ORIGINAL RESEARCH

Evaluation of vaginal cytology for detection of dysplasia, carcinoma-in-situ, Microinvasive carcinoma, Frank invasive carcinoma cervix

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ABSTRACT
Background: Carcinoma of cervix is the final step in the chain of events occurring in cervical epithelium at transformation zone.The present work aims at evaluation of vaginal cytology for detection of dysplasia, carcinoma-in-situ, Microinvasive carcinoma, Frank invasive carcinomacervix.
Material and Methods: In the present study 300 cases were selected from the outdoor department of Obstetrics and Gynecology of Darbhanga Medical college Hospital, Laheriasari. The cases selected were thoroughly examined and investigated. Cytological study and Histopathological study of positive smears were done.The lesions showing different grades of dysplastic lesions and malignant lesion were confirmed histopathologically.
Results: The mean age of cases studied was 32.23 years while the mean age of cases found cytological positive and chosen for histopathological examination was 36.75% years. All cases having cervical intraepithelial neoplasia (Dysplasia of various grades) were from the 30-50 years of age group. The mean age of CIN is 36.75 years. Dysplasia and carcinoma in situ have predilectionfor Hindu women especially those belonging to lower socioeconomic status, illiteracy, early marriage, high parity rate making them at high risk group. Among 20 cases of dysplasia diagnosed, 3 were from middle socio-economic group and other 19 belong to the lower socio- economic group. The occurrence of dysplasia and carcinoma-in-situ has a direct relationship with the number of sex partners of the female concerned, the incidence of these diseases rising with the number of sex partners. Although Muslim women had larger percentage of more than one marriage than the Hindu women but the incidence of dysplasia is less among them as found in the present study. Cervical cancer is extremely low in jews, but in Muslims it is higher than that of jews and lower than that of Hindus.The highly multiparous women were more prone to develop dysplasia. Most of the cases studied (65%) from parity status 4-5. Of all cases found dysplastic, 14 were among 4-5 parity status group and 2 cases of invasive carcinoma were from parity status 4-5.Among the presenting complain, intermenstrual bleeding, post- coital bleeding or both and leucorrhea, the highest percentage (79.33%) of women were suffering from leucorrhea. But the incidence rate of dysplasia 4.11% was lower in comparison to the incidence rate of

dysplasia 26.8% in intermenstrual bleeding. Both patients having invasive carcinoma had both intermenstrual bleeding and post coital bleeding as their presenting complain.5 percent cases of CIN (Dysplasia) were showed in healthy looking cervix. The rest 95 % of dysplasia (CIN) were found in unhealthy cervix. Three cases of CIN III (Severe dysplasia) were found in cervicitis with erosion, one cases of CIN III was found in cervicitis with vaginitis. 6 cases of CIN II was found with vaginitis with cervicitis, 1 case of CIN 1 was found with healthy cervix & 2 cases of invasive carcinoma was found with cervicitis with erosion. 0.66% of the study group showed invasive squamous cell carcinoma, which was found to be keratinizing, and large cell type. They all were confirmed histologically.
Conclusion:It was concluded that the load of dysplasia (mild, moderate & severe) & that of carcinoma cervix is quite high in our part of country
Keywords: dysplastic lesions, malignant lesion, histopathologically, cervix.

INTRODUCTION

Carcinoma of uterine cervix is the commonest malignancy of Indian woman. It takes up between 20-25% of all neoplasm of female genital tract[1].Carcinoma of cervix is the final step in the chain ofevents occurring in cervical epithelium at transformation zone. The transformation zone, when exposed to carcinogenicagents, undergoes a series of changes leading to progressive grade of dysplasia. These dysplastic changes may regress or persist and progress to cervical intraepithelial neoplasia (CIN) and invasive cancer.[2]George papanicolaou was the first to observe atypical cells which were not really malignant, in vaginal smears. These cells had a close relationship with cervical anaplasia there proceeding or associated with it. Several investigators have named these cells as hyperplasia, anaplasia, and pre-cancer-ous metaplasia. Prickle cell hyperplasia, basal cell hyperactivity, dysplasia and cervical intra-epithelial neoplasia. It was Regain who first coined term dysplasia in 1953 for these cells. The nuclear changes occurring in these cells with epithelial unrest were same in both early and advanced varieties of cervical intra- epithelial neoplasia. This observation led to the 'continuum concept' which has been confirmed by cytogenetics.[3]

The present work aims at evaluation of vaginal cytology for detection of dysplasia, carcinoma-in-situ, Microinvasive carcinoma, Frank invasive carcinomacervix.

The role of cytology in early detection of cancer cervix cannot be underestimated and the role of early diagnosis in management and prevention of cancer cervix is pivotal in medicare delivery system of any cancer, including cancer cervix. The present work gives clue to the epidemiological burden of cancer cervix in the society of North Bihar. It was also important because we do not have yet cancer registry system in our part of country.

MATERIAL AND METHODS

In the present study 300 cases were selected from the outdoor department of Obstetrics and Gynecology of Darbhanga Medical college Hospital, Laheriasari, who turned up in the department for her routine checkup or for some ailment. Selections of cases were at random irrespective of symptoms and signs. The cases selected were thoroughly examined and investigated. Demographic history was taken which include name, age, marital status, address, religion, occupation, socioeconomic status andnumber of marriage with reference to age of marriage. Clinical history include complaints and their duration with special reference to

1. **Bleeding per vaginum-** contact bleeding post–menopausal bleeding, passage of clots amount of bleeding colour,

2. **Vaginal discharge-**purulent or non purulent, amount, colour quantity, smell, whether mixed with blood etc.

History of Present illness, history of past illness, obstetrical history, menstrual history, family historyof tuberculosis, hypertension, diabetes, carcinomawasrecorded. General examination, gynecological examination, Internal examination (per speculum examination)-inspection of vaginal wall and cervix to note cervicitis, cervical erosions, cervical hypertrophy, cervical growth, cervical polyp, cystocele, rectocele, presence of cervical or vaginal discharge and inflammation or vaginal wall was recorded.Bimanual examination for finding any abnormality in genital organs was done. Rectal examination was done to note parametrical infiltration.Besides the routing investigations, the following studies were done:

a. Cytological study
b. Histopathological study of positive smears.

a) CYTOLOGICAL STUDY
MATERIALS USED
Calen glass slides, one pair of sterilized glass, a jar containing the fixative, Cusco's speculum, plastic aspiration pipette, disposable wooden spatula,shaped like Ayre's spatula, marker pencil, sterilized swab stick, papanicolaou stain, microscope, cover slips, xylol and mountant, chart for noting particulars, litmus paper and blotting paper.

SPECIMEN PREPARATION
The patient after being fully examined clinically and were placed in lithotomy position. After applying bivalve speculum, excess material lying at the os is gently removed, without touching the epithelium and discarded. The spatula was used by rotating it through 360. On the cervix, scraping the squamocolumnar, junction to make the smear, which contains, ectocervical, external os and endocervical components. On the same slide, using moistened non-absorbent cotton swab in place of cervical spatula material was obtained from endocervical canal, rolled onto the slide and fixed immediately. On a second slide with the use of a plastic aspiration pipette, material in the endocervical canal is aspirated and blown upon a glass slide upon which another slide was placed and then drawn apart both slides immediately being immersed in 95% ethyl alcohol. These slides having been properly marked previously yield a high detection (97%) of carcinoma of the cervix[4]. The endocervical component being added on the basis of the work of Gusburg & Moore, 1952[5] who found that carcinoma cervix grew into the endocervical canal earlier then on to the portion; the addition of this component thus facilitates the early diagnosis of carcinoma.

FIXATION
Immediate wet fixation, which gives highest diagnostic accuracy, was used 50% ethyl alcohol and 50%ether in a wide mouth jar was kept ready, into which the specimen rapidly and uniformly spread onto a slide was immediately immersed. Alcohols other than ethyl alcohol were avoided as they cause tinctorial artefacts and morphological distortions at timesobliteration valuable diagnostic nuclear detail.[6,7]

STAINING TECHNIQUE
The smears thus taken were stained by papanicolaou's method. The stains used was E.A. 36, Orange G, Alcohol, 0.25% aqueous HCL, Distilled water.

MICROSCOPIC ANALYSIS OF CYTOLOGICAL SMEAR
On being examined under microscope, the following components were identified: Epithelial cells, Superficial cells, Intermediate cells, parabasal cells, Bacteria, Trichomonas, Monilia, Red Cells, Mucus.

EVALUATION OF SLIDE
Slide thus prepared were classified as normal cell, inflammatory cell, Dysplasia which has been divided into cervical intraepithelial neoplasia I (CIN I), Cervical intraepithelial neoplasia II (CIN II), Cervical intraepithelial neoplasia III (CIN III), micro-invasive and invasive carcinoma. Dysplastic and malignant smear were considered positive smear. Patient with normal cytology were reassured and were advised to subject themselves for repeat cytology every one year, the patient with dysplastic lesion were requested to submit themselves for cytology every 6 months. The lesions showing different grades of dysplastic lesions and malignant lesion were confirmed histopathologically.

RESULTS
This study comprised of 300 cases selected from the patients attending outdoor department of obstetrics and Gynaecology, Darbhanga Medical College Hospital.

Table 1: Distribution of cases according to the religion and findings

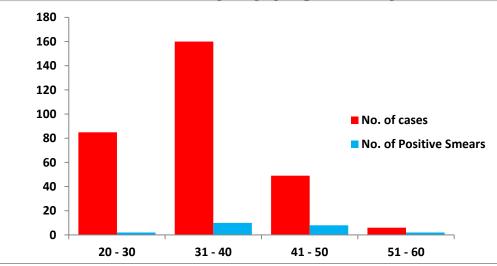| Religion	No. of Cases	Percentage	No of Positive Smears
Hindu	210	70%	16
Muslim	90	30	6

The Hindu predominated amongst the studies cases and 16 positive smears were found in Hindu females and 6 positive smears in Muslim female.

Table 2: Distribution of cases according to Socio-economic status and findings

Socio-Economic Status	No. of cases	Percentage	No. of Positive Smears
Middle	90	30.0	3
Lower	210	70.0	19

Most of the Cases were from lower Socio- economic group and most of the findings were also from them.

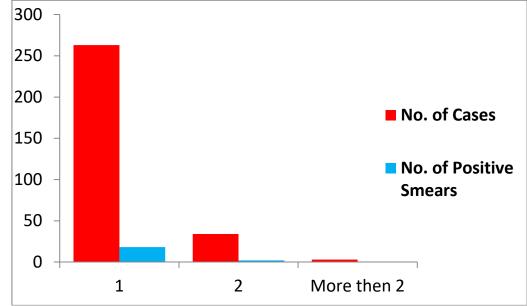**Graph 1: Distribution of cases according to age group and finding**

Most of the cases were from 31-40yrs age group and most of the findings were also from them.

Table 3:Distribution of cases according to marriage

No. of Marriage	Hindu	No.%	Muslim	No %	Total	No. %
1	195	92.8%	68	75.5%	263	87.66%
2	14	6.66%	20	22.2%	34	11.33%
More then 2	1	0.47%	2	2.3%	3	1.00%

Most of the cases had only one marriage but some of them had more than one of two especially Muslim.

Graph 2: Distribution of findings according to no. of marriage

Most of the cases had only one marriage but most of the findings were also from them.

Table 4: Distribution of cases according to age at marriage and findings

Age group at marriage	No. of cases	Percentage	No. of Positive smears
12 – 15	61	20.33%	13
16 – 18	189	63.33%	8
19 – 21	40	13.33%	1
More then 21	10	03.33%	0

Most of the cases of positive smear were found in 12 – 15 years age group at marriage.

Table 5: Distribution of cases findings according to parity status

Parity Status	No. of Cases	Percentage	No. of positive smears
1 – 3	90	30.00	2
4 – 5	195	65.00	16
More than 5	15	5.00	4

Most of the cases of positive smear were found in 4-5 times parity group.

Table 6: Distribution of cases and finding according to presenting complain.

Presenting complain	No. of Cases	Percentage	No. of positive smears
Intermenstrual bleeding	23	7.66%	6
Postal coital bleeding	5	1.66%	2
Both	34	11.33%	4
Leucorrhoea	238	79.33	10

Most of the cases of positive smear were found with compliant of Leucorrhoea.

Table 7: Distribution of cases and findings according to clinical appearance of cervix and external genetilia

Clinical appearance	No. of cases	Percentage	No of positive smears
Healthy cervix	190	63.33%	1
Healthy cervix with vaginitis	20	6.66%	0
Vaginitis	0	0%	0
Vaginitis with cervicitis	40	13.33	16
Cervicities with erosion	50	16.66	5

Most of the cases of positive smear were found with clinical appearance of Vaginitis with cervicitis.

Table 8: Distribution of cases according to cytological report

Nature of Cytological report	Grade	No. of cases	Percentage
Normal		156	52.00%
Infalmatary		122	40.66%
dysplasia		0	
	Grade I	10	3.33%
	Grade II	6	2%
	Grade III	4	1.33%
Malignant		2	0.66%

Maximum cases were having normal cytological report (52%).

Table 9: Distribution of cases according to Histopathological report

Nature of Histopathological report of total positive smear	No. of cases	Percentage
CIN I (Mild dysplasia)	10	3.33%
CIN II (Moderate dysplasia)	6	2%
CIN III (Severe dysplasia)	4	1.33%
Invasive carcinoma	2	0.66%

CIN I was found in 3.33% cases.

DISCUSSION

Out of 300 cases, histopathological examinations of the biopsy specimen were done in 22 cases. There were 210 (70%) Hindu patients and 90 (30.0%) Muslim patients in the present study. Positive smear was found in 16 patients in Hindu and 6 (Six) in Muslims showing Muslim women to be low risk patient. In the present series dysplastic changes were marked in Hindu females.

This finding the conformity with the result of Mana J Shah has found a very high incidence in Hindu (96.38%) than the Muslim.[8]

The difference in. the incidence can only be accounted by the fact that Muslim like Jews, practice circumcision. But lilienfield (Novak, 1985)[6,7] have found the incidence of carcinoma

cervix to be the same as in otherpopulation, claiming that circumcision does not confer any beneficial effect on the incidence of cancer but the Muslim population due to religious reason don't practice contraception having high parity, high incidence of more than one   marriage and they also belong to the high percentage of low socio-economic group are the factors that weight heavily against them.

Maximum number of patients (70.0%) belonged to poorer and almost illiterate strata of the society and the rest 30.0% belonged to middle class; none belonged to the upper class.

On analysis the incidence of dysplasia & cancer cervix found that 13.63 % belonged to the middle socio-economic group and 86.33% belonged to the lower socio-economic group. Similar findings have been recorded by most authors   Ferguson (1961)[9], and S.H. Chauhan et al (1987)[10]reported a very high incidence in the lower socio-economic group.

The cause of high incidence of carcinoma cervix and dysplasia in the lower socio-economic group are due to poverty and ignorance which result in poor sanitation inadequate hygiene. The significant factors of promiscuity and inadequate medical facilities also add to the total picture.

The bulk of the cases in this study have been drawn from the 30-40 years age group forming 58.33 of the total case studied. The rest of the 46.67 % belonged to the older or younger age group. The minimum number of cases belonged to the age group above 50 years. Incidentally, the bulk of the cases forming 53.33% of the total series also belonged to be most sexually active years of women's reproductive carrier. Dysplasia is a disease of sexually active period. All the cases of positive smear 81.81 % were found in age group 30-50 years showing that dysplasia and carcinoma cervix belong to older age group.

The mean age of the 300 cases was 32.23 years while the mean age of the 22 selected cases having histopathological examination was 36.75 years, showing an increase in the value of mean age in women having cervical abnormalities cytologically, which justified by histopathological examination.

Authorities on the subject have found similar values for the overall tendency of age of the patients.

The mean age of carcinoma in situ stated by Jeffcoat (1975) is below 35 years. [11]

In India, early marriage is the common social custom. Jeffcoate (1975) stated that lower the age at marriage greater is the risk of carcinoma cervix, because the epithelium of the early adolescent is likely to be disturbed by the large amounts of de-oxy-ribonucleic acids provided by the sperms, the ensuing dysplasia leading to carcinoma-in-situ in a few years which may progress to invasive carcinoma.[11]

Terris & Oalman (1960) found that over half the cases with carcinoma cervix had first intercourse before the age of 17 years.[12]

The early marriage in India could account for the earlier development of cancer cervix in Indian women. In the present study most of the females were married between 16-18 years of age group (63.33 %). None had been married before 12 and after 21 years only ten females were married.

Out of 22 cases positive smear 13 cases from females who married between 12 to 15 years of age group and eight cases from 16-18 years of age group and one in 19-21 age groups.

Martinez (1969)[13], and Kessler (1976)[14] investigated relationship of coitus with more than one Partner with that of carcinoma of cervix. They found a definite direct relationship.

In the present study, out of 300 cases 263 had one marriage and 34 cases had two marriage and rest three cases had more than two marriages.

Out of 22 positive smear cases 18 had one marriage and 4 were married twice.

Only 34 females who had married twice were included in the present study. Out of these 34 females, 4 developed positive smears showing that coitus with more than

one sexual partner is an important factor in the epidemiology of dysplastic changes of the cervix.

Jeffcoate (1975)[11] states that the married women having children is 2-4 times more likely to develop cancer cervix than the single women. But since most of women are highly multiparous in a country like India childbearing per se cannot be implicated as a direct etiological factor even but only severe as an indirect agent. Much childbearing implies much coitus (Jeffcoate, 1975) through many years premably starting from an early age of ten in poor socio-economic condition.

C. Swarna Kumar (1987) also found highest incidence in multiparous women.[15]

Kalpana mital et al (1988) found maximum number of cases dysplasia (40%) with parity IV.[16]

In the present series, 195 females had 4-5 children, 90 had 1-3 children and only 15 females had more than 5 children.

Two cases of positive smear were having 1-3children, sixteen cases of positive smear having 4-5 children while 4 cases of positive smear were having more than 5 children. So, multiparty also predisposes to cervical laceration,erosion etc. which may have an indirect relationship with the cervical dysplasia. So, the result of this similar to that of other workers.

The vaginitis in cases of malignant neoplasm could be accounted for the fact that the degeneration of cells serves as a good culture medium for invading organism (Novak, 1985).[6,7]

The rest of the cases could be considered as an incident finding being no higher than the rest of the population.

Mamta singh et al found an association of 87.5 % in cases of dysplasia.[17]

In the present series 60 cases, out of 300 having vaginitis. Positive smear was seen in 16 out of 60 females suffering from vaginitis. Rest of 240 females was not suffering from vaginitis. So, incidence of dysplasia is 72.7 associated with vaginitis in this study.

Gulati and chanra (1973) found 10.7% to 17.5% incidence of dysplasia with cervical erosion.[18]

In the present series of 300 cases 90 females were having cervicitis, 40 cases of cervicitis were associated with vaginitis and 50 cases with erosion. Out of these 90 cases 21 showed positive smear.

In the present series out of 300 cases 90 females had unhealthy looking cervix and rest had healthy looking cervix. Out of 90 females having unhealthy cervix 19 showed dysplastic changes and two showed invasive carcinoma. One case of dysplasia was noticed in female having healthy cervix.

C. Swarn Kumari found incidence of dysplasia in healthy looking cervix 34% and unhealthy-looking cervix 52.9%. [15]

On the close scuruitiny of presenting complain in the cases studies 7.66% had intermenstrual bleeding, 1.66% had post coital bleeding and 11.30% had intermenstrual with post coital bleeding 79.33% had leucorrhea.

Out of 23 females complaining intermenstrual bleeding six showed dysplasia in cervical smear i.e., 26.08 %. Out of 34 cases associated with intermenstrual bleeding andpost-coital bleeding both 3 had dysplasia and on had invasive Carcinoma. Of the 5 cases of post coital bleeding, one had dysplasia and one had invasive carcinoma.

But Hema Padmnabhan et al (1990) found 34 % cases of dysplasia in intermenstrual bleeding, 17 % cases of dysplasia in post coital bleeding and 68.7% cases of dysplasia were found in leucorrhoea.[19]

Out of 300 cases of cervical cytology result 156 cases (49.16%) were normal, 122 cases (46.66%) were inflammatory and dysplasia were found in 20 cases and invasive

carcinoma in 2 cases. Out of 20 cases of dysplastic cervix cervical smear examination were again followed up after a period of 1 month. In 16 cases, dysplastic change were still present but in 4 cases cellular characteristic returns towards normal repeated cytological examination. Out of 16 cases of persistent dysplastic changes biopsy specimen were taken after a gap of two months for histopathological examination. Invasive carcinoma was not found in any cases. In all 16 cases of different grades of cervical intraepithelial neoplasia were demonstrated.

Out of 238 cases of leucorrhea,10 had dysplastic changes in cervical smear. In the present series 52% of female had normal cervical cytology, 40.66% had inflammatory results and 6.66% had dysplastic changes. Of these dysplastic cases 10 were in group CIN I, 6 in CIN II & 4 in CIN III. Two cases had invasive carcinoma.

In the present series all the 20 cases of dysplasia were confirmed by histopathological report.

An incidence of dysplasia between 1.2 to 3.1 % was found by Murphy (1950)[20], Mackay (1959)[21].

The finding of the present study (3.33%) is almost in similar range to other workers.

In the present series, all the 20 cases of dysplasia were confirmed by histopathological report. Out of these ten cases were CIN I, 6 in CIN IIand 4 was in CIN III.

In summing up the discussion, it can be stated that cytological diagnosis is an important method for early diagnosis of cervical intraepithelial neoplasia including dysplasia and carcinoma in situ. The confirmation of this diagnosis can only be had from histopathological report after removing a biopsy specimen.

Once cervical intraepithelial neoplasia is Dysplasia is diagnosed by cervical smear examination, the patient was provided good assurance of satisfactory treatment and out-come.

CONCLUSION

It was concluded that the load of dysplasia (mild, moderate & severe) & that of carcinoma cervix is quite high in our part of country. Cytology is convenient, cheap, non-invasive technique which does not need specialized instruments and which can be done anywhere on out-door practice, which does not necessitate hospitalization. It is a very powerful tool to handle the problem of carcinoma cervix.

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