B. M .Patil Medical college. Information of a sensitive personal nature will not be a part of the medical records, but will be stored in the investigator's research file and identified only by a code number. The code key connecting names to numbers will be kept in a secured location. If the data are used for publication in the medical literature or for teaching purpose no names will be used. I understand that the relevant designated authority and permitted to have an access to my medical record and to the data produced by the study for audit purpose. However, they are required to maintain confidentiality.

Authorization to Publish Results:

When the results of the research are published or discussed, in a conference,

no information will be displayed that would disclose my identity. Any information that is obtained in connection with this study and that can be identified with me will remain confidential.

Refusal or Withdrawal of Participation:

I understand that my participation is voluntary and I may refuse to participate

or my withdraw consent and discontinue participation in the study at any time without prejudice to my present or future care at this hospital. I also understand that researcher may terminate my participation in the study at any time after I have been explained the reasons for doing so and has been helped to arrange for my continued care by my own physician, if this is appropriate.

Request for More Information:

I understand that, I may ask more questions about the study at any time. Researcher is available to answer my questions or concern in this research period. I understand that I will be informed of any significant new findings discovered during the course of this study, which might influence my continued participation.

STUDY SUBJECT CONSENT STATEMENT:

I confirm that Dr Shama Mallesh has explained to me the purpose of research, the study procedure, that I will undergo and the possible discomforts as well as benefits that I may experience in my own language. I have been explained all the above in detail in my own language and I understand the same . Therefore I agree to give consent to participate as a subject in this research project.

_____ _____

(Participant) Date

_____ _____

(witness to signature) Date

PROFORMA

Name of the participant :

OP No. :

Husbands Name :

Age :

Socio economic status :

Residential Address :

Phone No. :

History :

Chief complaints

- Intermenstrual bleeding Yes / No
- Other complaints (pain abdomen.backache) Yes/No
- Post coital bleeding Yes / No
- Post menopausal bleeding Yes / No
- Recurrent episodes of white discharge per vagian Yes / No
    - Discharge Profuse
    - Yellowish
    - Blood stained
    - Dirty brown discharge
    - Foul offensive odour.

Menstrual History : Age of Menarche:

Past Menstrual Cycle :

Present Menstrual Cycle : LMP-

Obstetric History : ML-

Obstetric score :-

Contraception using OC pills : Yes / No

Sterilization

Past History : H/o DM, HTN, TB, Previous surgeries & H/o STDs

Family History : H/o DM, HTN and TB in the family.

Personal History :

Smoking : Yes / No

Diet

General Physical Examination:

- BP
- PR
- Pallor
- Pedal edema

Systemic Examination:

- CVS :
- RS :
- P/A :
- P/S
- P/V

Investigations :

Hb% ;

Urine Routine :

RBS :

HBs Ag :

RVD :

USG :

Pap smear :

VIA :

Toludine blue test :

Colposcopy report :

Colposcopy guided biopsy report:

REMARKS:

KEY TO MASTER CHART

UID	-	Unique identity of the patient (OP/IP Number)
WD	-	White discharge
IMB	-	Intermenstrual bleeding
PMB	-	Postmenopausal bleeding
PCB	-	Postcoital bleeding
OTHERS	-	Pain abdomen,backache
VIA	-	Visual inspection by Acetic acid
P	-	Positive
N	-	Negative
Toluidine Blue Test		Results
Pap smear results		(PAPS)
ASCUS	-	Atypical squamous cells of unsignificance
N	-	Normal
INF	-	Inflammatory
LSIL	-	Low grade Squamous Intraepithelial Lesions
HSIL	-	High grade Squamous Intraepithelial Lesions
BV	-	Bacterial vaginosis
Colposcopy results		
Normal		
INF	-	Inflammation
Squamous metaplasia		(Sq. Metaplasia)
Cervicitis		
TZ	-	Transformation Zone
Punctations	-	Fine

Coarse

Mosaic pattern

Acetowhite areas (A-W) - Hazy/Faint

Dense

Malignancy

Unsatisfactory

Biopsy results

Normal

Cervicitis

Metaplasia

Mild dysplasia

Moderate/Severe dysplasia

SCC- Squamous Cell Carcinoma

MASTER CHART

Sl no	UID (OP/IP no)	Age	Parity	Inclusion criteria	VIA	TBDT	PAPS	Colposcopy	Biopsy
1	236064	33	P3L3	WD	P	N	Chronic cervicitis	Hazy aceto white areas	Mild dyplasia
2	21294	50	P4L3D1	WD	P	N	Chronic cervicitis	Hazy aceto white areas	Mild dysplasia
3	244319	41	P3L3	WD	P	N	Chronic cervicitis	Inflammation	Cervicitis
4	183055	30	P2L2A1	WD	P	N	Chronic cervicitis	Erosion	Cervicitis
5	100213	38	P5L5	WD	P	N	INF	Erosion	Cervicitis
6	100322	35	P2L2	WD	N	N	Normal	Normal cervix	Not done

7	103825	60	P2L2	WD	P	P	ASCUS	Intense aceto white areas	SCC
8	108778	38	P3L3	WD	N	N	INF	INF	Not done
9	299833	35	P3L1D2	Others-pain abdomen	P	N	Chronic cervicitis	Erosion	Cervicitis
10	112267	35	P2L2	WD	N	N	Normal study	Normal cervix	Not done
11	96413	59	P4L4	WD	N	N	Trichomoniasis	INF	Not done
12	9289	45	P7L7	WD	N	N	Normal	Normal study	Not done
13	111725	38	P2L2	WD	N	N	Candidiasis	Normal cervix	Not done
14	10287	65	P6L6	PMB-post menopausal bleeding	P	P	HSIL	Intense aceto white area with coarse punctations	SCC
15	115918	37	P2L2	WD	P	N	Acute cervicitis	Hazy aceto white areas	Mild dyplasia

16	11044	60	P5L5	Others-backache	P	N	Chronic cervicitis	INF	Metaplasia
17	119364	30	P2L2A2	WD	N	N	Chronic cervicitis	INF	Not done
18	11105	35	P2L2	WD	N	N	Normal smear	Normal cervix	Not done
19	123725	35	P3L3	IMB	P	P	BV	Dense aceto white area	Mod dysplasia
20	123225	52	P4L3D1	WD	P	N	BV	INF	Normal
21	11901	30	P5L4D1	WD	N	N	INF	INF	Not done
22	11834	38	P3L3	Others-pain abdomen	N	N	INF	INF	Not done
23	109593	65	P6L6	Others-backache	N	N	Atrophic vaginitis	Normal menopausal cervix	Normal
24	12478	35	P3L3	PCB-post coital bleeding	P	P	ASCUS	Dense aceto white area	Mod dysplasia

25	137269	30	P3L3	WD	N	N	Trichomoniasis	INF	Not done
26	12970	50	P5L4D1	WD	P	N	INF	INF	Metaplasia
27	115026	42	P3L3	WD	P	P	ASCUS	Intense aceto white area	Severe dysplasia
28	150897	35	P3L3	WD	N	N	Acute cervicitis	Erosion	Not done
29	150041	34	P4L4	PCB	N	N	Acute cervicitis	Squamous metaplasia	Not done
30	150030	42	P5L4D1	WD	P	P	Acute cervicitis	INF	Cervicitis
31	14175	31	P3L3A1	WD	P	N	INF	Hazy aceto white area	Mild dysplasia
32	14906	38	P3L3	WD	P	N	BV	Hazy aceto white areas	Mild dysplasia
33	161460	33	P1L1	Others-pain abdomen	N	N	Normal	Normal	Not done

34	183055	38	P2L2A1	Post coital bleeding	P	N	INF	INF	Not done
35	14058	40	P7L6D1	PCB-post coital bleeding	P	N	INF with mild atypia	Squamous metaplasia	Metaplasia
36	161450	33	P2L2A1	WD	N	N	Normal	INF	Not done
37	162055	45	P4L4	WD	N	N	Normal	INF	Not done
38	174854	38	P2L2A1	WD	N	N	Normal	Normal	Normal
39	170901	40	P3L3	Others-pain abdomen	N	N	Acute cervicitis	Normal	Not done
40	170412	46	P2L2	WD	N	N	Normal	INF	Not done
41	180010	32	P3L3	WD	P	N	BV	INF	Not done
42	181427	32	P1L1	WD	N	N	Acute cervicitis	Erosion	Not done
43	181803	32	P2L2	WD	N	N	Normal	Normal	Not done
44	181470	35	P2L2D1	Others-pain abdomen	N	N	Normal	INF	Not done

45	186628	33	P1L1	Others-pain abdomen	N	N	Acute cervicitis	INF	Not done
46	183146	50	P4L4	WD	N	N	Atropic smear	Normal	Not done
47	183339	40	P3L3	WD	P	N	Candidiasis	Erosion	Not done
48	17629	32	P5L4D1	WD	P	N	Candidiasis	Erosion	Not done
49	191332	41	P4L3D1 A1	Others-pain abdomen	N	N	Acute cervicitis	INF	Not done
50	17214	58	P3L2D1	Others-pain abdomen	P	N	Acute cervicitis	Erosion	Not done
51	191320	37	P3L3	PCB	P	N	Acute cervicitis	Erosion	Not done
52	195772	48	P3L3	WD	N	N	Acute cervicitis	INF	Not done
53	195454	30	P2L2	Others-pain abdomen	N	N	Normal	Normal	Not done
54	199099	35	P2L2	WD	N	N	Normal	Normal	Normal

55	214722	37	P2L2	WD	P	N	Acute cervicitis	Squamous metaplasia	Not done
56	215192	46	P1L1	WD	P	N	Acute cervicitis	INF	Not done
57	225501	39	P4L4	WD	N	N	Normal	Normal	Not done
58	228217	35	P4L4	WD	P	N	BV	INF	Not done
59	228031	30	P1L1A1	WD	N	N	Acute cervicitis	INF	Not done
60	227163	30	P3L2D1	WD	P	N	Candidiasis	Erosion	Not done
61	229108	36	P2L2	WD	N	N	Acute cervicitis	Normal	Not done
62	229000	30	P2L2	WD	N	N	BV	Normal	Not done
63	233514	36	P3L3	WD	P	N	Acute cervicitis	Erosion	Mild dysplasia
64	233433	30	P2L2A3	WD	P	N	Acute cervicitis	Hazy aceto white areas	Mild dysplasia
65	233252	33	P2L2	WD	P	N	Acute cervictis	Hazy aceto white areas	Mild dysplasia

66	235646	36	P1L1	WD	N	N	BV	Normal	Not done
67	236146	33	P2L2	WD	N	N	Normal	Normal	Not done
68	239669	33	P3L3	Others-pain abdomen	N	N	Normal	Normal	Not done
69	239576	39	P2L2	Others-pain abdomen	N	N	Normal	Normal	Not done
70	241925	30	P1L1	WD	N	N	Normal	Normal	Not done
71	22486	35	P4L4	WD	P	N	Acute cervictis	Hazy aceto white areas	Mild dysplasia
72	249433	30	P1L1A1	Others-bckache	N	N	Normal	Normal	Not done
73	251099	34	P2L2	Others-pain abdomen	N	N	Normal	Normal	Not done
74	250878	48	P3L3	WD	N	N	Normal	Normal	Not done
75	252074	30	P1L1	Others-backache	N	N	Normal	Normal	Not done

76	251807	30	P2L2	Others-backache	N	N	Normal	Normal	Not done
77	255068	45	P4L4A1	WD	P	N	INF atypia	Squamous metaplasia	Metaplasia
78	22795	34	P3L3	WD	N	N	Normal	INF	Not done
79	23110	35	P3L3	PCB	N	N	INF	Erosion	Not done
80	259740	40	P4L4	Others-backache	N	N	Normal	Erosion	Not done
81	251286	32	P4L4	WD	N	N	Normal	Normal	Not done
82	23069	38	P3L3	WD	N	N	Normal	Normal	Not done
83	265506	50	P5L5A2	WD	P	N	HSIL	Dense aceto white area	Mod dysplasia
84	24260	50	P6L5D1	WD	P	P	HSIL	Coarse irregular punctations	SCC
85	273878	40	P2L2A1	WD	P	N	BV	Dense aceto white areas	Mod dysplasia

86	274729	30	P2L3	WD	P	N	INF	Squamous metaplasia	Not done
87	21042	60	P8L8	PMB	P	P	HSIL	Intense aceto white areas	SCC
88	24577	50	P5L5	PMB	P	P	LSIL	Dense aceto white areas	Mod dysplasia
89	277807	30	P1L1	WD	P	N	INF	Squamous metaplasia	Not done
90	278467	35	P1L1	WD	N	N	Acute cervicitis	Erosion	Not done
91	278334	30	P4L4	WD	P	N	INF	Erosion	Not done
92	282874	36	P3L2D1	WD	N	N	INF	INF	Not done
93	283033	30	P1L1	IMB	N	N	INF	INF	Not done
94	283521	38	P3L2D1	WD	P	N	INF	Erosion	Not done

95	282971	34	P4L3D1 A1	WD	P	N	Acute cervicitis	Squamous metaplasia	Not done
96	283522	42	P3L3	WD	N	N	INF	INF	Not done
97	283036	39	P4L2D2	Others-pain abdomen	P	N	INF	Hazy aceto white areas	Mild dysplasia
98	283317	38	P3L3	IMB	N	N	INF	INF	Not done
99	294429	40	P3L3	WD	N	N	INF	INF	Not done
100	285249	30	P3L2D1	WD	N	N	Normal	Normal	Not done