A COMPARATIVE REVIEW OF ACRIDINE FLUORESCENCE AND PAPANICOLAOUS STAINING TECHNIQUE IN GENITAL CANCER CYTOLOGY

by

RAMA MISRA,* M.S.

REKHA MATHUR,** M.B.,B.S.

Late Prof. P. R. ATAL,† M.D.

and

MAMTA SINGH,*** M.D.

Cancer of the female genital tract is a major health problem in India, as it is the commonest malignancy in females and comprises nearly 80% of all malignancies in women. An early diagnosis of carcinoma is imperative, as it means all the difference between life and death, so a technique is desirable, which is simple, easy, quick cheap and reliable so that it can be used for mass screening purposes both in urban and rural areas.

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. The cytological diagnosis of carcinoma cervix by Papanicolaou staining was widely used by Grahams and Meig (1949), Novak (1949), Reagan and Moore (1951) and (1952) Wahi and Jain (1950, 1952) Ayre (1957) and Zinser (1957). However, the Papanicolaou staining technique requires a long time and expert personnel for the reading of the results. These difficulties were obliviated by using the Fluorescence microscopy technique which is much less time consuming. The use of the dye Acridine Orange further facilitates the reading of the results due to its polychromatic nature. This property serves to differentiate the two types of nucleic acids, R.N.A. (Ribonucleic acid) fluorescing a yellowish green colour and D.N.A. (Deoxyribonucleic acid) fluorescing a brilliant orange colour, the intensity of the stain depending upon the quantity of nucleic acid present.

Von Bertalanffy and Bicks (1956) evaluated the advantages of fluorescence microscopy over the routine Papanicolaou method, and found that in all clear cut normal cases, as well as in the cases with malignancy, the results were identical. Other workers, Dart and Turner (1959), Umirker, *et al* (1959), Liu (1961) and Walid and Magnano (1962) worked extensively on the diagnostic accuracy of the two techniques. They found, that either the fluorescence technique was just as good a diagnostic aid as the Papanicolaou smear technique, or that the latter technique proved a better diagnostic aid,

*Reader in Obstetrics & Gynaecology.

**Formerly Registrar in Obstetrics & Gynaecology.

†Formerly Professor and Head of the Dept. of Pathology.

***Lecturer in Pathology.

M.L.N. Medical College, Allahabad.

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though it was time consuming and required expert personnel for the reading of the results.

Material and Methods

The material for study was taken from patients with a clinical suspicion of diagnosis of carcinoma cervix, from the out patient departments of Obstetrics and Gynaecology, Kamla Nehru Memorial Hospital, Swaroop Rani Nehru Hospital and Dufferin Hospital, Allahabad.

In the present study, 458 smears were examined, out of which 246 smears were from 123 cases of malignancy of the female genital tract, and 212 smears were from 106 control cases. In each case cervical scrape smears were made from 4 different areas on the cervix by Ayre's spatula or if it was thought necessary, the smears were made from material collected by a pipette from the posterior fornix and cervical canal. These smears were fixed in 95% ethyl alcohol and were stained first by Acridine orange for fluorescent microscopy. Later on they were washed with 50% alcohol, stained by the Papanicolaou's stain and studied again. Thus an accurate comparison of results was made.

Observations and Discussion

TABLE I

Comparative Morphological Studies of Different Types of Cells

PAPANICOLAOU STAIN	*FLUORESCENCE TECHNIQUE*
THE DYSKARYOTIC CELL	
1. Cytoplasm: basophilic or eosinophilic	These cells stained darker and brighter in colour than other normal cells.
2. Nucleus: rounded or oval (a) Chromatin-clumped and smudgy. (b) Hyperchromasia present. (c) Nuclear envelope shrunken. (d) Nucleocytoplasmic ratio—normal.	
PREINVASIVE CARCINOMA	
1. Cells seen singly or in groups of dysplastic cells.	Cells seen singly or in groups with typical brilliant fluorescence.
2. Cytoplasm-scanty, cytoplasmic tail present-cellular borders sharp in outline.	Cytoplasm—brilliant yellow orange in colour.
3. Nucleus— (a) Large with coarsely granular chromatin arranged in clumps, often at the periphery. (b) Multinucleation present. (c) Nuclear membrane sharp in outline and thickened. (d) Nucleocytoplasmic ratio increased.	Nuclei—Yellowish green jagged in outline binucleation seen.

WELL DIFFERENTIATED SQUAMOUS CARCINOMA

Cytoplasm: Thin, dark pink or almost orange in colour due to hyalinization. Tadpole cells malignant pearls and phagocytosis seen.	**Cytoplasm:** Brilliant orange-yellow in colour. Secretory vacuoles seen in the cytoplasm.
Nuclei: Large with bizzare shapes coarsely clumped chromatin, nuclear membrane sharp in outline. Nucleocytoplasmic ratio altered.	**Nuclei:** Yellowish green and vesicular showing a good criteria of malignancy.

ADENOCARCINOMA

Cytoplasm — Hazy in outline and secretory vacuoles present.	*Cytoplasm:* Brilliant yellow orange in colour secretory vacuoles present in the cytoplasm.
Nuclei — Vesicular and hyperchromatic. Chromatin present in clumps with a sharp jagged outline.	*Nuclei* — Yellowish green vesicular, showing good morphological criteria of malignancy.

ANAPLASTIC CARCINOMA

1. Cell outline not clear.	Loss of typical fluorescence in the cytoplasm due to absence of cytoplasm.
NUCLEUS:	
(a) Nucleo-cytoplasmic ratio increased.	Nuclei — Yellowish green fluorescence usually obscured by the surrounding necrotic debris.
(b) Nuclear membrane-thick sharp and angulated.	
(c) Chromatin coarse and clumped.	
(d) Prominent nucleoli seen.	
(e) Bunches of naked nuclei seen.	

The fluorescence of malignant cell cytoplasm has been described to be a flaming red colour by various authors Von Bertalanffy *et al* (1958), Sussmann (1959), Kaplan *et al* (1960) and Liu (1961). In the present series of cases flaming red colour was not seen and the cytoplasm fluoresced a brilliant yellow orange colour only. The nuclei showed a yellowish green fluorescence becoming brilliant in colour in most of the carcinoma cells seen, and this staining is again consistent with the findings of the above authors. The cytomorphological details seen in the series compare favourably with the details of the cytological picture reported for the standard Papanicolaou technique.

Dyskaryosis in the present series was pin-pointed easily by the fluorescence technique, but the exact nature of the cells and the difference between dyskaryosis and malignancy was established only on the basis of cytomorphological details. Von Bertalanffy *et al* (1958) reported the same observations.

Out of 123 cases examined by the Papanicolaou technique, an accurate diagnosis was observed in 119 cases (96.7%), whereas fluorescent microscopy showed a diagnostic accuracy of 79.5% only, being positive for carcinoma in 78 out of 98 smears studied. There were no false positive reports with the Papanicolaou method but there were 4 false negative reports (3.25%). In contrast the fluores-

cent microscopy showed 20 false negative reports (20.4%) and 2 false positives (2.04%) Table II).

these cells which normally fluoresce brilliantly, are difficult to locate in a mass of necrotic debris and the loss of cyto-

TABLE II

Comparison of Diagnostic Accuracy of Fluorescence Microscopy and Papanicolaou Technique

Method of examination	No. of cases	Correct diagnosis	False diagnosis negative	False diagnosis positive
Papanicolau technique	123	119 (96.7%)	4 (3.25%)	0 (0%)
Fluorescence Microscopy	98	78 (79.5%)	20 (20.4%)	2 (2.04%)

However, other authors comparing these two techniques, have found very little statistical difference in the two techniques Kaplan *et al* (1960) found fluorescence microscopy a quicker method of diagnosis.

The large number of false negative reports by the fluorescence microscopy in this series, is explained by the presence of a large number of necrotic cancer cells in advanced carcinomata. The nuclei of

plasmic substance reduced the typical brilliant fluorescence of these cells. In these cases of advanced malignancy the Papanicolaou technique proved of better diagnostic value because its results were concentrated on the study of the morphological nuclear details. This statement is further proved by Table III which shows the percentage of error of detection of various types of carcinomata by the two techniques.

TABLE III A & III B

Showing Percentage of Error of Detection of Various Types of Carcinoma by the Two Techniques

III-A

Site of malignancy	Popanicolaou Technique		Invasive carcinoma	
	Pre-invasive	Squamous carcinoma	Adeno-carci-noma	Anaplastic carcinoma
Vulval	—	—	—	—
Vaginal	—	—	—	—
Cervical	—	6.7%	—	40.9%
Endometrial	—	—	—	—
Ovarian and tubal	—	—	—	—

III-B

Vulval	—	—	—	—
Vaginal	—	—	—	—
Cervical	—	13.6%	—	100%
Endometrial	—	—	—	—
Ovarian and tubal	—	—	—	—

With the Papanicolaou technique, the preinvasive squamous carcinoma, squamous carcinomata and adenocarcinoma were recognised and were diagnosed accurately. The percentage of error with squamous carcinomata was only 6.7% while the percentage of error was 40.9% in the anaplastic variety, possibly due to loss of cellular and nuclear details. With the fluorescence technique preinvasive carcinoma was diagnosed accurately while the percentage of error with squamous carcinomata was 13.6%. There was no percentage of error in the diagnosis of adenocarcinoma but with anaplastic carcinoma the diagnostic error was 100%.

In the control group of cases the diagnostic accuracy was almost equal with the two techniques (Table IV) hereby proving that fluorescence microscopy is just as good, if not better than the Papanicolaou technique for early detection of cervical lesions. It can therefore prove of value in mass screening programmes for early detection of genital carcinoma.

TABLE IV

Diagnostic Accuracy With the Two Techniques

Technique	No. of cases	Normal	Chronic cervicitis	Dysplasia		
				Mild	Moderate	Severe
Papanicolaou	106	40 (37.6%)	56 (52.8%)	4 (3.7%)	4 (3.7%)	2 (1.9%)
Fluorescence Microscopy	106	40 (37.6%)	55 (51.8%)	4 (3.7%)	4 (3.7%)	1 (0.9%)

Merits and Demerits of the two techniques

Merits	Demerits
Papanicolaou Technique	
Staining materials are easily available and the stained slides can be stored for many years. The cytomorphological details were easily recognised and most pathological laboratories are familiar with this technique.	The staining and scanning procedure required more than an hour's time and sometimes erythrocytes obstructed the morphological details. The nucleoli were at times not distinguishable from pseudonucleoli.
Fluorescence microscopy	
The staining and scanning of the slides did not take more than 15 minutes. Nucleoli stained a brilliant colour and were distinguished from the unstained pseudonucleoli. The brilliance of cytoplasmic staining helped in quick and easy detection of abnormal cells and cytomorphological details were also preserved for a detailed study.	The stains had to be kept at a given pH. The slides had to be examined wet, as drying often led to loss of fluorescence. Undifferentiated carcinoma was not recognised, due to loss of cytoplasm and the presence of necrotic debris. The initial cost of this equipment is high and continuous and haphazard usage of the mercury quartz bulb, may damage it. This bulb is not available in our country so is not easily replaceable.

Some authors, Von Bertalanffy *et al* (1958), claim that this technique and equipment can be handled by untrained persons, whereas Liu (1961) in his comparative study reported that this technique was neither easier nor required less experience and skill, than the Papanicolaou technique. This was also corroborated by the results of the present study. Liu (1961) also reported that the coolur and brilliance of cytoplasmic fluorescence was not specific for malignant cells. He stated, that this technique of fluorescence microscopy also showed poor morphological details, which led to establishment of false readings.

Conclusions and Summary

In the present series, fluorescence microscopy and the Papanicolaou technique were used to evaluate a total of 458 smears, 246 being smears from carcinoma of various sites in the female genital tract, and 212 smears being from control cases of carcinoma cervix and chronic cervicitis.

It was seen that with the Papanicolaou technique, the diagnostic accuracy was 96.7% and there was no false positive report. The false negative reports were 3.25%.

With the fluorescence microscopy the diagnostic accuracy was only 79.5% probably due to the large number of cases of undifferentiated carcinomata, presenting poor cytoplasmic detail.

However, fluorescence microscopy proved of good diagnostic value in detecting preinvasive and invasive cervical lesions.

The diagnostic accuracy in detecting early cervical lesions was almost identical with the two techniques.

The cost of equipment for fluorescence microscopy is high, spare parts are not available and these factors combined with a lack of trained personnel will be a big hindrance in the routine use of this technique.

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