EFFICACY OF TOLUIDINE BLUE STAIN TEST IN EARLY DETECTION OF CARCINOMA CERVIX

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SUMMARY

Two hundred and thirty two women presenting with symptoms suggestive of cervical disease were submitted to cytological study of cervical smear, toluidine blue stain test and directed wedge biopsy of the cervix. In all, 70% women had benign lesions and 30% had malignant lesions. None of the 154 women with grade 0 or 1 staining had positive cytological smears as against 56.3% of those with grade 2 or 3 staining. None of the lesions from grade 0 staining group revealed any significant histopathological finding. Malignant lesions were detected in 15.6% of grade 1,67.3% of grade 2 and all cases of grade 3 staining pattern. The overall sensitivity and specificity of the stain test in comparison with histopathology was around 89%. The test being simple, cheap and feasible even in field conditions can be recommended as a screening test for carcinoma cervix for rural women and also for taking directed biopsies from the cervix.

Introduction

Carcinoma of the cervix uteri is the commonest genital tract malignancy in females in India and has got a very high mortality mainly because of its late diagnosis. The lesion is known to exist in its preclinical microscopic noninvasive form for years preceeding the actual occurrence of invasive cancer.

Exploitative cytology as a screening test and colposcopically directed cervical biopsy for cases with abnormal cytological smear have made it possible to detect these early cervical intraepithelial neoplasia which can then be eradicated by relatively simple treatment. However, high cost and special expertise required for interpretation of these tests have made such early detection possible only at special institutions. Search for a simple, cheap, sensitive test for screening as well as for taking directed biopsy therefore has to be continued. Efficacy of Toluidine blue stain test in this respect is evaluated and presented in this paper.

Material and Methods

Two hundred and thirty-two women attending gynaecological outpatient depart-

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ment of Sassoon General Hospitals, Pune over a period of two and half years were included in the study. Most of them had symptoms suggestive of cervical disease. After thorough clinical examination, all patients were submitted to following tests.

1. *Cytological study of cervical smear.*

Smears were taken for each patient from cervical lips and from endocervical canal with the help of a cotton tipped applicator, which were immediately dipped in 1 : 1 solution of ether and 90% alcohol for fixing and then stained by Papanicolaou stain. The smears were graded as suggested by Papanicolaou (1948) (Grade I and II — Negative, Grade III — suspicious, Gr. IV and V positive.)

2. *Toluidine blue stain test*

Cervix was thoroughly cleaned with mucolytic solution of 1% acetic acid. It was then painted uniformly with 1% solution of toluidine blue. The stain was allowed to act for 1 minute and then removed by painting the cervix again with 1% acetic acid. The stained areas on the cervix were noted and graded as suggested by Shrotri *et al* (1978). Grade O -dye not retained, Gr. I-Violet, Grade 2-Pale blue and Gr. 3-deep blue. Grade O and 1 were regarded as benign or negative and grade 2 and 3 were considered as positive suggestive of malignancy.

3. *Directed Biopsy of Cervix*

A directed wedge biopsy was then taken from stained area including some unstained area also and the tissue was submitted to histopathological study.

Correlation of staining grade of toluidine blue was done with grading of cytopathological smears and also with findings on histopathological examination of biopsy specimens.

Observations

Out of 232 patients, 55% were in the third decade of life and another 35% were between fourth and fifth decade. Most of the patients were mutiparous, only 3.4% being nulliparous.

Seventy-five percent women presented with excessive vaginal discharge and low backache was experienced by nearly 45% women. Macroscopic lesions seen on the cervix are listed in Table 1, which shows that 20 patients had visible growth on cervix.

TABLE I

Macroscopic Appearance of Cervix

Sr. No.	Macroscopic appearance	No. of cases	Percentage
1.	No gross pathology	16	6.9
2.	Cervical erosion with endocervicitis	91	39.2
3.	Hypertropried unhealthy cervix	52	22.4
4.	Endocervicitis	27	11.6
5.	Hard nodular cervix	19	8.2
6.	Polyps	7	3.1
7.	Visible growth on cervix	20	8.6

Histopathological findings

In all, 70% women had benign lesions and 30% had malignant lesions. These were mild dysplasia in 11.6%, moderate dysplasia in 6% and severe dysplasia in 2.2% patients. Two patients were diagnosed as intraepithelial carcinoma of cervix and 1 had microinvasive carcinoma. In 20 patients clinically suspected to be having invasive carcinoma, the diagnosis was confirmed histopathologically.

Toluidine Blue Staining

One hundred and three (44.4%) women had grade O staining, 22% had grade I, another 22% had grade 2 and remaining 26 out of 232 women exhibited grade 3 staining. All 20 cases suspected to be having invasive carcinoma had grade 3 staining.

Stain test and cytopathology

Out of 232 smears, 74.6% were negative, 6.5% were suspicious and 19% were positive smears. Table II shows the correlation of results of stain test with cytopathological grading.

TABLE II

Correlation of Toludine Blue Stain Test Results with Cytopathological Grading

Staining grade	Cytopathological Grading					Total
	I	II	III	IV	V	
0	35	65	3	—	—	103
1	3	42	6	—	—	51
2	—	24	4	12	12	52
3	—	4	2	4	16	26
Total	38	135	15	16	28	232

Out of 154 women having grade O or 1 staining, none had positive smears, however 9 (5.8%) patients had suspicious smears.

Out of 78 patients having grade 2 or 3 staining, 44 (56.3%) had positive smears and 6 (7.7%) had suspicious smears. 36% women from this group had negative cytological smear.

Stain Test and Histopathology

Table III shows the correlation of results of toluidine blue staining with histopathological findings of biopsy specimens.

TABLE III

Correlation of Toludine Blue Stain Test Result with Histopathological Findings

Staining grade	Histopathological Report						
	Chr. Cervi.	Spider-midisation	Dysplasia			Cancer	Total
			Mild	Moderate	Severe		
0	89	14	—	—	—	—	103
1	14	29	6	2	—	—	51
2	12	5	21	11	—	3	52
3	—	—	—	1	5	20	26
Total	115	48	27	14	5	23	232

None of the lesions from grade O pattern revealed any significant histopathological finding. Thus, this grade can be considered as benign. Cytological grading of the same 103 patients also revealed no positive smear. However, 3 patients had suspicious smears. Thus 3% patients from this group were overdiagnosed by cytology.

Out of 51 patients exhibiting grade I staining, 6 had mild dysplasia and 2 had moderate dysplasia. Thus, by considering grade I staining as benign, 4% patients having moderate dysplasia were missed by the test. These 2 cases of moderate dysplasia as well as 4 out of 6 cases diagnosed as mild dysplasia had suspicious cytological smears. Hence if cytology is combined with grade I staining result, no patient having significant dysplasia is likely to be missed.

Thirty-five out of 52 (67.3%) cases manifesting grade 2 staining pattern had malignant lesions. Of these 3 patients were not suspected to have any gross pathology on cervix who on histopathology revealed carcinoma in situ in 2 cases and microinvasive carcinoma in one. As 17 patients with benign cervical lesions also had grade 2 staining, the specificity of this grade for diagnosing malignant lesions appears to be limited. However, detection of 3 clinically unsuspected cases of carcinoma justifies inclusion of this grade in positive test. Cytology revealed only 24 positive and 4 suspicious smears in these 52 patients, while histopathology reported 35 patients with malignant lesions. 14% being under diagnosed by cytology.

None of the 26 patients having grade 3, staining had benign lesions on histopathology. Twenty had invasive carcinoma (these had growth on cervix), 5 cases had severe dysplasia and 1 had moderate dysplasia.

Table IV denotes the sensitivity and specificity of the toluidine blue stain test in comparison with histopathological findings. The test had a sensitivity of 88.4% (false negative rate of 11.6%) and a specificity of 89.6% (false positive rate of 10.4%).

TABLE IV

Sensitivity and Specificity of Toludine Blue Stain Test in Comparison with Histopathological Character

Stain test	Histopathology		Total
	Positive	Negative	
Positive	61	17	78
Negative	8*	146	154
Total	69	163	232

$$\text{Sensitivity} = \frac{100 \times 61}{69} = 88.40\%$$

i.e. false negative rate $\pm = 11.6\%$

$$\text{Specificity} = \frac{100 \times 146}{163} = 89.57\%$$

i.e. false positive rate $= 10.4\%$.

* Six out of these 8 are mild dysplasia and 2 were moderate dysplasia, none belonged to category of CIN 3.

Discussion

Being a nuclear stain, the intensity of staining by Toluidine Blue appears to be related to the number of nuclei per unit area of cervical epithelium, the amount of chromatin and the size of nucleia in the cell. Richart (1963) described application of this test for in vivo deliniation of dysplasia and carcinoma in situ of the uterine cervix for which he found it to be much more accurate than Schiller's test. Shashiprabha *et al* (1973) Shashimani Pande *et al* (1977) and Shrotri *et al* (1978) have later reported similar observations.

The test has been reported to be reasonably sensitive but rather less specific for early detection of cervical neoplasm. Singh and Das (1982 and 1983) have combined this test with colposcopy and also have reported remarkable sensitivity of the stain test.

The observations in the present study suggested that with staining grade O and 1, presence of cervical malignancy can reasonably be excluded even at peripheral health centres.

The test is 100% sensitive for detecting CIN 3 lesions, microinvasive carcinoma and invasive carcinoma as no case with these lesions was missed by the test.

The test is extremely simple, cheap and feasible even at primary health center. Unlike colposcopy its interpretation does not require any expert person. Considering the high efficiency of the test alongwith these advantages, the test can be recommended for routine use as a screening test for all rural women who are at a greater risk of development of cervical cancer. In the hospital, it can certainly be used for taking directed biopsies.

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