EFFICACY OF TOUIDINE BLUE STAIN TEST IN EARLY DETECTION OF CANCER CERVIX—PRELIMINARY COMMUNICATION*

by

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Cancer cervix with a high mortality has headed the list of female cancers in India. However, its early preinvasive detection and prompt treatment can considerably improve the survival rate. Inspite of existing techniques a constant search for better tests continues.

Application of various dyes to the cervical epithelium in vivo in conjunction with colpomicroscopy has been tried for a number of years. Richard (1963) based on his experience with Toluidine blue expressed the possibility of delineation of cervical carcinoma in situ from normal epithelium because of differential staining. The stain has also shown some merit in detection of oral and vulval carcinoma (Shedd, 1965; Collins, 1966). The present study was undertaken to find out its utility in early diagnosis of cervical cancer. Initial observations are presented as a preliminary communication.

Material and Methods

One hundred and eight women who attended Sassoon General Hospitals for various gynaecological complaints were included in the study. After thorough clinical examination they were subjected to following tests.

1. *Cytological Study of Cervical Smear:* After introducing sterile Cusko's speculum smear material was collected from cervical lips and the endocervical canal with the help of cotton tipped applicator. Two smears prepared from this were immediately dipped in 1:1 solution of ether and 50% alcohol for fixing and stained by Papanicolaou technique.

2. *Toluidine Blue Stain Test:* After cleaning the cervix, the gross macroscopic appearance was noted down and the entire exposed surface was painted with 1% acqueous solution of dye with a cotton tipped applicator. After allowing it to dry for 3-4 minutes, decolourisation with 1% acetic acid was attempted and areas with stain retention were noted. Based on the pilot study initially conducted, following scheme of classification was followed.

3. *Punch Biopsy of Cervix:* For comparison, biopsy was taken from both the areas—with and without stain retention.

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TABLE I
Toluidine Blue Test—Grading

Grade	Appearance of tissue	Reading	Interpretation
0	Dye not retained	—	Normal
1	Violet	±	Inflammation
2	Pale Blue	±	Hyperplasia
3	Deep blue	++	Cancer

Results and Discussion

Being a nuclear stain, the intensity of staining by Toluidine Blue appeared to be related to the number of nuclei per unit area of cervical epithelium. Blue colour was initially retained by both neoplastic as well as columnar epithelium—, mucus and intense inflammatory exudate associated with erosion. However, after decolourization with acetic acid, the non-neoplastic epithelium either was decolourized or retained only a small quantity of dye- whereas localised retention was seen in neoplastic tissue.

TABLE II
Macroscopic Appearance of Cervix and Staining

Cervix	Staining grade 0	1	2	3	Total
No gross pathology	11	-	-	-	11
Endocervisitis	8	7	2	-	17
Endocervisitis with erosion	12	11	10	32	65
Polyps	5	-	-	-	5
Ulcer	-	1	-	-	1
Hard nodular cervix	-	-	-	6	3
Cancer Cervix with cauliflower growth	-	-	1	2	6
Total	36	19	13	40	108

Reports of cytopathology were available in 96 of 108 total cases studied.

TABLE III
Stain Test and Cytopathological Characters

Stain grading	Cytopathological Report: Normal	Inflammation	Epidermidical hyperplasia	Epidermidical dysplasia	Cancer	Total
0	3	28	2	-	-	33
1	-	11	7	-	-	18
2	-	5	6	1	-	12
3	-	15	10	6	2	33
Total	3	59	25	7	2	96

Above data indicated association between violet staining and inflammatory changes while deeper shades of blue seemed to be associated with significant clinical entities.

Similar observations were made after comparing the results of stain test with histopathological reports which were available only in 51 cases.

TABLE IV

Stain Test and Histopathological Character

Stain grading	Histopathological Report				Total
	Inflammation	Epidermidization	Dysplasia	Cancer	
0	7	-	-	-	7
1	9	2	-	-	11
2	5	-	1	2	8
3	11	9	3	2	25
Total	32	11	4	4	51

Sensitivity and specificity of stain test in comparison with cytopathological and histopathological changes were calculated. Only '2' and '3' staining grades were considered positive and dysplasia and cancer as significant entities.

TABLE V

Sensitivity and Specificity of Stain Test in Comparison With Histopathological Appearance

Stain test	Cytopathology		Total
	Positive	Negative	
Positive	9	36	45
Negative	0	51	51
Total	9	87	96

$$\text{Sensitivity} = \frac{100 \times 9}{9} = 100\%$$

$$\text{Specificity} = \frac{100 \times 51}{87} = 58.6\%$$

TABLE VI

Sensitivity and Specificity of Stain Test Comparison With Histopathological Appearance

Stain Test	Histopathology		Total
	Positive	Negative	
Positive	8	25	33
Negative	0	18	18
Total	8	43	51

$$\text{Sensitivity} = \frac{100 \times 8}{8} = 100\%$$

$$\text{Specificity} = \frac{100 \times 18}{43} = 42\%$$

The results thus indicated the stain test to be an extremely sensitive test for detection of malignancy, but with a limited specificity as many dye retained areas showed only inflammatory pathology. The utility of the test for early diagnosis of cancer, in a hospital well equipped with diagnostic facilities, seems to be low. However, the test, due to its remarkable sensitivity and extreme simplicity may serve as an useful tool for mass screening in the field, specially so in places like primary health centres where the medical officer can easily spot out the suspicious cases for further investigations. The test will also be useful in localising the site for biopsy due to retention of dye by neoplastic tissue.

References

1. Collins, C. G.: Advances in Obstetrics and Gynaecology, Edited by Marcus, S. L. and Marcus, C. C.: Vol. I, Published by Williams and Wilkins Co., Baltimore. **603: 1967.**
2. Collins, C. G.: J. Obst. & Gynec. **28: 158, 1966.**
3. Richard, R. M.: Am. J. Obst. & Gynec. **86: 703, 1963.**
4. Shedd, D. P., Hukill, P. B. and Bahn, S.: Am. J. Surgery. **110: 631, 1965.**