

# EVALUATION OF FLUORESCENT MICROSCOPY OF THE VAGINAL SMEARS AS A MASS SCREENING METHOD FOR THE DETECTION OF CANCER CERVIX

by

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## Introduction

Deaths from cancer of the cervix represent one important proportion of all cancer deaths and can be prevented if the diagnosis of the disease is made early. Neiburgs *et al* (1957) examined 27,899 smears from 17,761 cases between September, 1951-1955. The cases represented practically the whole population of the area above 19 years of age. One hundred and seventeen women (0.7%) were found to have cancer cells in the cervical smears and in 84 the diagnosis was confirmed by biopsy. From the repeated examinations of a restricted larger group over a period of 4 years it was found that 1.68 new cases of cancer cervix per 1,000 women arose in that population in U.S.A.

In one survey (1961) Tiagi and Sunanda Bai have also reported findings of two surveys in New York. Twenty thousand patients attending a clinic were investigated and incidence of carcinoma cervix of 1.5 per 1,000 was found. In an-

other large survey on 108,000 females, 393 intraepithelial carcinoma and 373 invasive cancer giving rate of 3.6 and 3.4 per thousand respectively was found. Papanicolaou technique is undoubtedly one of the early and reliable way of diagnosing cervical carcinoma but for the last two decades much emphasis has been laid on the use of fluorescent microscopy in exfoliative cytology. Friedman (1950) first reported the use of fluorescent dye for the study of exfoliated cells and used Berberine Sulphate, Acid Fuchsin and Acridine yellow. However, this technique as well as that of Mellore, Glassman and Papanicolaou (1952) proved to be too laborious. Von-Bertlanffy *et al* (1958) recommended the use of Acridine orange, and their technique was found to be simple, inexpensive and required considerably less time for staining.

The authors took this opportunity to present their observations of fluorescent staining technique using Acridine orange on the smears obtained from 300 patients. Papanicolaou stain will be presented separately.

## Material and Methods

Random collection of posterior vaginal pool smears from 300 cases was done from

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the patients attending the out-patient department of Obstetrics and Gynaecology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh.

A detailed history of each case was taken, physical and bimanual vaginal examination was done. Vaginal pool smear was obtained in each case by putting a drop of fluid from posterior fornix on the glass slide and spreading it with the gloved fifth finger in one stroke. Slides were immediately immersed in the jar containing equal parts of 95% alcohol and ether for fixation. After fixation slides were stained by fluorescent staining technique, using Acridine orange as dye, dried and then mounted under a cover glass in a drop of phosphate buffer (PH 6.0) and were examined under fluorescent microscope.

#### Observations

The age of the patients varied from 18-50 years maximum cases (71.6%) belonged to the age group of 20-40 years (Table I). Vaginal smears of 300 patients were examined and the various exfoliated cells seen in vaginal smear under fluorescent microscope appeared as follows:

(i) *Epithelial Cells*: The superficial and intermediate squamous appeared greyish yellow, the parabasal cells also showed yellowish green fluorescence in both nuclei and cytoplasm. Small parabasal cells gave orange pink fluorescence. Endocervical and endometrial cells had an orange pink fluorescence in the cytoplasm.

(ii) *Red Blood Cells*: appeared as small round shadows.

(iii) *Leucocytes*: Fluorescent, a bright yellowish green colour; in inflammatory smears they formed the most brilliant clusters.

(iv) *Mucus*: Strands of mucus gave bright yellowish green fluorescent.

(v) *Micro Organisms*: Majority of cocci and bacilli gave bright orange red colour. Trichomonas appeared as pear shaped bodies of brick red colour scattered in the smear (Fig. 1). Yeast cells and hyphae were seen in marked clarity and gave brilliant red fluorescence. (Fig. 2).

(vi) *Malignant Cells*: Malignant cells depicted brick red fluorescence in the cytoplasm and a brilliant yellowish green in the nucleus (Fig. 3).

TABLE I

Showing the Grading of Smears Stained with Acridine Orange Technique in Relation to Different Age Groups

Age group	Total No. of cases	Grades*					Incidence of cancer
		I	II	III	IV	V	
20-30 Yrs. (including the age of 18-20 yrs)	143	89	49	2	3	-	Nil
31-40 yrs.	90	29	51	3	5	2	2.22%
41-50 yrs.	45	21	21	-	2	1	2.22%
50 & above	22	11	7	-	1	3	13.64%
Total no. of cases 300	300	150	128	5	11	6	2.00

\* Grade: I—Negative, II—Inflammatory smear, III—Inconclusive, IV—Suspicious for malignancy, V—Positive for malignancy.

Out of 300 smears 128 (40.0%) showed inflammatory reactions (non-specific 90, monilia 22 and trichomonas 16).

The smears examined were grouped according to the standard Papanicolaou classification as shown in table.

#### Discussion

In the present study the incidence of cancer cervix was 2% (0.20 per 1000 female). Out of 143 cases belonging to the age group of 20-30 years none had cancer smear. The incidence was 2.22% in the age groups of 31-40 years and 41-50 years. There was, however, high incidence (13.64) amongst 22 cases of age group of above 50 years. Similar observations have been made by Tiagi and Sunanda Bai (1961) who have reported 6.89% incidence of cancer cervix in 29 cases above 50 years of age as compared to 2.55% in the age group of 31-40 years and 3.63% in the age group of 41-50 years.

High incidence in the age group of above 50 years is probably due to less number of cases studied in that group. The overall incidence is much below as compared to studies conducted in U.S.A. (4.6% by Stewart *et al*, 1966 and 4.2% by Haenseal and Hillhouse 1959).

Acridine orange technique was found to be more convenient, rapid and accurate for screening the cervical smears as compared to Papanicolaou technique. Moreover, this technique is less expensive and the malignant cells could be recognised at a glance as their morphologi-

cal characteristics were very prominent.

However, one should always remember that for interpreting the cytological results more experience is necessary.

#### Summary

Cervical smears obtained from 300 cases attending the outpatient department of Gynaecology Section were stained with Acridine orange technique. The overall incidence of cancer cervix was found to be 0.2 per 1000 females. The technique was found to be easy, rapid and less expensive. The cancer cells were picked up more easily as compared to Papanicolaou stained smears.

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See Figs. on Art Paper III-IV