

EARLY DIAGNOSIS OF PRE-INVASIVE CARCINOMA OF THE CERVIX BY FLUORESCENT MICROSCOPY

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SUMMARY

Five hundred married women were included in this study. The incidence of cervical malignancy was found to be 0.8%. The false positive rate was 0.2%. There was one false negative 0.2% but the false negative rate cannot be definitely commented as negative smears patients were not further followed.

The acridine orange fluorescent technique is a simple and a rapid method and can be used as a prescreening method to eliminate the large number of negative slides, while the abnormal smears may be repeated and if necessary subjected to the conventional methods of Papanicolaou staining and cervical biopsy.

Introduction

Cancer of the uterine cervix is a dreadful cause of mortality amongst women. Prevention of this disease has been the fundamental object of those concerned with the patient care since times immemorial. Short of absolute prevention, the next best course is to detect this fatal disease in its earliest phase and then to remove it. Exfoliative cytology is one technique which is being used extensively for this purpose. In 1946 Papanicolaou and Traut recognised the diagnostic value of the stained vaginal smears in cancer of the uterus. However, this technique requires a long time and expert personnel for the

reading of the results. These difficulties were overcome by using fluorescent microscopy and acridine orange recommended by Von Bertalanffy *et al* (1958). It is rapid and simple method for early diagnosis of pre-invasive as well as invasive carcinoma of cervix.

Material and Methods

Five hundred married women attending the outpatient department of Government Hospital for Women, Amritsar were included in this study. Cases were taken at random with exclusion of frank cases of carcinoma of the cervix. Most of the patients included in this study had come to the hospital for symptoms like vaginal discharge menstrual disorders, low backache, post coital bleeding, post menopausal

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bleeding. Some had visited the hospital for antenatal care (Table I).

TABLE I
Distribution of Cases According to Clinical Diagnosis

Clinical diagnosis	No. of cases
Cervical erosion	80
Chronic cervicitis	47
Suspicious cervix	12
Endocervicitis	17
Vaginitis	40
Pregnancy	84
Pelvic inflammation	20
Prolapse	16
Dysfunctional uterine bleeding	30
Sterility	16
Urinary tract infection	20
Cervical polyp	8
Gynaecologically normal	30
Miscellaneous	30
Total	500

A complete clinical examination of the patients was done. Two smears were prepared (on numbered clean glass slides) from each patient one from the posterior fornix of the vagina and other from the ectocervix using non-absorbant cotton swab sticks. After fixation and drying, staining of the smears was done with acridine orange according to the method described by Von Bertalanffy *et al* (1958). The smears were then grouped into the following categories:

(I) *Negative*: Cells showing green fluorescence with nuclei Brilliant whitish green, normal nucleocytoplasmic ratio (NIC), normal cell outline and no suspicious or atypical cells.

(II) *Inconclusive*: Atypical cells with a normal N/C ratio but with abnormal cytoplasmic fluorescence.

(III) *Suspicious*: Cells with extremely abnormal cytoplasmic fluorescence or cells

with increased nuclear fluorescence and with morphologic criteria suggestive of malignancy i.e. cells with intense red fluorescence that absconded the nuclei, cells with nuclei that fluoresced brighter than nuclei of the neighbouring cells, cells with nuclei showing marked variation of size and shape with or without brighter fluorescence.

(IV) *Positive*: Malignant cells giving flaming reddish orange fluorescence, with increased N/C ratio and cytoplasm containing reddish granules and nuclei in various shades of yellow, yellow-orange, yellow green. Nucleoli in brilliant orange colour.

In group II and III repeat smears were prepared. If the smear report repeatedly fell in II and III category, confirmation by cervical biopsy was done. Smears distinctly positive were directly followed by cervical biopsy. The necessary treatment was then advised to the patient.

Results

On 1st smear examination there were 445 patients with negative smear (89%) while abnormal smears were found in 55 patients. One of these negative smears turned out to be false negative when cervical biopsy was done on strong clinical grounds.

Table II—Cervical examination and smear report:

Out of 500 patients, 337 (67.4%) had healthy cervix, 80 (16%) had cervical erosion and 59 (11.8%) had unhealthy cervixes which included both chronic cervicitis and suspicious cervix. Eight (1.6%) had cervical polyp and 16 (3.2%) cases had prolapse.

Abnormal smears were found in 55 patients. Out of these 55 abnormal smears, 44 (8.8%) were inconclusive. Six (1.2%)

TABLE II
Cervical Examination Smear Report

State of cervix	Total Pts.	Smear report			
		Negative	Inconclusive	Suspicious	Positive
Normal	337	319	12	2	1
Cervical erosion	80	64	15	2	2
Unhealthy cervix (Chronic cervicitis and suspicious cervix)	59	41	14	2	2
Cervical polyp	8	5	3	—	—
Prolapse	16	16	—	—	—
Total	500	445	44	6	5

suspicious and 5 (1%) were positive smears.

When repeat smears were prepared in 55 cases with initial abnormal smears, a further 35 cases were eliminated as negative, while 20 (4%) still continued to be abnormal; out of which 9 (1.8%) were inconclusive, 6 (1.2%) were suspicious

and 5 (1%) were positive.

Table III Follow up and further study in 20 cases with abnormal smears.

(a) *Inconclusive smears*: Out of 9 inconclusive smears, cervical biopsy was done in 7 cases. Histopathology revealed chronic cervicitis in all the 7 cases. In 2

TABLE III
Follow-up of the 20 Abnormal Cases

Repeat smear report	Total Pts.	Total No. of cervical biopsies	Biopsy report	Remaining patients in whom cervical biopsy was not done
Inconclusive	9	7	Chronic cervicitis	One was a pregnant patient with negative smear in post-partum period. Other case had a big polyp projecting from the cervix. No biopsy was done but panhysterectomy done. No malignancy on H.P.E.
Suspicious	6	5	Chronic cervicitis	A pregnant patient with negative smear in post-partum period
		1	Severe dysplasia	
Positive	5	4	Chronic cervicitis	Ovarian malignancy proved on H.P.E. of tumour removed
		3	Epidermoid carcinoma	
Negative	1	1	Epidermoid carcinoma	

cases cervical biopsy was not done. Of these 1 was pregnant. Repeat smears in the postpartum period became negative. Other case had a big cervical polyp for which panhysterectomy was done and whole specimen was sent for histopathological examination and it showed no evidence of malignancy.

(b) *Suspicious smears*: Out of 6 cases with suspicious smears, cervical biopsy

was done on the clinical grounds and in view of age of the patient i.e. history of postmenopausal bleeding, unhealthy cervix which bled on touch. H.P.E. revealed epidermoid carcinoma.

Thus there was one false positive and one false negative case.

Table IV: Smear report in various age groups:

TABLE IV
Smear Report in Various Age Groups

Age group (years)	Total Pts.	1st smear report			
		Negative	Inconclusive	Suspicious	Positive
Below 20	14	14	—	—	—
21-30	250	230	23	—	1
31-40	140	124	11	3	—
41-50	10	56	7	2	3
51-60	22	17	3	1	1
61-70	4	4	—	—	—
Total	500	445	44	6	5

was done in 5. Histopathology revealed chronic cervicitis in 4 cases and severe dysplasia in 1 case. The remaining 1 case, was pregnant, and cervical biopsy was not done and repeat smears became negative in the postpartum period.

(c) *Positive smears*: Out of 5 cases with positive smears, cervical biopsy was done in 4. Histopathological report revealed chronic cervicitis in 1 case and epidermoid carcinoma in 3 cases. The fifth case in which cervical biopsy was not done was a case of ovarian tumour which was removed by laparotomy. On Histopathological examination the ovarian tumour proved to be carcinoma of low malignant potential (See Fig. 1). Repeat smears were negative after the operation.

(d) *Negative smear*: In one case cer-

Table IV shows that in the present study inconclusive smears were found to be more common in younger age group i.e. 21-30 years. Out of a total of 44 inconclusive smears, 23 were found in this group. There was no suspicious smear. One smear was positive which showed ovarian malignancy on histopathological examination of removed tumour. Suspicious smears were more common in 31-60 years. Positive smears were more common in older age group 41-60 years i.e. 4 cases.

Table V: Smear report and gravidity:

It is apparent from Table V that gravidity did not significantly influence the inconclusive smears but suspicious and positive smears were found more commonly in multigravidas.

TABLE V
Smear Report and Gravidity

Gravidity	Total Pts.	Smear report			
		Negative	Incon- clusive	Suspicious	Positive
Nulliparous	43	37	6	—	—
Gravida 1	60	57	3	—	—
Gravida 2	87	73	12	2	—
Gravida 3	83	71	9	1	2
Gravida 4	80	73	3	2	2
Gravida 5	54	46	7	1	—
Gravida 6 and above	93	88	4	—	1
Total:	500	445	44	6	5

Discussion

The present study included a series of 500 female patients. Total 5 cases of malignancy were detected. Of these 4 were epidermoid carcinoma of cervix. Three were detected as positive smears while in the 4th one a false negative smear was obtained. Cervical biopsy was done on clinical grounds. The fifth one was a case of ovarian malignancy. In the present series, 4 cases of carcinoma of cervix were detected (80%). These findings are in agreement with the findings of Kawaske and Wilson (1963) who in a series of 1357 cases detected 11 cases of cervical malignancy (0.80%). Sussman (1959) detected 4 cases of malignancy of the cervix in a total of 1050 cases (0.38%) lower than in the present study. Of the 500 patients, 44 (8.8%) were labelled as inconclusive and the results are in agreement with those of Sussman (12%). Six (1.2%) cases were labelled as suspicious and the results are in agreement with the studies of Holland and Ackermann (1961) 1.6%. The results of this study with respect to the suspicious smears are in disagreement with those of Culling and Vassar (1961) and Liu (1961) who reported 9% and 23% suspicious smears, respectively. The large

group of inconclusive and suspicious smears were due to many factors as trichomonal or monilial infection of vagina, pregnancy, chronic cervicitis, cervical erosion and cervical dysplasia.

In the present study, the false positive rate was 0.2% i.e. 1 of the 500 cases. The results are in agreement with the false positive results reported by Kaplan *et al* (1960) 0.20%, Von Bertalanffy *et al* (1958) 0.3%. The false positive results disagree with those reported by Framp-ton (1963) i.e. 0.81% and Culling and Vasser (1961) 4%, Lawhagen *et al* (1966) 4.6% and Stevenson (1964) 6.1%.

The false positive rates ranged in literature from 0.2 to 33% (Using both increased cytoplasmic fluorescence and morphological features).

In the present series, there was 1 false negative case (0.2%) in which cervical biopsy was done on clinical grounds and it turned out to be epidermoid carcinoma. But as all the negative smear cases were not followed, no definite comment can be made on the total false negative in the series. Kaplan *et al* (1960) reported a very low false negative rate of 0.05% in total of 2000 cases. Von Bertalanffy *et al* (1958) found a false negative rate of

0.3%. The false negative rate reported by Rocky (1963) was 1.4%, while that reported by Betts *et al* (1962) ranged from 5.7%.

In this study, one false positive case was clinically diagnosed as a case of benign ovarian cyst which even on laparotomy appeared to be benign papilliferous cyst which had undergone torsion. Right sided ovariectomy was done as the patient was young (28 years). Histopathological report of the tumour was (appearances are those of a serous ovarian tumour of borderline malignancy, Carcinoma of low malignant potential. Epithelial proliferation, papillary formation present and pleomorphism present. Mitosis infrequent. No stromal invasion (Fig. 1). It is interesting to note that in this patient pap smears were negative repeatedly while acridine orange smears were positive. patient was kept under observation. Repeat smear on 10th post-operative day were negative and subsequently monthly repeat smears remained negative. She became pregnant and delivered normally. After that we lost track of her.

In the present study, no case of carcinoma in situ was detected. Considering the false negative rate of this method to be low (from the reports in the literature, it is unlikely that carcinoma in situ of the cervix would have been missed. Probably within these 500 cases there was no case of carcinoma in situ. This is likely as there is no uniform distribution of the disease as such. Diagnostic accuracy of the method is high. In total 96 of the slides screened, 89% turned out as negative on first smear examination, 7% on repeat smear examination, in agreement with the results of Holland and Ackerman (1961), 97%, Sussman (1959) 98.2%, Kaplan *et al* (1960) 94.8%; Kawaske and Wilson

(1963) 97.5% negative slides. Lowhagen *et al* (1966) studied 3270 cases 96.3% were negative.

Von Bertalanffy and Bicks (1958) evaluated the advantages of fluorescent microscopy over the routine Papanicolaou's method and found very little statistical difference in the two techniques. Kaplan *et al* (1960) found fluorescent microscopy a quicker method of diagnosis. Misra *et al* (1979) found that diagnostic accuracy of fluorescent microscopy in detecting early cervical lesions was almost identical with the Papanicolaou's technique.

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See Fig. on Art Paper II