THE PROBLEM OF VAGINAL ADENOSIS

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The National Cooperative Diethylstilbestrol Adenosis (DESAD) Projtct has completed the major portion of its enrollment phase with the examinaton of more than 3000 daughters of women taking synthetic monsteroidal estrogens (denoted DES) during pregnancies occurring from the early 1940.s to the mid 1960's. The aims of the Project were to fill urgent needs for information on the prevalence and incidence of structural and epithelial abnormalities or neoplastic changes and their complications in these young women. The primary group of abnormal findings have been designated vaginal apithelial changes (VEC), a designation which includes first the colposcopic observation of columnar epihtelium, glands, Nabothun cysts, white apithelum, leukoplakia, mosaic patterns, punctation, or the gross observation of nonstaining areas to iodine in the vagina. Also, to mett our criteria for VEC, these observations must be confirmed by a direct biopsy which shows squamous metaplasia, adenosis, and a variety of gross structural anomalies. Cervical ectropion is the presence of glandular (columnar) epithelium or its mucinous products in the portio vaginals of the cervix. The histopathology of cervical ectropion is similar, but not identical of vaginal adenosis. Vaginal adenosis la defined as the presence of glandular (columnar) epithelium or its mucinous products in the vagina. However, at the present time, clinically detectable adenosis is largely confined to young women who have been exposed to DES in utero. The frecuency of this disorder in this population has ranged from 35 to 90 porcent in the various reported series. Des-related adenosis is most commonly seen on the anterior wall of the upper pontion of the vagina, where it is typically continuos with an area cervical ectropion.

The columnar epithelium chracerizing vaginal adenois may be composed of cells resembling those of the endocervix or of mucin-free cells with dark cytoplasm. Vaginal adenoisis, like cervical ectropion, heals as a result of squamous metaplasia of both the surface epithelium and the glands. Squamous metaplasia, the presence of which accounts for the colpospically observed transformation zone in cases of vaginal adenoisis, is a very common feature in biopsy specimens showing this disorder, having been reported in over 80 percent of the cases.

The restriction of the designation "adenosis" to cases in which columnar epithelium or its secondary products are identificated specifically in the vagina resultd in the acquisition of more precise and comparable scientific data. On very rare occasions, intrauterine exposure to DES or chemically related mon-steroidal estrogens is followerd seven to 27 years later by the appearance of adencarcinoma of the vagina or cervic, almost always of the clear cell type. About 60 percent are vaginal and the reaminder are cervical in location. In addition to local spread, these tumors metastasize via lymphatics and blood vessels. There is abundant evidence of the mullerian nature of the clear cell adenocarcinoma. Almost all clear cell adenocarcinomas of the vagina and cervix hace been shown to be accompanied by adenosis and ectropion respectively. The bulk of the evidence links this tumor to the endometrial or endometriod type of cell. The nature of the cells that make up the clear cell adenocarcinoma has long been a mystery, but at both light and electron microscopical levels they resemble closely the hobnail cells and clear glycogen rich cells found in the endometrial glands during pregnancy. Hobnail cells characterize the so-called Arias Stella phenomenon. The peak age of detection of clear cell adenocarcinoma of the cervix and vagina at 19 years in the Registry cases suggests pubertal hormonal influence but evidence for this role is lacking. 2

Robboy et al evaluated squamous cell abnormalities of the vagina and cervix in 1424 women exposed to diethylstilbestrol in utero. The dysplastic epithelial changes were almost always mild in women with no prior history of dysplasia and were slightly more frequent in the cervix than the vagina. Severe dysplasia and carcinoma in situ were encountered only in those subjects specifically referred becauce of those abnormalities. The mos* common problem in the diagnosis of these squamous cell changes for dysplastic squamous cells. Discordance between biopsy and cytology was commonplace in the detection and follow-up of dysplasia, especially when it was mild. Colposcopically directed biopsies did not increase the frequency of confirmation of cytologic findings. These data suggest that both cytology and biopsy of abnormal segments of the vagina and cervix remain an integral part of the examination of the DES-exposed female during long-term follow-up studies.3

Prenatal exposure to diethylstilbestrol is associated with both malignant and nonmalignant abnormalities of the vagina and cervix in young females. Until recently most attention has focused on the rare clear cell adenocarcinoma and the benign lesions, vaginal adenosis and cervical ectropion, which are more frequent. Cytologic and histologic studies of the lower genita tract of the great majority od DES-exposed women examined have also revealed the presence of metaplastic squamous epithelium, which is a manifestation of the healing phase of both adenosis and ectropion and theoreotically subject to the same range of pathologic changes that is seen in the metaplastic epithelium of the cervix of the

unexposed female. Recently, the findings of squamous cell dysplasia in a small number of subjects has led some investigators to speculate that the incidence of in situ and invasive squamous cell carcinoma of the lower genital tract will rise greatly as the exposed population reaches the peak age at which these lesions are encountered in the cervix of the unexposed woman. This prediction has precipitated controversy regarding the frequency of dyplasia and carcinoma in situ in exposed females and the validity of the prediction itself.

Six type of squamous cell abnormalities were identified in the histologic and cytologic specimens: 1) mature squamous metaplasia; 2) immature squamous metaplasia; 3) mild dysplasia; 4) moderate dysplasia; 5) severe dysplasia and 6) carcinoma in situ. The prevalence of dysplasia was $2.1^{o}6O$ and the dysplasia originated equally in the cervix and vagina, which in most instante was mild. There was no instance where both were abnormal simultaneously (0 of 35 instances). The results of examination of 1424 young females exposed prenatally to DES with follow-up for varying periods up to six years indicate that dysplasia of the vagina and dysplasia and carcinoma in sity of the cervix occur but infrequently.[3]

The distinction between the madure and inmature forms of squamous metaplasia on the one hand and dysplasia on the other is important to make because metaplastic squamous cells are encountered commonly in DES-exposed females. These cells, which are believed to represent a reparative phenomenon, are often erroneously interpreted ad dyspalastic and on extremely rare occasions have been misinterpreted as clear cell adenocarcinoma or microglandular hyperplasia. Atypical glandular cells have also been confused with squamous metaplasia and dysplasia, but probably arise from atypical adenosis and ectropion.

It has been stated that DES-exposed subjects will develop unusually high rates of dysplasia because of extensive abnormalities of the vagina. Ywt, the fact that every instance of carcinoma in situ in Robboy's study involved the cervix (3 of 3 cases), as did almost all instances of moderate and severe dysplasia, raises the possibility of factors other than DES exposure that may be involved. Although it has been taught in the past that dysplasia rarely develops in teenage girls, it has benn shown that high grades of dusplasia of the cervix do occur in young females who presumably have not been exposed to DES. In a sexually active population all grades of squamous cell abnormalities in the cervix were encountered in up to $75^{o}/o$ of subjects and moderate dysplasia or worse in $3^{o}/o$. An unexpected finding of this study was the discrepancy between biopsy and cytology in the detection of dysplasia. In most studies of cervical dysplasia and carcinoma in situ in the unexposed population, cytologic smears are used to screen for abnormalities and biopsy to confirm the diagnosis. The site of biopsy is selected on the basis of a failure of the mucosa to stain with iodine or more specifically on the basis of characteristic colposcopic abnormalities. As much larger areas of the cervix and vagina typically fail to stain with iodine and are abnormal by colposcopic criteria in the population of DES-exposed females, the difficulty in obtaining bipsy confirmation of abnormal smears may be explai-

ned in part by the problem in the identification of the optimal site for biopsy.

Once dusplasia had been detected by giopsy in this series of cases, it was initially expected that repeated meticulous cytologic examinations would confirm the diagnosis, particulary since the dysplasia always involved the squamous epithelium covering the surface of the vagina or the exocervix. Since neither cytology nor biopsy alone after iodine staining or colposcopy is uniformly reliable at the present time in the diagnosis of squamous cell dysplasia in patients exposed prenatally to DES, it is recommended that cytology with biopsy of abnormal segments of the vagina and cervix remain at the present time an integral part of the examination of the DES-exposed female. Long-team follow-up of the population will be important to elucidate the fate of dysplasia once it is detected and the incidence of carcinoma in situ and invasive squamous cell carcinoma in this population.

The following examination procedures are used for DES-exposed females: 1) vulvar inspection; 2) vaginal palpation; 3) vaginal inspection: 4) cytology (separate slides) of the vagina and cervix; 5) colposcopy (optional); 6) iodine stain (initial visit); 7) tissue sampling; and 8) bi-manual.

The epithelium of the vagina must be carefully inspected. Grossly adenosis may appear red and granular, whereas squamous metaplasia may be almost indistinguishable from mature squamous epithelium. Vividly red focal areas should arouse suspicion for clear cell adenocarcinoma. Following gross inspection, cutology is taken. Following cytology there are two tests that are usually performed, colposcopy or Lugol's staining. The squamous cell changes, noted so frequently in the DES-exposed offspring, are confusing and make the colposcopic differentiation of benign and neoplastic changes difficult. Following evaluation, the speculum should be reinserted and the tissues stained with half-strength aqueous Lugol's solution. The major benefits of staining the tissue with iodine are to confirm the extent of epithelial change, as noted by colposcopy, or to determine this boundary when colposcopy is not being employed. Glycogen poor tissue that will not stain with iodine includes adenosis, inmature metaplasia, clear cell adenocarcinoma, dysplasia, and carcinoma in situ.

The following findings require biopsy: 1) indurated or unucually firm or cystic areas in the vagina or on the cervix; 2) discrete areas that appear of different color and texture than surrounding tissue, i.e., focal, red, irregular and 3) highly abnormal colposcopic findings, i.e., heavy mosaic, punctation, atypical vessels.

It is not necessary to randomly sample non-staining areas of the vagina or the cervix. Studies have been carried out where this was done and the lack of significant pathologic findings negates the validity of such an approach. Follow-up examination varies from six months to a year. The important steps at the follow-up examination include palpation, inspection and cytology. It is now recognized that adenosis is displaced by immature metaplastic squamous epithelium. Both of these tissues fail to take iodine and both can be detected

through iodine staining as well as colposcopy. Neither iodine staining nor colposcopy is necesary for routine follow-up examination. 4

Evaluation of stilbestrol exposed girls has two major objectives: 1) to screen for malignancy in cervico-vaginal epithelium and 2) to diagnose and follow-up frequent non-malignant changes in the cervico-vaginal epithelium. For the first objective, careful inspection and observation of the vagina and cervix, cytology, Schiller test, and biopsies of all abnormal areas are the most important steps in diagnosis. The value of coloposcopy in the diagnosis of clear cell adenocarcinoma in stilbestrol exposed girls is not yet established. To date, there are no described specific colposcopic changes compatible with clear cell adenocarcinoma. It is possible that a small deep lesion, below the vaginal epithelium, can be missed by colposcopy because the overlying epithelium looks normal. For diagnosis and follow-up of stilbestrol exposed patients with benign changes in the cervico-vaginal epithelium, colposcopy is a valuable adjunct to the byneco-logical examination.

The advantages of colposcopy in evaluation of stilbestrol exposed girls are similar as in the evaluation of cervical lesions in stilbestrol unexposed girls. Colposcopically, it is possible to localize abnormal changes in the cervix or vagina and to evaluate the extent of the lesion. With a colposcopically directed biopsy, it is possible to sample the most suspicious area for histologic examination. Therefore, a large number of blind biopsies from iodine negative areas in the vagina are unnecessary. Colposcopy is ideal for follow-up of benign changes in cervicovaginal epithelium because it can evaluate changes in the entire abnormal area, not only in randomly selected spots for tissue examination. Colposcopic examination of stilbestrol exposed girls, however, requires a certain amount of expertise because the interpretation of colposcopical changes in DES-exposed women is more difficult than in normal subjects. 5

Vaginal adenosis, by definition, is the presence of columnar epithelium in the vagina. When the columnar epithelium is present on the surface of the vagina, a reddich area is visible before the acetic acid test. The sudden change from the diffuse reddish area to grape-like structures after the acetic acid test is dramatic, and the columnar epithelium in vaginal adenosis is easily recognizable.

Exposure of the human fetus to DES before the 18th week of pregnancy is associated in women with developmental anomalies of the vagina and cervix. In a few such women clear cell adenocarcinomas have appeared, usually during adolescence around the age of 19 years, in the cervix or vagina. When carcinoma is found, coexistent benign adenosis is the rule. The behavior of these tumors is comparable with that of other cancers at this location. They invade and metastasize along the lymphatic either by surgery or radiation therapy. The usual preference for wurgery is based on its potential for resection of the entire tumor bed and tissues at risk, including pelvic lymphatics and nodes, with the capacity also of sparing the ovaries and simultaneously reconstructing a vagina which is satisfactory for coitus. Since the individuals at risk can be identified by their clinical history, it is feasible and proper to carry out the kind of detailed

screening examination on them that can detect both the morphologic anomalies so frequently being found and the much rarer early carcinomas. Cytology, Schiller testing and colposcopy and biopsy are all readily available and useful in this pursuit. 6

The Registry for Research on Hormonal Transplacental Carcinogenesis collects epidemiologic, clinical, and pathologic data on all cases of clear cell adenocarcinoma of the genital tract in women born in 1940 or later. Cases of other histological types of genital cancer are also accessioned if the patient has a history of prenatal hormone exposure. Between 1970 and 1976, 333 cases of clear cell adenocarcinoma were accessioned. Approximately two-thirds of the completely investigated cases had histories of prenatal exposure to stilbestrol (DES) or similar compunds. A recent analysis of ageincidence showed a peak at age 19, and an estimate of the risk of clear cell adenocarcinoma of 0.14 to 1.4 per 1,000 DES-exposed for the detection of clear cell adenocarcinoma, but a thorugh pelvic examination is vital to accurate diagnosis. Both surgery and radiation have been effectiva in treating these tumors, but most cases have been followed for less than five years. Spread to reagional pelvic nodes has been observed in several cases of small or superficial tumors, and recurrences in the lungs or supraclavicular areas have been observed more frequently in these cases than in squamous cell carcinomas. Prenatal DES exposure did not appear to increase the frequency of kidney and ureteral abnormalities, ad judged by the result of intravenous pyelograms.

Clear cell adenocarcinoma of the vagina and cervix is diagnosed on pelvic examination by careful direct inspection and palpation, with biopsies of grossly suspicious lesions. Although colposcopy has been advocated as a useful diagnostic method in the evaluation of the DES-exposed, it has not been particularly useful in the detection of clear cell adenocarcinoma. A few carcinomas have been overlooked on colposcopic examination because the tumor was on the posterior vaginal wall or behind a partially obstructing transverse ridge. The pelvic examination must be done meticulously, because carcinomas of the anterior or posterior vaginal wall can be obscured by the blades of a speculum. Screening examinations are usually begun once and exposed female has begun to menstruate or by the age of 14 years, even if she has not begun menstruation.

Cytology has proved useful in the diagnosis of clear cell adenocarcinoma, with a positive or suspicious report recorded in about two-thirds of the cases. In a few instances, an abnormal cytological report led to diagnosis of cancer in an asymtomatic female. However, a false negative smear report was received in over 30 percent of the cases in which a clear cell adenocarcinoma was present. The high proportion of false negative smears will undoubtedly decline when optimal techiniques for obtaining the smear are in more widespread usage and pathologists and cytotechnologists become more aware of the cytological features of this tumor. It is probable, however, that a significant but smaller number of false negative result will persist.

Most of the patients with clear cell adenocarcinoma had abnormal bleeding or discharge as a presenting sympton. However, about 20 percent had no symptoms

when the tumor was detected on routine pelvic examination. Many of these patients sought medical advice because of a history of prenatal DES exposure.

Surgery and radiation have been used to treat clear cell adenocarcinomas and both have resulted in eradication of the tumor in a high proportion of the cases. However, the follow-up of most of the cases is still too short for the calculation of a meaningful five year survival for comparing the efficacy of various nodes of therapy at this time. The finding of regional lymph node metastases in approximately 16 percent of Stage I carcinomas treated by operation emphasizes the importance of considering sites of potential spread as well as the primary tumor in any treatment plan. For early stage tumors, especially of the upper vagina and cervix, radical hysterectomy, vaginectomy, and pelvic lymph node dissection with replacement of the vagina with a split-thickness skin graft has been successful in a high percent of cases. Ovarian function has been preserved in most such cases. Radiation therapy, either in the form of internal application combined with pelvic radiation or transvaginal radiation after lymph node dissection and transposition of the ovaries, has also been employed successfully in a few cases.

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