

CHAPTER - V

HISTOLOGICAL AND HISTOCHEMICAL OBSERVATIONS
AND DISCUSSION ON MUCOSUBSTANCES IN NORMAL
AND CANCEROUS VAGINA

The present literature about vaginal cancer showed that, as compare to other genital organ cancers vaginal cancer showed very less occurrence of malignancy. Due to less occurrence of the vaginal malignancy least attention was paid to it. The work uptill now did purely about its therapy that is radiation as well as chemotherapy. Hence very less attention paid about its histochemistry. So present investigation is carried, out in which normal and cancerous vagina was taken, and histochemical observations taken and summarised in table number 6 and 7. From these both normal and cancerous vagina histochemical observations, mucopolysacchrides were compared. The histochemical and histochemical observations in normal and cancerous vagina given hereafter and illustrated photomicrographically in plate No.7, Figs 1 to 8.

OBSERVATIONS

I) Histological observations of normal Vagina.

Anatomically the vagina is the last part of genital tract. This portion some what long as compare to cervix uteri or corpus uteri. Histologically the vaginal wall consist of three layers viz. an outer fibrous, a middle muscular, and inner mucus. The chief interested layer for the present investigation is mucus layer, made with stratified squamous epithelium. The epithelium showed small papillae, so that the basement membrane on which the stratified epithelium rests also showed irregular wavy outline.

There are normally no glands in the vagina,

though rarely glands observed that are from cervix. For histological observations the sections of 5 μ were taken And the Hematoxylene - Eosin staining technique was did and observed. Stratified squamous epithelium and connective tissue observed as shown in Fig.1.

II) Histochemical Observations of Normal Vagina.

From the normal Vagina the stratified epithelium is of more interest for the present investigation because the cancerous condition is of arises from stratified epithelium. Hence histochemical site for present investigation is stratified squamous epithelium of normal vagina and squamous cell carcinoma. The histochemical observation of normal and cancerous vagina are given in table No.5:

A) Stratified Squamous Epithelium. This histochemical site showed intense PAS activity (Fig.2) and that activity was slightly diminished after saliva digestion. This reaction showed presence of glycogen in it. The PAS intensity was slightly diminished after phenylhydrazine treatment, showed probable presence of acidic mucosubstances and neutral mucins. This histochemical site showed moderate alcianophilia with AB pH 1.0 (Fig.3) showed probable presence of sulfomucins. When stained with AB pH 1.0 - PAS showed bluish pink staining. This site also showed

metachromasia at azure A at 1.5 pH levels. The alcianophilia was also observed with critical electrolyte concentrations techniques at 0.1 M Mg^{++} and 0.2 M Mg^{++} concentration. Hence it was concluded that it showed presence of sulfomucins.

This histochemical site showed also moderate alcianophilia with AB pH 2.5 (Fig.4). Bluish pink staining was observed with AB pH 2.5 - PAS technique showed probable presence of carboxy mucins. That was also confirmed after sequential staining method like AF-AB pH 2.5 as well as by CI - PAS technique. The alcianophilia gets restored after mild and active methylation. After acid hydrolysis there was no change in alcianophilia hence this site showed probable presence of hyaluronic acid. That was also confirmed after hyaluronidase digestion. After sialidase digestion no change in alcianophilia hence sialic acid absent.

From these all above histochemical reactions it was confirmed that there was presence of neutral mucin sulfomucins, hyaluronic acid and glycogen but absence of sialic acid.

B) Connective Tissue. The region just under the stratified squamous epithelium is connective tissue or stroma. This histochemical site showed moderate PAS reactivity (Fig.2). The PAS reactivity was reduced to half after

prior phenylhydrazine treatment. Hence there was probable presence of acidic mucins. After saliva or amylase digestion the PAS activity gets decreased slightly, hence there must be presence of glycogen.

Moderate alcianophilia was observed with AB pH 1.0 (Fig.3). Bluish pink staining was observed with AB pH 1.0 - PAS. Pink staining observed with AF and bluish pink with sequential staining technique AF - AB pH 2.5. These reactions showed presence of sulfomucins. Metachromasia also observed with azure A at all pH levels 1.5 to 4.5. The alcianophilia was observed with critical electrolyte concentration at 0.1 M and 0.2 M Mg^{++} concentrations.

This histochemical site showed alcianophilia at AB pH 2.5 (Fig.4). And bluish pink staining was observed with sequential staining AB pH 2.5-PAS. The staining reaction was also observed with CI and CI-PAS. The alcianophilia was restored after demethylation at 37° C and 60° C. Alcianophilia not affected by acid hydrolysis at 60° C showed probable presence of hyaluronic acid. The presence of hyaluronic acid confirmed after hyaluronidase digestion. No change in alcianophilia after sialidase digestion. These reactions showed presence of hyaluronic acid and absence of sialic acid.

Hence in the connective tissue presence of neutral mucins, glycogen, sulfomucins and hyaluronic acid and

absence of sialic acid.

III) Histological Observations of Epidermoid Type of Cancer

The vaginal cancer at present is very rare. The stratified squamous epithelium showed malignancy called epidermoid or squamous cell carcinoma. Squamous cell carcinoma showed over growth and invade in the connective tissue. The nucleus showed more chromatin material which was observed from Hematoxylene-Esoin staining technique as shown in Fig.5. The cytoplasm of these malignant squamous cell became very less. The cells showed necrotic changes. The cells showed many shapes like polygonal, cuboidal, coloumner type. The stroma is very scanty or sometimes abundant. Most of the characters of squamous cell carcinoma were like that of the squamous cell carcinoma of cervix, uteri-keratin pearls were also developed. Sometimes. The cells showed great compactness and hyperchromatic nucleus (Fig.6).

IV) Histochemical Observations of Vaginal Epidermoid Cancer.

Various histochemical methods were done. The observations were given in table in table No.5. The squamous cell carcinoma or epidermoid cancer show moderate PAS reactivity. The PAS activity was diminished with prior phenylhydrazine treatment, showed probable presence of acidic mucosubstances. The PAS staining intensity was not

diminished by diastase or amylase digestion demonstrate absence of glycogen.

The histochemical site showed alcianophilia AB pH 1.0. The sequential staining method AB pH 1.0 - PAS (Fig.7) showed bluish pink staining. The pink staining was observed with AF and pinkish blue with AF-AB pH 2.5. The metachromasia was observed with all pH levels from 1.5 to 4.5 of azure A, staining technique. Alcianophilia was observed with critical electrolyte concentration at 0.1 M Mg^{++} concentration. These results showed presence of sulfomucins.

Weak alcianophilia was observed with AB pH 2.5 staining technique. The sequential staining technique AB pH 2.5 - PAS (Fig.8) showed bluish pink staining. These primary reactions showed probable presence of carboxymucins. Blue staining was also observed with CI and bluish pink with CI - PAS sequential staining technique. Pink staining also observed with AF and pinkish blue with AF-ABpH 2.5. The alcianophilia was restored after demethylation at 37° C and 60° C. These reactions showed probable presence of carboxymucins. After acid hydrolysis the alcianophilia gets stored showed presence of hyaluronic acid. That was also confirmed by hyaluronidase digestion. The basophilia also slightly reduced by sialidase digestion.

metachromasia, critical electrolyte staining as well as modification of alcianophilia by methylation and demethylation, acid hydrolysis, enzyme digestion showed similar mucosubstances found in other organ system.

2. Distribution of Mucosubstances In Normal Vagina.

The present investigation was carried out to understand the nature of mucosubstances in normal vagina. Histologically the vagina differentiated into stratified squamous epithelium or mucosa and connective tissue. From above mentioned all histochemical reaction it is observed that in the stratified epithelium or mucosa layer having neutral mucins, sulfomucins and carboxymucins are of hyaluronic acid type were confirmed.

In the present investigation the mucosubstances of connective tissue or fibrous layer were studied. In this presence of neutral mucins, acidic mucins, sulfomucins and carboxymucins of hyaluronic acid confirmed and presence of glycogen.

3. Comparative Distribution of Mucosubstances In Normal Vagina.

This chapter deals with comparison between the present investigation and related past literature. The presence of glycogen has been reported in vaginal epithelium of woman (Matter, 1966; Toth and Gimes, 1966; Moursi et al., 1971; Gregoire et al., 1971). The present investigation showed presence of glycogen in normal vagina of woman.

The glycogen presence is also recorded previously many scientists. Sani (1954) demonstrated glycogen in superficial strata of the vaginal epithelium in woman, slightly less amount of glycogen was found in the lower layer but it was absent in the basal layer. Glycogen was found to decrease in vaginal epithelium with progressive menopause and only traces are seen in old age. Burgos and Wisolcki (1956) reported on cyclic change in glycogen and mucus in vagina.

Festisov (1966) observed very little glycogen in the superficial epithelial layers in sheep vagina during the resting period, which was increased during the preovulatory period and decreased during pregnancy. Giardinelli and Renis (1957) observed glycogen in the intermediate layers of human vaginal epithelium and there was no apparent decrease in it during the secretory phase of the cycle. Cymerya (1966) reported that in vaginal epithelial cells the staining for glycogen was most intense in the ovular phase and least intense in the ovulatory phase & least intense just before menstruation. During the pregnancy the intensity increased reaching maximum at 10th week followed by reduction after parturition. Lapan and Friedman (1950) described increase in glycogen content in human vagina, during pregnancy and decreased after parturition.

Forborg (1962) reported on the occurrence of PAS positive material in the vaginal epithelium of albino rats. Acidic mucoproteins or polysaccharides have been demonstrated in vaginal mucosal epithelium in guinea pigs. (Gibb 1969 Damova, and Takeva, 1970), rat (Ejamont, 1968). The acidic mucosubstances in vaginal epithelium were identified as hyaluronic acid in woman (Matten, 1955) and sialic acid in mouse (Spicer, 1960; Warren and Spicer 1961). The present investigation also found the same observations. In the striated epithelium sulfomucins and hyaluronic acids present. The presence of neutral mucins also demonstrated by histochemical reactions. The presence of neutral mucopolysaccharides has been reported in vaginal epithelium of rat (Ejamont, 1965), Cow (Igumnov, 1967). Woman (Toth and Gimes, 1966).

4) Distribution of Mucosubstances In Epidermoid or Squamous Cell Carcinoma of Vagina.

Epidermoid or squamous cell carcinoma was nothing but the cancer of striated squamous epithelium. The vast majority of primary vaginal carcinomas are squamous cell neoplasm which exhibit histological appearance similar to that of cervical cancer. The squamous carcinoma showed moderate PAS reactivity which reduced to half prior to phenylhydrazine treatment showed presence of neutral as well as acidic mucins. The glycogen was absent as there was no reduction in staining intensity after salivardigestion in PAS. technique. The presence of sulfomucins,

hyaluronic acid and sialic acid also confirmed.

3. Comparative Distribution of Mucosubstances In Cancerous Vagina

Very scanty material about mucopolysaccharides in vagina, ulva, present in cancerous condition. A adenocarcinoma of vagina studied clinically by Clement et al (1982). The cells resembles about its cytology as that of cancerous cervix. This cancerous condition also studied electromicrographically by Okagaki Takashi et al (1976). The tumour cell material show positive for mucicarmine, AB and PAS stains.

The present investigation reveals that in cancerous (epidermoid) the neutral, acidic type - hyaluronic acid, sialic as well as sulfomucins present.