

## TOLUIDINE BLUE IN DELINEATING AREAS OF DYSPLASIAS AND CARCINOMA OF CERVIX

by

S. SASHI PRABHA,\* M.B.B.S., D.G.O.

K. KAUSALYA,\*\* M.B.B.S.

R. S. REDDY,\* M.D., D.G.O.

and

C. R. R. M. REDDY,\*\* M.D.

The difficulties in the study of dysplasias and carcinoma-in-situ of cervix are due to lack of definite clinical criteria for these lesions and also due to the need of a reliable guide to indicate the exact site for a biopsy. Either multiple punch biopsies or cold-knife conization were used for doing biopsy. Whilst in the former the choice of site is difficult in the latter the choice of the outer limit of the cone is difficult to judge. One of the aids to choose the site for biopsy is macroscopic staining with toluidine blue, which has been done for delineating areas of dysplasia and carcinoma of oral cavity with great success (Niebel and Chomet, 1964; Shedd *et al*, 1967; Strong *et al*, 1968; Reddy *et al*, 1972). It was felt that the same procedure could be extended to the cervix for delineating areas for doing biopsy and also screening cases of carcinoma of cervix.

### *Material and Methods*

The procedure of staining is as follows. The patient is put in a lithotomy position and the cervix is exposed by a Sim's speculum. The mucus is completely removed by using cotton swabs dipped in 1% acetic acid. The macroscopic lesion

of the cervix is then noted. The cervix is then painted with 1% toluidine blue solution with a cotton tipped applicator. The stain is then removed by using cotton swabs dipped in 1% acetic acid. Stained areas of the cervix are then noted. The staining has been divided into the following types viz. blue staining, dark royal blue staining and areas which stain violet. The blue and dark royal blue stained areas were present in the squamous lined areas. The violet stained areas were always present in the columnar celled areas. The violet stained areas were taken as negative, and blue and dark royal blue stained areas were taken as positive and biopsied.

One hundred and sixty cases with various types of cervical lesions were stained and later biopsied. Fifteen of the 160 cases revealed a healthy cervix and the rest had unhealthy looking cervixes. Irrespective of whether they stained positive or not, the cervix was biopsied using a punch.

The sections were stained with H & E. Changes in the squamous lining with respect to atypia, carcinoma-in-situ, micro-invasive carcinoma or frank carcinoma were looked for. Apart from the above, hyperplasia of squamous epithelium, basal cell hyperplasia, reserve cell hyperplasia, squamous metaplasia of glands and regenerative hyperplasia were look-

\*Department of Obst. & Gynec.

\*\*Department of Pathology, Andhra Medical College, Visakhapatnam.

Received for publication on 22-5-72.

ed for according to criteria laid down by Govan *et al* (1966).

### Results

Fifty of the 160 cases did not take the stain. Thirty-seven had areas which were stained light blue, 32 stained dark blue and 41 stained violet.

(A) Of the blue stained 37 cases, the cervix was healthy in 5, while 32 cases had an unhealthy cervix.

Histopathologically the following types were seen in this group:

Cervix was normal	10 cases.
Mild dysplasia (Fig. 1)	21 cases.
Moderate dysplasia	2 cases.
Severe dysplasia (Fig. 2)	2 cases.
Carcinoma-in-situ (Figs. 3 & 4)	2 cases.

In 18 of the 21 cases with mild dysplasia, cervix was clinically unhealthy and 3 were healthy. The cervix was unhealthy in all the cases of moderate, and severe dysplasias and the cases of carcinoma-in-situ.

In 10 cases out of the 37 stained blue, the cervix was histologically normal giving a figure of 27% false positives. But this is more than compensated for with the advantage that 2 cases of carcinoma-in-situ were brought out when not clinically suspected.

(B) All the 32 cervixes having dark royal blue stain, were clinically unhealthy. Thirty out of these 32 were diagnosed as malignant clinically, but two were thought to be only erosions. On biopsy all the 32 were frank squamous cell carcinomas. The important finding in this group was the finding of the two carcinomas which were clinically not suspected.

(C) Clinically all these 41 cases staining violet were erosions of cervix and belonged to the group of unhealthy cervixes.

Seven out of these 41 cases histologically showed mild dysplasia. Thus 17% of the cases may show false negative. But all these may not be truly false negative, because the stain may not penetrate into the folds of the mucosa in erosions and thus not give the blue stain.

(D) Unstained cases were 50.

Ten out of the 50 unstained cases had a healthy cervix and the remaining 40 had unhealthy cervixes.

On biopsy, 8 out of these 50 cases showed mild dysplasia, giving 16% false negative. All the 8 were from unhealthy cervixes.

### Comment

Toluidine blue is a member of the thiazine group of metachromatic dyes. It is soluble in water (upto 3.5%) and also in alcohol (upto 0.5%). Both the D.N.A. and R.N.A. of the cells can fix toluidine blue. Toluidine blue can stain 3 to 4 or more cells deep of the squamous lining when the cells are loosened as in tumours. Parakeratotic areas may stain as the cells contain nuclei in the keratin. The stain can only delineate the superficial squamous epithelium. The stain can be useful in dysplastic areas where there could be mild spongiosis and the stain percolates in between the cells and stain the cells.

It was found to be quite useful in the study of oral carcinoma and precancerous lesions (Reddy *et al*, 1972). Richart (1963) found it to be useful for the study of cervical lesions and he was of the opinion that toluidine blue staining was more useful than Schillers test.

In the present study the following points are well brought out. Two cases of carcinoma unsuspected clinically were shown up as dark royal blue stained areas by toluidine blue staining. Two cases of carcinoma-in-situ unsuspected

clinically were shown up as toluidine blue positive areas. All the carcinomas stained deep royal blue and there were no false positives or false negatives in this group. All the moderate dysplasias, severe dysplasias and carcinomas-in-situ also were toluidine blue positive and no false positives or negatives were seen in this group also. Most of the mild dysplasias were toluidine blue positive. In this group there were 27% false positives and 16% false negatives. False positives or false negatives should not matter much as mild dysplasia is not of much importance clinically or pathologically. The number of false positives could be reduced by more thorough removal of mucus in the beginning and a better removal of the stain by 1% acetic acid. The few false negatives seen in the erosion group (violet stained) could be due to the fact that the stain might not have percolated into the folds of the mucosa in which there was dysplasia. The most important feature of this stain is the ease with which the staining can be done and the ease with which the area to be biopsied is delineated. In none of the lesions which matter, like moderate and severe dysplasia, carcinomas-in-situ and carcinomas, there was false positives or negatives showing that this staining procedure aids in pin pointing the biopsy site.

### Summary

Staining of the cervix with toluidine blue in 160 cases and later biopsy of the positive areas have shown that it is a very useful procedure in delineating carcinomas, carcinomas-in-situ, severe dysplastic areas and moderate dysplastic areas.

### Acknowledgement

Our thanks are due to K. Ch. Appalawamy, Madhava Rao and P. Nagabhushana Rao for the help rendered.

### References

1. Govan, A. D. T., Haines, R. M., Langley, F. A., Taylor, C. W. and Woodcock, A. S.: *The Jour. Obst. & Gynec. of Brit. Emp.* 73: 883, 1966.
2. Niebel, N. H. and Chomet, B.: *J. Amer. Dent. Assoc.* 68: 801, 1964.
3. Reddy, C. R. R. M., Ramulu, C., Sundareswar, B., Raju, M. V. S., Gopal, R. and Sarma, R.: *Bull. Wld. Hlth. Org.* (Sent for publication).
4. Richart, R. M.: *Amer. Jour. Obst. & Gynec.* 86: 703, 1963.
5. Shedd, D. P., Hukill, P. B., Bahn, S. and Ferraro, R. H.: *Arch. Surg.* 95: 16, 1967.
6. Strong, M. S., Vanghan, C. W. and Ineze: *Arch. Otolaryng.* 87: 527, 1968.

*See Figs. on Art Paper V*