BETA-GLUCURONIDASE ACTIVITY IN VAGINAL FLUID WITH SPECIAL REFERENCE TO CANCER CERVIX

by

NAWAL KISHORE, M.S., F.A.C.S., (U.S.A.)

SNEH SINGH, M.B.B.S.

The problem of cancer cervix occupies an important place in gynaecological practice and research. Even in apparently favourable cases results of treatment are disappointing as the diagnosis is not made early. Any procedure which might facilitate earlier diagnosis or indicate most appropriate therapy for individual patient merits careful consideration. Diagnosis of carcinoma-in-situ and early invasive carcinoma is being made by cytological studies of vaginal smears and surface biopsies, but the technique, being time-consuming and calling for considerable skill and experience, is practicable only in limited number of specialized laboratories. Neither cytology nor histology can fortell the ultimate behaviour of any given lesion labelled as carcinoma-in situ or intraepithelial carcinoma. Biochemical tests like estimation of Beta-glucuronidase activity in vaginal fluid and vaginal fluid alpha-Mannosidase, seems to offer potential advantages. As a diagnostic screening procedure a biochemical test would obviate personal element, which is so important in interpretation of cytological and histological tests. Moreover, as observed by James Lawson such a test might provide useful information about the activity of epithelial abnormalities. It might serve to differentiate progressive from static or reversible lesion.

Beta-glucuronidase is an enzyme widely distributed in mammalian tissue whose properties, functions and behaviour have been reviewed by Fishman, Anlyen, Spinger, Brunetti in 1940-1950 and Odall and Burt in 1948-50. The function of Beta-glucuronidase has not yet been fully established. Not only is it essential for release of active hormone from steroid hormone glucuronidase in certain organs, but it also plays some role in catabolism of glucuronic acid containing poly-saccharides. The enzyme is present intra-cellularly in cytoplasmic granules and is evenly distributed in all mammalian tissues. Activity varies in different tissues and species and is found to be enhanced in tissue growth, repair, proliferation and malignancy as found by Fishman Kasdon and Homburgeer in 1950. The evidence at hand suggests some inter — relationship between Beta-glucuronidase activity, steroid hormone growth phenomenon, cellular proliferation and malignancy.

Observations on cancer tissue

Department of Obstetrics & Gynaecology S. N. Medical College, Agra.

Received for publication on 11-2-63.

showed marked difference in concentration of enzyme level in normal and cancerous tissue. Odell and Burt, in 1949, found striking difference in enzyme activity in vaginal fluid of normal and cancer cervix patients. This led them to state that estimation of this enzyme activity could be used as a diagnostic test for cancer cervix.

In our series of cases we have tried to determine (1) enzyme activity in different age groups in non-pregnant, non-cancerous patients, excluding all cases of vaginitis of any type and (2) to determine enzyme level in cancer cervix patients.

Material and Method

Cases were selected from patients attending gynaecological out-patient department of S. N. Hospital, Agra.

Method of Collection of Specimen

Vaginal fluid specimen was secured before any vaginal examination (digital or instrumental) had been made or lubricating jelly used. Douches were interdicted for 12 hours before specimen was obtained. Standard Papanicolaou glass pipette was used (16 Cm, 0.6 Cm. diameter) with distal end polished to reduce the capillary bore to approximately 1/3 of original diameter. A standard Papanicolaou 2 ounce rubber suction bulb was attached to clean dry pipette.

Vaginal fluid was obtained by compressing the bulb and inserting the pipette high into the posterior vaginal vault. Fluid was collected by releasing the bulb and slowly sweeping the pipette across vaginal wall while it was withdrawn. Fluid was then expelled from pipette into carefully weighed standard Wasserman's tubes (10 cm., long and 1.3 cm. diameter). The specimen was kept at 4°C till the time of analysis.

Procedure of Test (Fishman Homberger 1950)

Weight of the tube with vaginal fluid was recorded and the contents were mixed thoroughly. From well homoginate, 0.1 c.c. portion were pipetted first into 2 Wasserman's tubes containing 0.8 c.c. of 1/10 M acetate buffer (PH 4.5) and 0.1 c.c. 1/100 M phenolphthalein glucuronidase and then into their d tube containing 0.8 c.c. of 1/10 M acetate buffer. These tubes were incubated for 2 hours at 38°C and reaction was stopped by placing the digest in boiling water for 1 minute. 1½ c.c. of water was added to the digest and after centrifuging 2 c.c. of each supernatents were transferred to colorimeter tubes 2.5 c.c. of alkaline glycine reagent (PH 10.45), 1.0 c.c. of 5% trichloracetic acid and water to complete 6 c.c. volume was added in order given. 0.1 c.c. of 0.01 M phenolphthalein glucuronidase was added to the control tube which was used in colorimeter to obtain the setting at 0. After thorough mixing, readings of other tubes were taken with 540 filter. Optical density values were substituted in phenolphthalein calibration curve.

Calculations

$$\frac{\text{(Micrograin phenolphthalein liberated in digest X 100)}}{\text{Hours of incubation X weight of fluid analysed)}} = \text{Unit glucuronidase/gm. vaginal fluid.}$$

Precautions

(1) Gravimetric method of collection of sample was observed.

(2) Specimen was kept at 4°C before estimation was done.

(3) Mixing of blood with the specimen was avoided.

Observations

Fifty-two non-cancerous non-pregnant, pre-menopausal cases were examined. Out of them 38 cases were between 15 and 35 years of age and 14 were of age group 36 — 55.

Forty-four patients with invasive cervical cancer proved clinically and histologically were examined and total of 47 vaginal fluid specimens were estimated for enzyme.

Table 1 gives mean levels of Beta-glucuronidase in vaginal fluid in different age groups.

Beta-glucuronidase activity in vaginal fluid of non-pregnant and non-cancerous cases is depicted in Table II along with Fishman series.

Table III shows Beta-glucuronidase activity in vaginal fluid of untreated cancer cervix cases along with finding of Fishman.

TABLE III

Beta glucuronidase activity. Units per gm. of vaginal fluid	Our findings (No. of cases)	Fishman series (No. of cases)
0-100	1	5
101-200	7	5
201-300	4	4
301-400	7	6
401-500	7	5
501-600	5	5
601-700	5	6
701-800	3	5
801-900	2	3
901-1000	2	5
1001-1500	2	5
1501-2000	1	3
2001-2500	1	0

Comments

A total of 52 non-cancerous pre-menopausal women were examined, 19 cases of postmenopausal age, and 44 cases of invasive carcinoma were also studied.

TABLE I

Age group	No. of cases examined	Mean average of enzyme in units per gm. of vaginal fluid	80% level
15-35	38	182.36	250 units
36-55	14	257.8	300 „ „
15-55	52	220.08	276 „ „
Total			

TABLE II

	20-40 years			41-60 years					
	No. of cases	80%	Mean value	No. of cases	80% value	Mean	No. of cases	80% value	Mean
Our findings	41	250	170.8	11	350	285.72	19	700	507.8
Fishman series	190	250	192.2	98	400	299.7	162	850	525.6

Table I reveals that the age of the patients examined ranged from 15 to 55, the average values for enzyme varies from 182.36 units/gm. to 257.8 gm. If 200 units is taken as the dividing line between normal and suspected cancerous level the number of false positives comes to 34.2% in younger age group and 64.3% in older age group. However, if we take 300 units as a demarcating line as suggested by Fishman et al, our percentage for false positives falls to 16.3% in younger age group and 21.42% in older group as shown in Table IV below.

TABLE IV

Age group	No. of cases examined	No. of cases having value more than 300 units/gm. vaginal fluid	Per cent of false positive cases
15-35	38	6	16.3
36-55	14	3	21.42
Total	52	9	17.3

In the postmenopausal women the mean value for enzyme was found to be 507.8 units per gram of vaginal fluid. Taking 300 units as measure we find 63.1% false positive cases. Comparing our result with Fishman and Kasdor we find our values are lower; of course our series were comparatively smaller.

Table III gives our values as found in cases of cancer along with Fishman's series. Out of 44 patients 9 cases gave values below 300 units per gram thus giving a false negative result of 20.4%. We also find that values over 800 units per gm. were seen only in 8 cases out of 44 i.e. 18.5%. A critical study of Table II reveals that the number of cases are evenly distributed upto 1500 units per gm. of vaginal fluid in Fishman series; however in our series the number of cases gradually fell as higher levels are seen. This might be due to the fact that in our series maximum number of cases is between 40 to 55 years of age, while in Fishman series age group goes up to 90 years.

A point worth commenting in our series is low value in women having sterility. We found that out of 9 cases of sterility, in 80% the mean value for enzyme was 104 units per gm., the average figure for all sterility cases being 81.6. units per gm.

Relationship between cellular activity, mitosis and enzyme activity has been accepted by all the workers. The increased activity of Beta-glucuronidase in genital cancer in the luteal phase of the cycle and in pregnancy has been due to its close association with cellular growth. Fishman et al stated that the high Beta-glucuronidase activity may be associated with decreased ovarian function. Cytological studies have shown no lowering of hormone level in cancer cases. Thus it is difficult to believe that high enzymatic level is due to deficient oestrogen level. There is no doubt as seen in figures already stated that there is signi-

ficant increase of Beta-glucuronidase in vaginal fluid in case of cancer cervix. However, the test has numerous limitations in its practical utility. Although we can not draw any definite conclusion in this small series of experiments it appears that in present form the test has a limited utility.

Summary and Conclusion

Fifty-two cases of non-cancerous pre-menopausal women, 19 cases of post-menopausal women and 44 cases of invasive carcinoma were studied to find out Beta-glucuronidase activity in vaginal fluid.

1. In the premenopausal women the value varied from 182.36 unit per gm. to 257.8 units per gm. If 300 units is taken as the demarcating line the number of false positives was 16.3% in younger age and 21.42% in older age.

2. In the post-menopausal women however with same standard the value comes to 507.8 units per gm.

3. In cases of cancer there were 20.4% false negatives.

4. A fact worth noting was that lower the age of patient after development of cancer, comparatively low figures of Beta-glucuronidase were found.

5. Women who were sterile gave low figures of Beta-glucuronidase activity. The average was 81.6 units per gram.

It appears from the study mentioned above that in its present form the test has very limited utility in the early diagnosis of cancer cervix.

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