



Abnormal pap smear detection by application of revised Bethesda system in commercial sex workers and a control group – A comparative study.

KEYWORDS

Cervical cancer, papanicolaou (pap) smear, Bethesda system.

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ABSTRACT

Background – Cervical cancer is a major public health hurdle in the area of women's health. The most common cause of cervical cancer is the human papilloma virus (HPV). Human papilloma virus has various genotypes, with HPV 16 and HPV 18 being the major etiological factor causing carcinoma of the cervix. Early screening and detection by papanicolaou smears (PAP) is an effective method for identifying premalignant and malignant lesions. In case of existing pre- malignant lesions /cervical dysplasia's found with HPV 16 or 18, appropriate follow up can be done to prevent it from developing into a neoplasm.

Aims and Objectives –

1) Primary aim - To study various abnormal cervical cytology reports as detected by pap smear tests, using the Bethesda system in women at a tertiary care hospital

2) Secondary aim - To discuss the importance of pap smear in cervical cancer screening program.

Materials and Methods - Our study is a prospective study, based on 101 women who attended the out-patient department of obstetrics and gynecology at a tertiary care hospital in age group 20-40 years with chief complaints of white/foul vaginal discharge, post-coital bleeding, low back pain, irregular menstruation etc. 60 women, who were tested, of the total no of women, were commercial sex workers, thus being a high risk group for HPV infection. All women underwent conventional cytology. For all the abnormal smears, further cervical biopsies were done and final diagnosis was done on the basis of histopathology (gold standard).

Results – In all these patients, 16 patients presented with normal smears out of which 2 belonged to the category of commercial sex workers (3.33%) and 14 being from the normal/control group (34.15%). 44 women presented with inflammatory smears out of which 30 were commercial sex workers (50%) and 14 from the control group (34.15%). A total of 11 women presented with infectious etiology with 6 being commercial sex workers (10%) and 5 (12.2%) being in the control group. A total of 8 patients presented with low grade squamous intra epithelial lesion (LSIL) with 7 (11.7%) being commercial sex workers and 1 (2.44%) patient belonging to the control group. A Total of 7 patients presented with high grade squamous intraepithelial lesion (HSIL) with 6 (10%) being commercial sex workers and 1 (2.44%) belonging to the control group. 9 patients in total presented with atypical squamous cells of undetermined significance (ASCUS) with 6 (10%) being commercial sex workers and 3 (7.32%) belonging to the control group. Squamous cell carcinoma (SCC) presence was found only in 1 (1.7%) commercial sex worker.

Conclusion – We conclude that HSIL, LSIL, SCC and sexually related infections are comparatively more common in vulnerable groups such as sex workers due to a variety of factors such as multiple sexual partners and poor genital hygiene. Early screening and follow up interventions are highly needed for them along with health education for risk factors and to emphasize on the importance of pap smear screening.

Introduction –

Cervical cancer is a leading public health threat in the field of gynecological oncology. Cancer of the cervix is a leading site of malignancy following cancer of the breast. Infection with oncogenic human papilloma virus (HPV) is known to cause carcinoma of the cervix. HPV has various sub-types and HPV 16, 18 are known to cause cervical cancer. Cervical cancer is the second most common cancer among women worldwide with an estimated 527,624 new cases and 265,653 deaths with overall incidence: mortality ratio of 52 percent.⁽¹⁾ In a developing nation like India, cancer of the cervix is the second most common number one killer among women. It is estimated that during 2012, 122,644 new cases of cancer cervix occurred in the country, with an incidence rate of 22 per lakh population and about 67,477 women died of the disease, mortality rate of 12.4 per lakh population.⁽²⁾ It comes to 20.7 percent of cancer deaths in women and 9.9 of total cancer deaths in the country.⁽³⁾ The symptoms of carcinoma of the cervix could range from whitish and foul smelling vaginal discharge, post coital bleeding, intermenstrual bleeding, and pain in the lower abdomen. The various risk factors associated with cancer of the cervix are age group 25-45, early marriage, early coitus, early childbirth with poor birth spacing and multiparity, multiple sex partners, drug abuse and smoking, the use of oral/hormonal contraceptive pills, low socio-economic class and poor genital hygiene and immunocompromised patients (HIV positive). Early screening for cervical cancer has become accepted globally and is known to reduce the global cervical cancer burden. The pap smear test helps detect various abnormalities of cervical cytology and pre-

cancerous and cancerous lesions. current policy states that all women should have a pap test (cervical smear) at the beginning of sexual activity and then every 3 years recommended.⁽⁴⁾ Preventive oncology focuses on early screening of cancers, and thus pap smear is an important diagnostic tool to detect any abnormal cervical cytology which could be an underlying premalignant or malignant lesion. If diagnosed early and treated accurately further progression to cervical carcinoma can be inhibited. The revised Bethesda system is a standard nomenclature used for studying abnormal cervical cytology and various conditions such as infections such as trichomonas vaginalis, candida, bacterial infections etc. . In addition it is also useful for the study of epithelial cell disorders, cervical dysplasia's, premalignant conditions such as Low squamous intra-epithelial lesion (LSIL), High squamous intra-epithelial lesion (HSIL), Atypical squamous cells of undetermined significance (ASCUS), Squamous cell carcinoma (SCC) and many more.

Materials and Methods –

This study was conducted on 101 pap smears from patients who presented with erosion, chronic cervicitis, whitish /foul smelling vaginal discharge, post-coital bleeding, low back pain, irregular menstruation. Age group of the women was 20-40 years. Out of the total women tested, 60 were commercial sex workers, making them a high risk group for cervical cancer and HPV infection due to multiple sex partners. Post history taking and clinical examination, Pap smear was taken for cervical cytology. For detection of cervical cytology, a medscand sampling kit was used with patients being told to lie in a dorsal

position and speculum being inserted vaginally to expose the cervix. Endo-cervical sampling using a cytobrush rotated to 180 degrees was done After fixation and staining with papanicolou , each smear was carefully studied. Reporting of the slides was done as per the revised Bethesda system.

Results –

Our study was carried out over a period of one year with the age group of patients being aged 20-40. A total of 101 smears were studied and interpreted in this period, as per the revised Bethesda system for reporting cervical cytology abnormalities. Out of the 101 women, 60 were commercial sex workers thereby forming a vulnerable group for cervical cancer /HPV infection due to multiple sex partners and early coitus.

The most common findings on cervical examination were as follows -

Table 1 – Findings on cervical examination

Condition	No. Of Cases
Cervical erosion	34
Chronic cervicitis	71
Hypertrophied cervix	19
Cervix bleeds to touch	12

Cervical erosion (33.67 %), chronic cervicitis (70.3%), hypertrophied cervix (18.8%), cervix bleeds to touch (11.9%) with common symptoms being vaginal discharge and irregular bleeding.

In all these patients, 16 patients presented with normal smears out of which 2 belonged to the category of commercial sex workers (3.33%) and 14 being from the normal/control group (34.15%). 44 women presented with inflammatory smears out of which 30 were commercial sex workers (50%) and 14 from the control group (34.15%). A total of 11 women presented with infectious etiology with 6 being commercial sex workers (10%) and 5 (12.2%) being in the control group. A total of 8 patients presented with LSIL with 7 (11.7%) being commercial sex workers and 1(2.44%) patient belonging to the control group. A Total of 7 patients presented with HSIL with 6 (10%) being commercial sex workers and 1 (2.44%) belonging to the control group. 9 patients in total presented with atypical squamous cells of undetermined significance with 6(10%) being commercial sex workers and 3 (7.32%) belonging to the control group. SCC presence was found only in 1(1.7%) commercial sex worker. 5 patients presented with unsatisfactory smear with the division ratio being 2 (3.33%) in commercial sex workers and 3 (7.32%) in the control group. Thereby, a total of 58(96.7%) smears from the commercial sex workers group were satisfactory and 38 (92.7%) from the control group were satisfactory.

Table 2 – Cervical cytology in Commercial sex workers and Control group

Pap-Cytology	Commercial Sex Workers	Control Group
Normal	2(3.33%)	14(34.15%)
Inflammatory	30(50%)	14(34.15%)
Infectious etiology	6(10%)	5(12.2%)
LSIL	7(11.7%)	1(2.44%)
HSIL	6(10%)	1(2.44%)
ASCUS	6(10%)	3(7.32%)
Squamous cell carcinoma	1(1.7%)	0
Unsatisfactory	2(3.33%)	3(7.32%)
Satisfactory	58(96.7%)	38(92.7%)

Specimen Adequacy:

- 1)Satisfactory smears – Presence of endo-cervical /transformation zone component.
- 2)Unsatisfactory smears – Absence of endo-cervical/transformation zone component. Specimen rejected and not processed because specimen was not labeled.

The sensitivity and specificity of the specimens was as follows – Sensitivity refers to the test's ability to correctly detect patients who have the concerned disease/illness.

$$\begin{aligned} \text{sensitivity} &= \frac{\text{number of true positives}}{\text{number of true positives} + \text{number of false negatives}} \\ &= \frac{\text{number of true positives}}{\text{total number of sick individuals in population}} \\ &= \text{probability of a positive test given that the patient has the disease} \end{aligned}$$

Sensitivity in context however differs from precision or positive predictive value which is the ratio of true positives to combined true and false positives and is a conclusive statement about the proportion of actual positives in the population being tested as it is about the test.

Specificity

Specificity relates to the test's ability to correctly detect patients without the concerned diseased condition like a medical test for diagnosing a disease.

$$\begin{aligned} \text{specificity} &= \frac{\text{number of true negatives}}{\text{number of true negatives} + \text{number of false positives}} \\ &= \frac{\text{number of true negatives}}{\text{total number of well individuals in population}} \\ &= \text{probability of a negative test given that the patient is well} \end{aligned}$$

A positive result in a test with high specificity is useful for ruling in disease. The test rarely gives positive results in healthy patients. A test with 100% specificity will read negative, and accurately exclude disease from all healthy patients. A positive result signifies a high probability of the presence of disease.

Positive predictive value

The positive predictive value (PPV) is defined as

$$\text{PPV} = \frac{\text{number of true positives}}{\text{number of true positives} + \text{number of false positives}} = \frac{\text{number of true positives}}{\text{number of positive calls}}$$

Where a "true positive" is the event that the test makes a positive prediction, and the subject has a positive result under the gold standard, and a "false positive" is the event that the test makes a positive prediction, and the subject has a negative result under the gold standard.

Negative predictive value

The negative predictive value is defined as:

$$\text{NPV} = \frac{\text{number of true negatives}}{\text{number of true negatives} + \text{number of false negatives}} = \frac{\text{number of true negatives}}{\text{number of negative calls}}$$

Where a "true negative" is the event that the test makes a negative prediction, and the subject has a negative result under the gold standard, and a "false negative" is the event that the test makes a negative prediction, and the subject has a positive result under the gold standard.

Diagnostic Efficiency –

Diagnostic efficiency of a test can be calculated as well using the 2x2 table. With total diagnostic accuracy, we are comparing the "true" findings (true-positive and true-negative) to all possible diagnostic findings (true-positive, true-negative, false-positive, and false-negative.

$$\text{Diagnostic Efficiency} = \frac{\text{True Positive} + \text{True Negative}}{\text{True Positive} + \text{False Positive} + \text{True Negative} + \text{False Negative}}$$

Table 3 - Diagnosis by Histo-Pathology (Gold Standard)

Pap Smear	Positive	Negative
Positive	14	6
Negative	2	38
Total	16	44

Table 4- Showing sensitivity, specificity, PPV and NPV in comparison

Pap Comparison	Sensitivity	Specificity	PPV	NPV	Diagnostic Efficiency
InduVerma et al	78.57%	88.67%	64.71%	94%	81.15%
Our Study	87.5%	86.4%	70%	95%	86.7%

Table 5 – Comparison of epithelial cell abnormalities as per revised Bethesda System along with other studies.

Study	HSIL	LSIL	SCC
Bal MS et al (2012)	0.7	2.7	1.3
Altaf FJ and Mufti ST (2012)	0.9	2.7	0.06
Mulazim et al(2012)	2.2	4.6	1.4
InduVerma et al (2014)	0.8	5.6	0.8
Our Study	6 (10%)	7(11.7%)	1(1.7%)
1)Commercial Sex Workers	1(2.44%)	1(2.44%)	0
2)Normal/Control Group			

Discussion –

Carcinoma of the cervix is preventable with early screening, diagnostic and therapeutic procedures being taken into consideration. Preventive oncology focuses on early screening and intervention to detect any pre malignant lesion and curb further progression. The papanicolaou test, commonly known as pap smear is used world- wide for early screening for cervical cancer and has lead to a decline in the global ratio of cervical cancer morbidity and mortality. The pap smear is a cost effective and valuable tool for cervical cytology and diagnosis. The USPSTF recommends screening for cervical cancer in women aged 21 to 65 years with cytology (papanicolaou smear) every 3 years or, for women aged 30 to 65 years who want to lengthen the screening interval, screening with a combination of cytology and HPV testing every 5 years .⁽⁵⁾ Early screening should thus be emphasized globally, especially in low resource settings and vulnerable groups such as commercial sex workers. Introduction of screening to populations naive to screening reduces cervical cancer rates by 60% to 90% within 3 years of implementation.⁽⁶⁾

The bethesda system is accepted globally for reporting cervical cytology in an unhealthy cervix. The bethesda system thereby focuses on –

- 1) The need for a standard system of nomenclature among various diagnostic laboratories.
- 2) Criteria for specimen adequacy
- 3) Further evaluation of the system if indicated.

Our study was conducted on 101 women attending the outpatient department of a tertiary care hospital in Navi Mumbai. A detailed history was taken and most of them presented with common symptoms such as vaginal/foul smelling discharge, irregular menstruation, painful coitus, low back ache etc.

Out of the 101 women, 60 were commercial sex workers thus forming a highly vulnerable group for cervical cancer and papilloma virus infection while the rest 41 formed the control group. The age group of patients included in the study was between 20-40 years. Majority of the patients belonged to low and middle socio- economic class as per the modified kuppusswamy scale. Conventional cervical cytology was done on all women. A total of 58(96.7%) smears were found satisfactory from the vulnerable group(commercial sex workers) thereby showing the presence of endo-cervical/transformation zone component whereas 38(92.7%) smears from the control group were satisfactory showing presence of endo- cervical/transformation zone component. For confirmation of the cytology report all women underwent colposcopic examination and biopsies were taken in those with abnormalities. Final diagnosis was made according to the gold standard (histopathology) reports.

InduVerma et al⁽⁷⁾ found overall sensitivity 87.5%, specificity 86.4%, positive predictive value 70%, negative predictive value 95% and diagnostic efficiency 86.7%. In comparison to InduVerma, we found relatively similar findings with overall sensitivity, specificity, Positive predictive value, negative predictive value and diagnostic efficiency of our study being 78.57%, 88.67%, 64.71%, 94%, 81.15% respectively.

In our study epithelial cell abnormalities as per revised Bethesda system were such that HSIL was seen in 6(10%) of sex workers, LSIL in 7 (11.7%) and SCC in 1(1.7%). In the control group HSIL was seen in 1 (2.44%), LSIL in 1(2.44%) and none had SCC. In comparison to our study, Bal MS et al⁽⁸⁾ found HSIL, LSIL, SCC ratio as 0.7, 2.7,1.3 respectively. Altaf FJ et al⁽⁹⁾ found HSIL, LSIL, SCC to be 0.9 , 2.7, 0.06 respectively. Mulazim et al⁽¹⁰⁾ found the same to be 2.2,4.6,1.4 respectively. Indu Verma et al⁽⁷⁾ reported 0.8, 5.6 and 0.8 respectively for the same.

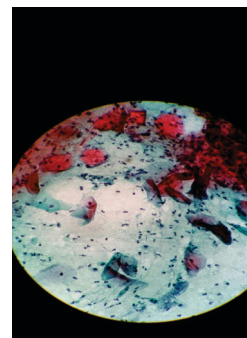
Pap smear and annual cervical cancer screening is thus highly beneficial to detect various abnormalities. However a high rate of false negative smears and of atypical squamous cells diagnoses have led to the development of new diagnostic techniques.⁽¹¹⁾

Conclusion –

Women who have unhealthy cervix on examination need cervical cytology evaluation to help detect and pre- malignant and malignant lesion of the cervix. Early screening for the same should be emphasized upon especially for women in low income/resource settings and vulnerable groups such as commercial sex workers. The bethesda system is accepted globally for reporting cervical cytology and also lays down the need of a standard system of nomenclature for diagnostic laboratories along with criteria for specimen adequacy and further evaluation. Various factors taken into consideration such as ensuring a proper technique for sample selection accompanied by long term measures implemented at base level to improve compliance with cervical cancer screening, health care advocacy and screening for unscreened, low resource and vulnerable population can surely go a long way in improving the detection of cervical cancer and its precursors such as cervical dysplasia. It is widely accepted that detection and treatment of HPV-related dysplastic epithelial change in the form of CIN-2 and CIN-3 can prevent the development of invasive cervical cancer in individual patients.⁽¹²⁾

In addition, post the above measures the concerned doctor should ensure that the treatment of the condition follows acceptable guidelines emphasizing on cytologic, colposcopic and histologic correlation.

We conclude that HSIL, LSIL, SCC and sexually related infections are comparatively more common in vulnerable groups such as sex workers due to a variety of factors such as multiple sexual partners and poor genital hygiene. Early screening and follow up interventions are highly needed for them along with health education for risk factors and to emphasize on the importance of pap smear screening.

**Figure 1 - Inflammatory Smear**

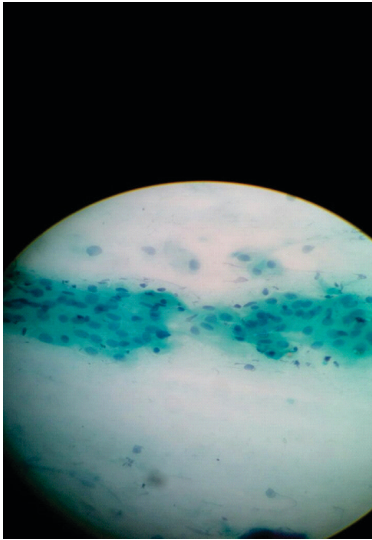


Figure 2 - High Squamous Intra-Epithelial Lesion on Pap Smear

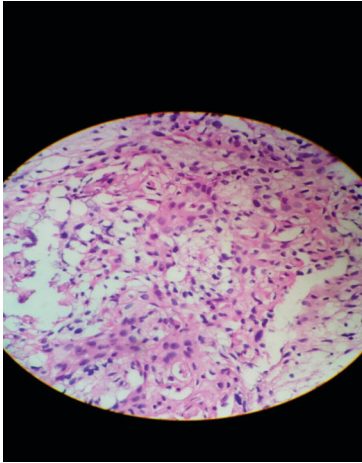


Figure 3 - Squamous Cell Carcinoma on Histopathology

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