

IN VIVO STAINING OF DYSPLASIA AND CARCINOMA OF UTERINE CERVIX

by

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Introduction

The difficulties encountered by the pathologist in the diagnosis of early malignant lesions of the uterine cervix are mainly due to inadequate biopsy samples. The gross appearance of a cervix harbouring dysplasia or carcinoma in situ is frequently normal. Schiller's iodine test introduced into the gynaecological clinics as early as 1928, is very helpful in delineating areas of normal epithelium. However Richart in 1963 carefully evaluated the Schiller's test by means of colpomicroscopy and found a significant abnormality with the test. The present study is undertaken to evaluate the effectiveness of Toludine Blue Test over Schiller's iodine test, if any, in delineating areas of dysplastic or carcinomatous epithelium of uterine cervix.

Materials and Methods

Biopsies were taken from patients who attended the Gynaecological out-patient of the S.A.T. Hospital, Medical College, Trivandrum to delineate the atypical

areas for punch biopsy, seventy-three cases were stained with Schiller's iodine and 72 cases with Toludine blue.

Schillers Iodine

The cervix was exposed by Sim's speculum and mucus was completely removed by using cotton swab dipped in one per cent Acetic acid. Then the cervix was painted with Schiller's iodine.

Unstained areas were noted as Schiller positive and stained (mahogany brown) areas as Schiller negative (Fig. 2). Biopsies were taken from Schiller positive areas and also from Schiller negative areas to assess the accuracy of the test.

Toludine Blue

After removing the mucus with 1% Acetic acid, the cervix was painted with 1% aqueous Toludine blue solution using a cotton swab. After 5-8 mts. the excess dye was removed using cotton swab dipped in 1% acetic acid and stained areas of the cervix were noted. Areas which had taken up dark royal blue colour were taken as positive areas. (Fig. 3).

Observation

Of the 73 cases which were examined after staining with Schillers iodine, 39 cases were Schiller negative, i.e. they were stained mahogany brown, 30 cases did not take up any stain and 4 cases

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TABLE I
Findings of *in Vivo* Staining of 72 Cases of Uterine Cervix With Toluidine Blue

	Dys- plasia	Carcinoma in situ and early invasive carcinoma	Normal and Benign lesions	Total
Schiller positive	8	17	9	34
Schiller negative	16	1	22	39
False positive	— 12.3%			
False negative	— 23.2%			

showed a faint staining, i.e. 34 cases were schiller positive.

Of the 34 Schiller positive cases the histological diagnosis of the cervix is given below:

Carcinoma	..	17
Dysplasia	..	8
Benign lesions		
Squamous metaplasia	..	4
Metaplasia with reserve cell hyperplasia	..	3
Chronic cervicitis	..	2

It shows that epithelial abnormalities like squamous metaplasia and reserve cell hyperplasia can give false positive reaction.

metaplasia 5, chronic cervicitis 10 and reserve cell proliferation 3. Thus it gave a false positive rate of 28.5%. However, there was not a single false negative case.

Discussion

Schiller's iodine test is widely accepted as a method for delineating areas of neoplasia of the uterine cervix. Normal epithelium is rich in glycogen which combines with iodine to form a mahogany brown colour. The test is based on schiller's original concept that glycogen is absent in neoplastic epithelium. Later on Foraker *et al* (1956) demonstrated glycogen in 13 out of 21 cases of carcinoma of the cervix. Columnar epithelium and metaplastic epithelium fail to

TABLE II
Findings of *in Vivo* Staining of 72 Cases of Uterine Cervix With Toluidine Blue

Finding	Total	Dys- plasia	Carcinoma in situ and early invasive carcinoma	Normal and benign lesions
Negative	9	0	0	9
Positive	63	31	14	18
False positive	28.5 percentage			
False negative	Nil			

Of the 72 cases of cervix studied by staining with toluidine blue, 63 cases were toluidine blue positive. Of these, 18 were benign lesions like squamous

take up the stain. In the present study 16 cases of dysplasia and 1 case of carcinoma in situ gave false negative results.

Richart (1963) carefully evaluated the

Schiller's test by means of colpomicroscopy and found that about one quarter of patients with carcinoma in situ and nearly one half of the patients with dysplasia failed to display a significant abnormality with the test.

Toluidine blue dye is a metachromatic nuclear stain. Cervical epithelium is stained by this dye in vivo with an affinity and intensity that appears to be closely related to the number of nuclei per unit area. After the removal of excess stain the retained stained area is composed of dysplastic or neoplastic epithelium. Sometimes there may be areas of epithelium retaining the dye but which are not neoplastic such as columnar epithelium or areas of intense inflammatory infiltration. However, these areas can be differentiated from the neoplastic epithelium by their tinctoral qualities. The columnar epithelium and inflammatory areas stain dirty violet and the neoplastic epithelium stains shades of royal blue. Since the intensity of the stain is correlated with the nuclear density, the severity of the neoplastic process can be judged roughly from the shades of blue, which ranges from a pale royal blue in mild dysplasia to a very intense royal blue in carcinoma in situ. In the application of the toluidine blue stain the most important part is the removal of excess dye with one per cent acetic acid. The acetic acid should be applied uniformly but lightly over the cervix in order to remove the stain from all areas at about the same rate. Areas of mild dysplasia may be very sensitive to decolourisation, if the acetic acid is applied too liberally or too vigorously. Areas of moderate or severe dysplasia or carcinoma in situ are not sensitive to the decolourisation.

In the present study no false negative case was found though there were 27.7%

of false positive cases. The accuracy of diagnosis for dysplasia and carcinoma in situ was 72.3%. Of the 2 cases of carcinoma in situ, 1 case was missed in cytology. However, since the patient had irregular bleeding, she was subjected to fractional curettage. On painting the cervix with toluidine blue, there was a small area of intense royal blue staining on the posterior lip of the cervix which was biopsied and was proved to be a carcinoma in situ.

Toluidine blue test is widely accepted in delineating areas of dysplasia and carcinoma of oral cavity with great success. Shedd *et al* (1967), Strong and Vanghan (1968) Prabha *et al* (1973) used the toluidine blue test for delineating areas of cervical dysplasias and carcinoma. They have showed that it is a very useful simple procedure for the purpose. According to them the number of false positives could be reduced by more thorough removal of mucus in the beginning and a better removal of the stain with one per cent acetic acid.

The advantage of toluidine blue over schiller's iodine is that the number of false negative in the case of the first test is nil, whereas the Schiller's test gives 23.2% false negative cases. The percentage of accuracy for diagnosing dysplasia and carcinoma for toluidine blue test is 72.3% and that for Schiller's iodine test 64.8%.

Conclusions

(1) Toluidine blue is proved to be advantageous over Schiller's iodine to delineate areas of dysplastic and neoplastic epithelium.

(2) The percentage of accuracy of diagnosing dysplasia and carcinoma for toluidine blue is 72.3% and that for Schiller's iodine is 64.5%.

(3) False negative results are nil for Toluidine blue test, whereas Schiller's iodine gave a false negative rate of 23.2%.

As the material is cheap and the test is simple and non-traumatic to the patient in vivo staining with toluidine blue can be utilised in all gynaecological clinics. Even in the absence of sophisticated instruments such as colposcopy and colpomicroscopy, neoplastic epithelium can be delineated for adequate biopsy and also for adequate excision during radical surgery.

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See Figs. on Art Paper V