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Cervicalcytopathology: Evaluation of its Efficacy in Detecting Cervical Precancerous and Cancerous Lesions, As Evidenced by Colposcopic Biopsy

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Abstract: ***Aim: To correlate the cytological findings with that of the histopathological findings in preinvasive and invasive cancerous lesions of the cervix and to assess the accuracy of the paptest as a screening procedure for cervical cancer. Materials and Methods : The present study was conducted at MAHARAJAH's INSTITUTE OF MEDICAL SCIENCES, in the Outpatient department of OBSTETRICS AND GYNAECOLOGY, over a period of 2 years, from JUNE 2010 – JULY 2012. Results: Of the 500 subjects studied, biopsy was normal in 394 (78.8%) women i.e, they had either an inflammatory smear or a normal smear.106 women (21.2%) had a positive biopsy, i.e., they had a premalignant or malignant lesion on biopsy.Of those with the disease,67 women had CIN I. (13.4%),19 women had CIN II (3.8%),20 women had malignancy on biopsy.(4%) Conclusion: Even though the sensitivity of pap seems to be low, this can be overcome by a regular programme of papsmear screening with appropriate follow up. The regimen of repeated cytologic screenings, and follow up of abnormal results not only increases the sensitivity of Pap smear but also decreases the incidence of cervical cancer. Papsmear is an often cited example of a successful program of secondary prevention. Pap smear, a relatively simple and inexpensive procedure, is still the initial step of cervical cancer screening of women.***

Keywords: Cervical Precancerous, Cancerous Lesions, Colposcopic Biopsy. Pap Smear

1. Introduction

Cancer cervix, a preventable disease continues to be a cause of great concern to women's health, being associated with agonizing morbidity and high mortality. Approximately 493,100 new cases and more than 273,000 deaths occur each year, among women worldwide. All over the world cervical cancer is the second most common cancer in women, after breast cancer and in developing countries it is considered as the most fatal malignancy in women.

Incidence of cervical cancer in developed and developing countries is 83,000 and 409,000 respectively. Number of deaths due to cervical cancer in developed and developing countries are 40,000 and 234,000 respectively. In Africa, its estimated that there are 36,900 new cases each year. In South Africa 32.7% of all cancers are seen in black women.

India, which accounts for one sixth of the World's population also bears one fifth of the World's burden of cervical cancer. It is the most common cancer among women in India. There are approximately 130,000 new cases of cervical cancer in India per year and the disease is reported to be responsible for almost 20% of all female deaths.

India's cervical cancer age standardized incidence rate (30.7/100,000) and age standardised mortality rate (17.4/100,000) are the highest in South Central Asia. Data from Mumbai, suggest that there may have been a slight decline in cervical cancer incidence in recent years. However the absolute incidence is still very high especially in rural areas where cancer cervix accounts for more than half of cancers among women and the number of cases grows due to high population rate.

Simultaneously, there is also evidence that India is on the verge of a HIV epidemic. The Indian National Aids Control Organisation estimates that the number of people living with HIV is approximately 5.1million (38% of whom were women). This suggests cause for concern 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.

Global evidence demonstrates that the key to reducing cervical cancer morbidity and mortality is early detection coupled with timely treatment of cervical pre cancerous lesions. Cervical cytology, often referred to as Pap smear, is perhaps the most well known of available screening methods.

Cytological screening was recommended as part of routine medical examination in gynaecological practice in context of health maintenance program, with the set goal of health for all by 2000A.D.

Developed countries like USA have witnessed a marked decline in the incidence of invasive cancer from 44 cases/100,000 in 1947 to fewer than 8/100,000 in 2002. Much of the credit for this decline goes to detection of pre invasive disease, by organised Pap smear Screening Program.

While evidence of effective screening programs can be seen throughout the developed World, the burden and impact of

the disease remains high in developing countries where 85% of disease related deaths occur.

Similar screening program was introduced in India in the 1950s. Cytological services in India are still a luxury. Though in existence for over half a century, these services are not even touching the fringe of the problem that they are meant to solve, namely the problem of cancer cervix. Although performing a Pap test may seem relatively simple, from both a clinical and programmatic perspective, a large number of steps are required to take an adequate smear, process and analyse the specimen and inform patients of results. If any of these steps are unreliable or logistically burdensome, the entire screening program could break down and with it, the potential for any public health benefit. Unfortunately many, if not all of these steps can be problematic in many developing countries. For example, whatever cytology screening services that do exist in such resource limited settings are usually offered in urban settings, by a small private sector or at referral centres. And even in these settings, trained cytotechnicians and cytopathologists are scarce and turn around times for processing and reading specimens are slow. Such screening programmes requires systems for transportations, communications and follow up that are beyond the capacity of health care infrastructure in most less developed countries. Lack of political will, poor organisational backup, financial constrains and priority given to other health issues like population explosions, ignorance of masses about the diseases and consequences are other important reasons for setback.

This lead to a gloomy picture that today India bears 18% of the burden of invasive cancer cervix in the world and 80-85% of the cases are detected in advanced stage III and IV, when it is too late for treatment.

WHO states that cancer cervix is a totally preventable disease. Preventable but not prevented is the glaring reality in India. Given this reality and the fact that screening by Pap smear (cytological) does have pitfalls and the fact that burden of disease is highest in low resource settings, if screening and subsequent treatment is to have a measurable affect on the burden of disease borne by women and the health care systems, a regular screening with Pap smear and proper follow up is the best approach.

The present study was undertaken to assess the sensitivity and specificity of Pap smear in picking up pre-invasive or invasive cancer in an abnormal cervix by taking the histopathology as the reference standard.

2. Materials and Methods

Source of Data
The present study was conducted at MAHARAJAH's INSTITUTE OF MEDICAL SCIENCES, in the Outpatient department of OBSTETRICS AND GYNAECOLOGY, over a period of 2 years, from JUNE 2010 – JULY 2012. The patients for the study were consenting women, according to the inclusion and exclusion criteria.

Sample Size
The study includes 500 women.

Study Design
It is a hospital based prospective analytical study, conducted on 500 gynaecological patients, to evaluate and compare Papsmear with cervical biopsy.

All the women falling into the eligible group were subjected to papsmear by the conventional technique, using an Ayres's spatula, following which a cervical biopsy was taken from the aceto white and abnormal areas on colposcopy, with a punch biopsy forceps.

Both the test samples were sent to the pathology lab for reporting.

Inclusion Criteria
1) Women with abnormal vaginal discharge.
2) Women with menstrual abnormalities
3) Women with complaints like contact bleeding, or bleeding on straining or post coital bleeding.
4) Women with intermenstrual bleeding.
5) Women with postmenopausal bleeding.
6) Women with erosion of the cervix on per speculum examination.
7) Women with cervical polyps, nabothian follicles on speculum examination.

Exclusion Criteria
1) Already established case of carcinoma cervix.
2) Presence of evident growth.
3) Bleeding per vaginum.
4) Pregnant women.
5) Women with post hysterectomy status.
6) Women with previous history of treatment for cervical carcinoma.

3. Method of Collection

After taking a detailed history with all the pertinent information regarding patient profile in terms of Age, Age at marriage, Age at first child birth, Parity, Contraceptive use, Smoking habbits and after taking her consent, the specimen was collected after examination.
1) Patient was put in dorsal lithotomy position.
2) An unaided visual inspection of the cervix was performed under good illumination & the squamo-columnar junction visualized.
3) No vaginal examination or application of the lubricant should be performed prior to obtaining the sample.
4) This is followed by taking a papsmear using a wooden Ayre's spatula. The pointed end of the Ayre's spatula was inserted into the cervical os in a nulliparous cervix and the rounded end of the spatula inserted into the patulous os of a parous woman.
5) The Ayre's spatula was then rotated 360 degrees around the entire ectocervix while maintaining tight contact with the ectocervical surface. The surface cells are gently scraped from the transformation zone, squamo columnar junction and the endocervical canal.
6) The specimen was then transferred to a glass slide, which was immediately placed in a bottle of 95%

ethanol fixative and transported to the laboratory, where the smears were stained by papanicolaou stain

7) Papanicolaou stain results in well stained nuclear chromatin, differential cytoplasmic counterstaining and cytoplasmic transparency.
8) The mucus is first swabbed off with cotton swabs moistened with normal saline. the cervix and vagina are then thoroughly moistened with normal saline in order to view the vascular patterns. 3 % acetic acid is applied with a cotton swab and waited for 2-3 minutes. Under Colposcopic guidance the acetowhite areas and the most suspicious areas were then biopsied.
9) The tissue obtained was fixed by 10% formalin and sent to the lab where it was stained with Eosin and Hematoxylin.

Reporting

Cytology was reported by the pathologist as per the Bethesda III system and the histopathology was reported in the CIN system.

Threshold for Positivity

Cytology was considered positive if it showed LSIL or a more severe lesion.
Biopsy was considered positive if it showed CIN 1 and above.

Reference standard and definition of true positive lesion :

This study was taken up to evaluate the diagnostic accuracy of papsmear by comparing it against the gold standard or the reference standard, the biopsy. In biopsy, CIN 1, 2, 3 or higher lesions were considered as true positive.

4. Statistical Software

Statistical data pertaining to sensitivity, specificity and efficacy rate of cervical cytology were calculated. Statistical software namely SPSS – 11.0 and Systat 8.0 were used for the analysis of the data and Microsoft word and excel have been used to generate graphs and tables.

5. Results

Table 1: Age distribution of subjects

Age (yrs)	Total number of Subjects	Papsmear Positive.	Biopsy Positive.
≤ 20	8 (1.6)	–	–
21-30	165 (33)	20 (22.7)	24 (22.6)
31-40	191 (38.2)	15 (17.04)	10 (9.43)
41-50	104 (20.8)	38 (43.18)	57 (53.77)
51-60	22 (4.4)	10 (11.36)	8 (7.54)
>60	10 (2)	5 (5.68)	7 (6.60)
Total	500	88	106

(Figures in parenthesis indicate percentage.)

The above table shows the age distribution of the subjects studied.

Maximum number of subjects were in the age group of 31-40 yrs (38.2%).

53.7% of positive cases were detected in women of 41-50yrs age.

Mean age of the study group was 38.41 ± 9.92

Statistical analysis shows that the incidence of precancerous or cancerous lesions in women >40 yrs was almost twice its incidence in younger women.

Table 2: Age at Marriage of Subjects.

Age at marriage	Total number of subjects.	Papsmear positive	Biopsy positive.
≤15 yrs	158 (31.6)	50 (56.8)	51 (48.1)
16 – 20	246 (49.2)	30 (34.1)	46 (43.3)
21 – 25	88 (17.6)	8 (9.1)	8 (7.5)
26 – 30	80 (1.5)	–	1 (0.9)
Total.	500	88	106

(figures in parenthesis indicate percentage.)

This table shows age at marriage of the subjects.

About 246 women were married between the ages of 16 and 20 yrs.(i.e 49.2%).

Majority of the positive cases (91.4%) were detected in women who were married at an early age (< 20 yrs).

Mean age at marriage of the study group was 16.81 ± 3.29

The incidence of precancerous and cancerous lesions is 1.5 times higher in women married before 20 yrs than those married at a later age.

Table 3: Distribution of Marital Life Among Subjects

Yrs of marriage.	Total number of subjects	Papsmear positive.	Biopsy positive.
1 – 10	140 (28)	9 (9.7)	21 (20)
11 – 20	195 (39)	27 (31)	40 (38.2)
21 – 30	125 (25)	30 (34)	29 (27. 1)
31 – 40	26 (5.3)	12 (13.5)	10 (9.7)
41 – 50	12 (2.4)	9 (9.7)	5 (4.4)
> 51	2 (0.4)	1 (0.97)	1 (0.9)
Total	500	88	106

(figures in parenthesis indicate percentages.)

The above table shows the distribution of marital life among the subjects. Maximum number of subjects had a marital life of 11- 20 yrs (39%).

Mean marital life was 17.8 ± 9.96

Statistical analysis shows that women with marital life greater than 30 yrs had 2 times higher risk for developing precancerous cancerous lesions of the cervix.

Table 4: Parity Distribution of Subjects

Parity	Total number of subjects.	Pap positive	Biopsy positive.
Nullipara	21 (4.1)	2 (1.9)	2 (2.2)
1 – 2	257 (51.3)	32 (36.8)	49 (46.2)
3 – 4	199 (39.8)	44 (50)	47 (44.4)
> 4	23 (4.6)	10 (11.6)	8 (7.5)
Total	500	88	106

(figures in parenthesis indicate percentage)

This table shows that majority of patients were of para 1 or 2.

Highest parity in the study group was 8.

Women with parity greater than 5 were at alomost 3 times higher risk to develop precancerous or cancerous lesions compared to nulliparas.

Table 5: Contraception Practice among Subjects

Type of contraception	Total number of subjects.	Pap positive.	Biopsy positive.
Tubectomy.	348 (69.5)	55 (62.1)	74 (70.2)
OCP	18 (3.6)	3 (2.9)	5 (4.8)
Condoms	24 (4.8)	3 (3.8)	3 (2.6)
Vasectomy	7 (1.3)	–	2 (1.8)
Cu-T	5 (1)	–	1 (0.9)
None	98 (19.5)	27 (31)	21 (20)
Total	500	88	106

(figures in parenthesis indicate percentage).

The above table shows that maximum number of patients included in the study were tubectomised (69.5%). Out of 106 biopsy positive cases, only 2.6 % were using barrier contraceptives and almost 97 % were using other methods, all exposed to the risk of sexually transmitted diseases.

Table 6: Distribution of Cases Based on Socioeconomic Status

Socio economic status	Total number of cases	Pap positive.	Biopsy positive.
Upper	15 (3)	1 (1.9)	1 (0.94)
Middle	175 (35)	15 (17.4)	30 (28)
Lower	310 (62)	72 (81.5)	75 (70.6)
Total	500	88	106

The table shows that maximum number of patients were from the lower socio economic status. (62%)

81.5 % of the positive cases were in women from the low socioeconomic status.

Women belonging to socio economic status were at 2.6 times higher risk to develop precancerous lesions compared to those of higher socioeconomic status.

Table 7: Distribution of symptoms among subjects

Symptoms	No of subjects.	Pap positive	Biopsy positive
WDPV	387	54	93
MI	258	38	88
PAIN ABDOMEN	70	13	6
PCB	23	9	14

This table shows presentation of various complaints by the subjects.

White discharge was the most common complaint in the studied group (77.4%)

Majority of the patients had more than one complaint.

However, patients presenting with post coital bleeding were at higher risk for developing cervical precancerous or cancerous lesions.

Table 8: Distribution of Cases By PAP Smear

NEGATIVE / INFLAMMATORY	412	82.4 %
ASCUS	17	3.3 %
LSIL	43	8.6 %
HSIL	14	2.7 %
MALIGNANCY	14	2.8 %
TOTAL	500	100 %

All 500 patients were subjected to Papsmear test.

82.4 % of the smears were negative or inflammatory smears.

11.9 % of the smears were graded as ASCUS or LSIL.

2.7 % were reported as HSIL and 2.8 % were positive for malignancy.

So, out of the 500 cases studied, 88 cases were positive on papsmear.

And 412 were either negative or had an inflammatory smear.

Table 9: Distribution of Cases By Biopsy

NORMAL	394 (78.8)
CIN 1	67 (13.4)
CIN 2, 3	19 (3.8)
MALIGNANCY	20 (4)
TOTAL	500

(Figures in parenthesis indicate percentages.)

Of the 500 subjects studied, biopsy was normal in 394 (78.8%) women i.e, they had either an inflammatory smear or a normal smear.

106 women (21.2%) had a positive biopsy, i.e, they had a premalignant or malignant lesion on biopsy.

Of those with the disease,
67 women had CIN I. (13.4%)
19 women had CIN II (3.8%)
20 women had malignancy on biopsy.(4%)

Table 10: Statistical Analysis: Correlation of PAPSMEAR and Biopsy

PAPSMEAR	BIOPSY		Total
	positive	negative	
Positive	68	20	88
Negative	38	374	412
Total	106	394	500

Sensitivity of PAP Test: 64%
Specificity: 95%
Positive Predictive Value: 77 %
Negative Predictive Value: 90.7 %
Accuracy: 88.4%

6. Discussion

Table 11: Comparision of diagnostic values of papsmear of various studies

Study	Sensitivity	Specificity	Reference Standard
Goel et al (2003)	50%	97%	Colposcopy
Hendrik S Cronje et al	53.30%	94.60%	Colposcopy /Biopsy
Doh et al (2001)	47.70%	94.20%	Biopsy
R.Sankaranarayanan et al (multicenteric -2003)	72.30%	97.90%	Colposcopy /Biopsy
P.S. Basu et al (2003)	29.50%	92.30%	Colposcopy /Biopsy
Present study	64%	94.90%	Colposcopy /Biopsy

The risk of cervical cancer continues to be still high in many developing countries, including India, where 70 % of the affected patients present late and also because screening programmes are not being routinely conducted.

In India, screening by papsmear is not being effectively conducted due to several limitations like inadequate coverage of a large population, lack of awareness and lack of proper follow up.

As a screening test pap has been foundto have a low sensitivity, between 50% and 80%, thereby resulting in a high false negative rate of 9 - 40%.the sensitivity is even lower in developing countries. The possible reason for this may be the large percentage of cervicitis and inflammatory smears, which mask mild dysplasia.

However, the specificity of cytological evaluation is high
In the present study, the sensitivity of cytology is 64% and the specificity is 94.9%.This is similar to the study conducted by Hendrik S Cronje et al, where the sensitivity was 53.3% and specificity was 94.6%.

A study by Doh et al (2001) showed a sensitivity of 47.7% and specificity of 94.2%.

A study conducted by Goel et al showed that Pap test has a sensitivity of 50% and a specificity of 97%.

A multicenteric study by Sankaranarayan et al showed a sensitivity of Pap smear ranging from 36.6% to 72.3% and a specificity ranging from 87.2% to 98.6%.

The sensitivity of cytological examination still continues to be a subject of debate.Other studies also varied in the sensitivity rates while the specificity rates were similar to the present study.

Higher sensitivity reported in some studies may be because of

- Variations in the quality of specimen collection
- Lower threshold for positivity of pap smear in the study.

The very low sensitivity of papsmear in some studies may be because of high percentage of inflammatory smears. Fahey et al has reported a sensitivity of 58% and specificity of 69%.

Also, unless histologic condition is determined on all the screening negatives, in addition to the screen positives, the sensitivity will be unrealistically high.This statement has been confirmed in a recent review of sensitivity of cytologic examination by Nanda et al.

So, to avoid this, all the subjects included in the study, ie, both the negatives and positives on papsmear were subjected to cervical biopsy and the biopsy was taken as the reference standard in the study.

The mean age of our study population is 34 yrs.The incidence of cervical lesions after the age of 60 yrs was almost 7%. and about 32% of the cervical precancerous or cancerous lesions occurred before the age of 40 yrs.Hence, from this data, the WHO recommendation to screen for cervical cancer before the age of 35yrs has been justified. Early screening is mandatory to detect premalignant lesions and to prevent incidence of cervical cancer.

The mean age at marriage of this study was 16yrs, which is an important risk factor for cancer cervix. 51% of the subjects were married at an age less than 16 yrs.this study shows that these women are at 1.5 times higher risk for precancerous lesions compared to those married after 20 yrs of age. Early marriage is still prevalent in India particularly in the rural setup. Education and stringent laws are important to tackle this social problem

Long duration of marital life and hence, prolonged sexually active period has been implicated as a risk factor. This study has shown that women with marital life >30 yrs have 2 times higher risk for precancerous lesions or cancver cervix.

High parity (para 5 or more) has also shown significant association with precancer. These women are at 2.6times higher risk for precancerous lesions compared to nulliparity.

69.5 % of the patients were tubectomised and only 2.6% were using barrier contraception. The association of use of oral contraceptives with the development of cervical cancer cannot be proved because of their use for a short duration.

81.5% of the lesions were seen in women belonging to low socio-economic status (as per the B.G Prasad classification). The reasons for this may be early age at marriage, malnutrition, multiparity, all of which are mostly predominant in the lower classes.

Eventhough white discharge is present in 77.7% of the participants, this complaint is not significantly associated with the risk of precancerous lesions compared to the history of post coital bleeding, which was significantly associated with precancerous lesions and invasive cancer. Hence, these patients should be promptly evaluated for presence of malignancy.

7. Limitations of the Study

In this study, sample is selected from the population attending the OPD This population is not representative of the general population

Hence when this test is used for screening in general population, the estimated sensitivity and specificity may not be achievable.

8. Summary and Conclusion

The present study was conducted with the aim to evaluate the sensitivity, specificity, positive predictive value and negative predictive value of pap smear in detection of CIN and to evaluate its diagnostic value.

The confirmation of the true positive cases was done by cervical biopsy.

This study includes a total of 500 patients attending the OP department of Gynecology.

Patients with abnormal vaginal discharge, menstrual abnormalities and with an abnormal cervix on speculum examination were subjected to papsmear.

Simultaneously, cervical biopsy was also taken from these women.

The mean age of the study group was 34.8±9.9 yrs.
The mean age at marriage was 16.8 ± 3.29 yrs.
Mean marital life was 17.8 ± 9.96 yrs.
Majority of the patients were para 1 or 2.

Early age at coitus,multiparity, and low socioeconomic status have 1.5, 2.6, and 2.6 times higher risk respectively for developing precancerous or cancer cervix.hence, these are significant risk factors.

Out of the 500 patients, pap test was positive in 88 women.

Of the 106 cases positive on biopsy, pap was positive in 68 women.

When biopsy was taken as a reference standard, Pap smear has a sensitivity of 64 %
Specificity of 94.9 %.
Positive predictive value of 77 %.
Negative predictive value of 90.7 %
Accuracy of 88.4 %

9. Conclusion

The prevalence of HIV / AIDS in women, in developing countries like ours makes the need for a national screening programme for cervical cancer, even more urgent.

With cervical cancer, early screening is the ounce of prevention that can cure.

No woman should be denied of this opportunity.

Pap test in the past was the successful cervical cancer screening programme and still continues to be. No test is 100 % accurate and it is possible for the papsmear to miss the presence of cancer.However, abnormal cells missed on one test are likely to be spotted during the next test without any significant danger.

90-95% of the false negatives of the test are due to inadequate sampling and improper slide preparation..The sensitivity can be improved by correcting these errors.

Whereas, a single test yields as much as 10 – 15 % false negative reading, it is reduced to only 1 % with 3 repeated tests.
A yearly negative pap smear for 3 years is assuring.

So, even though the sensitivity of pap seems to be low, this can be overcome by a regular programme of papsmear screening with appropriate follow up.

The regimen of **repeated cytologic screenings, and follow up of abnormal results** not only increases the sensitivity of Pap smear but also decreases the incidence of cervical cancer.

Papsmear is an often cited example of a successfulprogram of secondary prevention.
Pap smear, a relatively simple and inexpensive procedure, is still the initial step of cervical cancer screening of women.

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