

Cellular Dysplasia in Urine : Cytologic Follow up Study on 220 Cases

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Abstract

The aim of the present study is to characterize the cytomorphologic features and natural history of urinary dysplasia. It represents a follow up study of a cytologic screening project done on a rural community in Dakahliya governorate during the years 1976-1982. The material included 120 cases of mild dysplasia, 80 cases of moderate dysplasia and 30 cases of marked dysplasia. Cytologic follow up of these cases was done 10 to 42 months from the initial screening (with a median duration of 25 months). On rescreening of mild dysplasia, 80 cases reverted to normal, 38 cases remained as mild dysplasia two cases progressed to moderate dysplasia, but none developed malignancy. Conversely, in the group with moderate dysplasia, 22 cases became normal, 27 reverted to mild dysplasia, 27 remained as moderate dysplasia, 8 cases progressed to marked dysplasia and one case developed carcinoma. Also, in the group with marked dysplasia, only one case became normal and 4 reverted to mild dysplasia, and 6 to moderate dysplasia, 3 remained as marked dysplasia and one case developed carcinoma. Accordingly, cytologic follow up is recommended for cases with moderate or marked urinary dysplasia for early detection of bladder cancer.

Introduction

DYSPLASIA describes atypical cytomorphologic changes intermediate between normal cells and malignant cells (Koss, 1979). This cellular atypia includes a spectrum of changes which vary in their morphologic

as well as biologic behavior. The majority of the dysplastic changes revert to normal with proper treatment. Few cases, however, may progress to malignancy if left untreated hence the clinical importance of dysplasia.

Most of the previous studies on dysplasia were done on the uterine cervix (Peterson, 1956; Fox, 1967; Hall and Walton, 1968), whereas dysplasias of other organ sites received little attention. Only few reports are available on dysplasia of the respiratory tract (Kern, 1965; Saccomanno et al, 1965) and the urinary system (Kern, 1975; Koss et al, 1977). The present report describes the cytologic features and natural history of urinary dysplasia associated with schistosomiasis. The malignant potential of these changes was evaluated through follow up study and correlated with the degree of cytomorphologic atypia.

Material and Methods

During the years, 1976 to 1982, a cytologic screening project was conducted in a rural Egyptian community in Dakahlia governorate. This population was heavily infested with schistosomiasis with 67% of the individuals having urinary and/or intestinal types of the disease. Details of this project and its yield in cancer detection were previously reported by the authors (El-Bolkainy and Chu, 1981; El-Bolkainy et al, 1982).

In the initial screening of 8744 individuals, cellular atypia of various degrees were observed, including 784 mild dysplasia, 105 moderate dysplasia and 20 cases of marked dysplasia. Rescreening was made for all cases with moderate and marked dysplasia and for a random sample of 150 individuals with mild dysplasia. The time interval between initial and subsequent screening varied between 10 to 42

months, with a median duration of 25 months. The majority of the studied subjects were adults with 94.4% over the age of 20 years.

Urine samples, up to 100 ml, were collected in plastic bottles containing 30 ml of 95% ethyl alcohol as a fixative. They were transported on the same day of collection to the National Cancer Institute, Cairo, where smears were prepared and stained by the standard Papanicolaou method. Atypical nuclear and/or cytoplasmic changes in transitional or squamous metaplastic cells, but not amounting to malignancy, defined dysplasia. Cellular atypia was classified into three degrees with increasing severity (Tawfik et al, 1981). For cases with advanced dysplasia (moderate or marked degrees), complete urologic investigations including cystoscopic biopsies were performed at the Department of Urology, Mansoura University Hospital, located two to five miles from the field site.

Results

It was possible to examine a total of 220 individuals out of the target number of 275 subjects, a participation rate of 80%. The cause of non-response included: Absence 12 cases, change of address 16, travel abroad 8, military service 9, refusal to participate 6 and death from causes other than bladder cancer 4 individuals. The age and sex of the groups at the time of subsequent cytologic examination are presented in Table 1. The age and sex distribution was not statistically significant among the different groups.

TABLE 1
Age and Sex Distribution

	No. of cases	Males	Females	Median Age
Mild Dysplasia	120	72	48	42
Moderate Dysplasia	85	53	32	45
Marked Dysplasia	15	9	6	44

In mild transitional dysplasia the nucleus is slightly enlarged and slightly hyperchromatic (Fig. 1 A) as compared to normal transitional cells (Fig. 1 B). However, the chromatin is granular and evenly distributed, and the cytoplasm is relatively abundant. Dysplastic binucleated cells may be seen (Fig. 1 C) but the two nuclei appear similar in size and shape. In marked dysplasia the atypical changes are more prominent, the nuclear-cytoplasmic ratio is increased, and the nuclear membrane is folded or wrinkled (Fig. 1 D). Histologically, dysplasia shows atypical cells with loss of polarity and crowded appearance, but with little change in the surface, or «umbrella, cell» layer (Fig. 1 E). Dysplastic transitional Cells with prominent intracytoplasmic vacuoles are not uncommon (Fig. 1 F).

Squamous dysplasia is recognized by keratinization in the cytoplasm which appears dense and eosinophilic with refractile border. The degree of dysplasia is denoted by the extent of nuclear atypia as well as the amount of cytoplasm (Fig. 2 A,B,D, E). Koilocytotic change with perinuclear vacuolation may be observed (Fig. 2 E). Histologically, the epithelial atypia and hyperplasia in mild dysplasia are limited to the deeper layers (Fig. 2 C), Whereas in marked dysplasia they involve most of the epithelial layers (Fig. 2 F).

The cytologic findings on subsequent examination are presented in Table 2. Mild dysplasia showed a tendency to revert to normal or remain as such, whereas moderate and marked dysplasia showed a tendency to progress either to a higher degree of atypia or cancer. These transition tendencies among the groups were statistically significant. The tendency of advanced dysplasia to progress was higher in individuals over the age of 30 year than younger subjects, but this difference was not statistically significant (Table 3). Again, progression of advanced dysplasia was more marked in males than females but the difference was not statistically significant (Table 4).

Two patients with bladder cancer were detected by cytology on subsequent screening. The first patient was a male farmer aged 43 who had moderate squamous dysplasia at initial screening. Subsequent screening done 29 months later was positive for squamous carcinoma, which was proven by biopsy to be low grade squamous carcinoma, in situ. The second patient was a male farmer aged 47 years with cytologic diagnosis of marked squamous dysplasia in the initial screening. Subsequent cytologic examination, 26 months later, was positive for cancer and the biopsy showed invasive squamous cell carcinoma grade 1, stage P2.

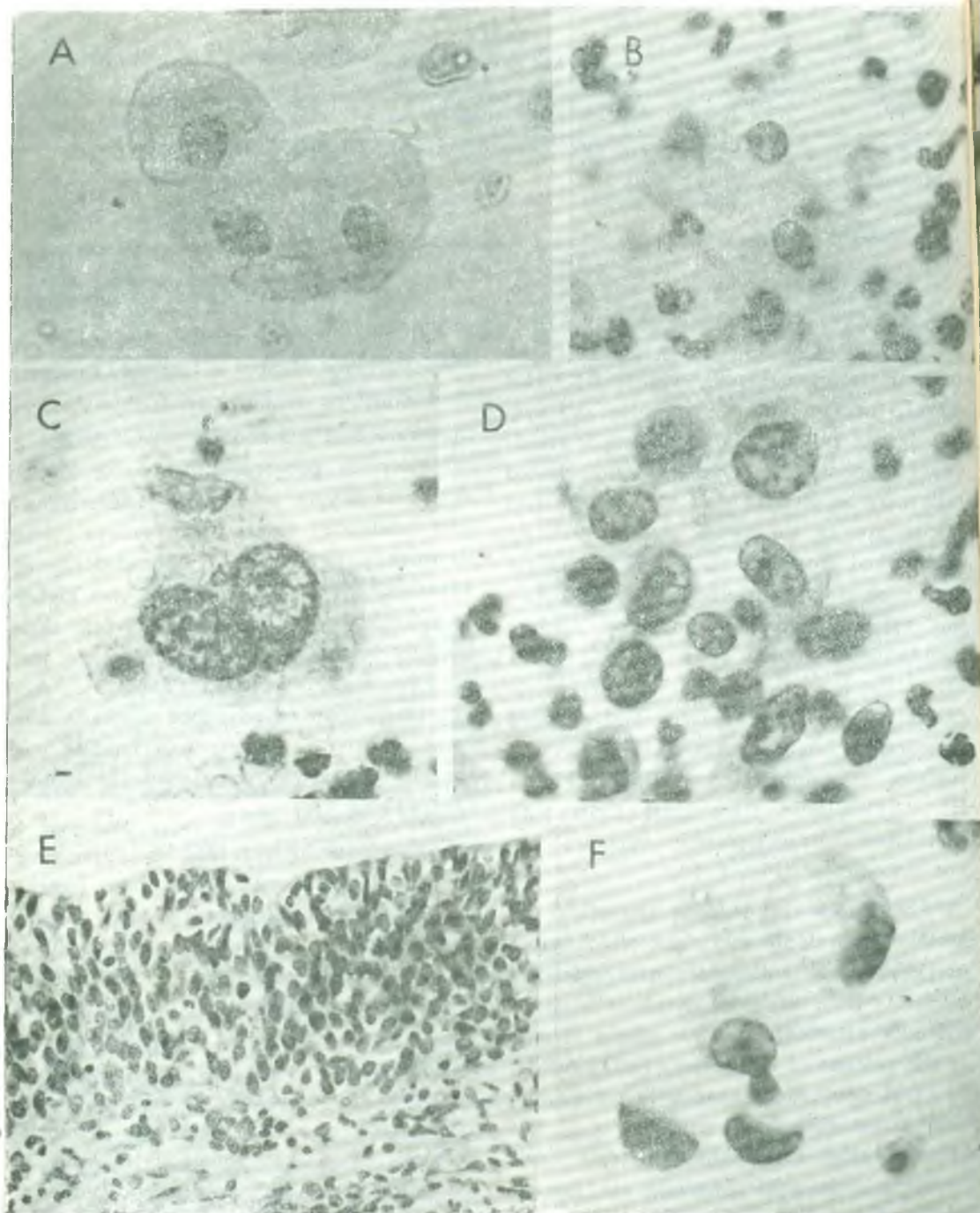


Fig. 1 Transitional Cell Dysplasia. A) Mild dysplasia (X1000), B) Normal transitional cells (X1000), C) Moderate dysplasia, binucleated cell (X1000), D) Marked dysplasia exfoliated in sheets (X1000). E) Histology of marked transitional dysplasia (X400), and F) Marked dysplasia, cytoplasmic vacuolation (X1000).

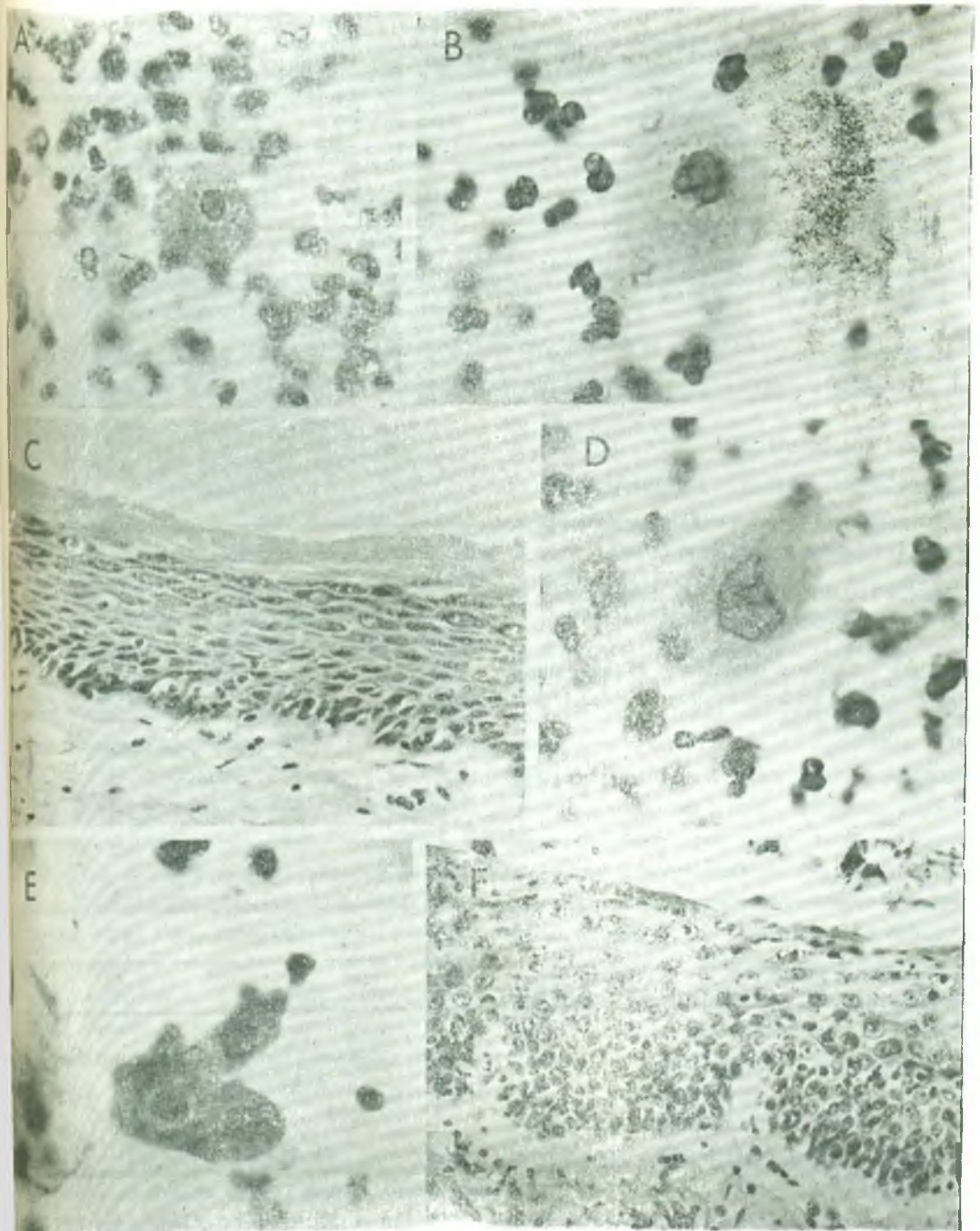


Fig. 2. Squamous Cell Dysplasia. A) Squamous metaplastic cell with mild dysplasia (X600). B) Moderate squamous dysplasia (X1000). C) Histology of moderate squamous dysplasia with keratinization (X400). D) Marked dysplasia (X1000). E) Marked dysplasia with perinuclear vacuoles (X600) and F) Histology of marked squamous dysplasia (X400).

TABLE 2
Cytologic Findings in Follow up of 220 Cases of Urinary Dysplasia

Initial screening	Rescreening ^(a)				
	Negative	Mild Dysplasia	Moderate Dysplasia	Marked Dysplasia	Carcinoma
Mild dysplasia ^(b) (120 cases)	80	38	2	—	—
Moderate Dysplasia (85 cases)	22	27	27	8	1
Marked Dysplasia ^(c) (15 cases)	1	4	6	3	1

(a) Rescreening was done 10—42 months from initial screening (median duration of 25 months).

(b) Transition significantly different from moderate and severe forms (RIDIT Analysis, $Z = 9.828$, $P < 0.001$).

(c) Transitional probabilities of this group are significantly different from moderate dysplasia with more tendency to progression and less tendency to regression (RIDIT Analysis, $Z = 2.253$, $P < 0.05$).

TABLE 3
Outcome of Advanced^(a) Dysplasia in Relation to Age.

Age at Initial Screening / Years	No. of Cases	Progression ^(b)	
		No.	%
Below 30	20	—	0.0
30 and above	80	10 ^(c)	12.5

(a) Includes moderate and marked Dysplasia.

(b) Progress to a higher degree of dysplasia (8 cases) or cancer (2 cases).

(c) Difference is not statistically significant ($X^2 = 1.56$).

TABLE 4
Outcome of Advanced^(a) Dysplasia in Relation to Sex.

	No. of cases	Progression ^(b)	
		No.	%
Males	64	9	14.1
Females	36	1	2.8 ^(c)

(a) Includes moderate and marked dysplasia.

(b) Progression to a higher degree of dysplasia or development of malignancy.

(c) Difference is marked but statistically not significant ($X^2 = 2.1$).

Discussion

Papanicolaou in 1954 was the first to introduce the term dyskaryosis to describe abnormal cytologic pattern associated with early carcinoma of the uterine cervix. It implies a state of abnormal nuclei but the cytoplasm is normally differentiated. Recently, the definition has been modified to include atypical changes both in the nucleus and or cytoplasm as a result of abnormal differentiation (Takahashi, 1981). Various nomenclatures have been given to this state including: atypical hyperplasia, borderline change and dysplasia. In current terminology, the term dysplasia, meaning abnormality of development, is internationally preferred (Christopherson, 1977). These recent concepts in terminology and definition were adopted in our studies (Tawfik et al, 1981, El-Bolkainy et al 1982).

The cytologic diagnosis of cellular dysplasia is rather difficult and liable to subjective variation, hence it is important to clearly define the diagnostic criteria of this condition. The nucleus in dysplasia

is characterized by enlarged size, hyperchromatic, granular evenly-distributed chromatin, ground glass parachromatin, inprominent nucleoli, folding or wrinkling of nuclear membrane which appears uniform in thickness (Takahashi, 1981). These changes must be distinguished from malignancy and other cellular alterations simulating malignancy. The most significant distinguishing characteristics for malignancy are: prominent and irregular nucleoli, unevenly-distributed very coarse chromatin, parachromatin clearing and thick nuclear membrane with angulations (Kalnins et al, 1970). It is also important to distinguish dysplastic changes from hyperplastic degenerative changes in transitional cells which are rather common phenomena. In benign hyperplasia, the nucleus is slightly enlarged but the shape is regular, chromatin finely granular and nucleoli may be prominent (Takahashi, 1981). Degenerated transitional cells, particularly the so called «Decoy» cells may simulate atypical cells in view of their scanty cytoplasm. However, the nuclei of «decoy» cells are

pyknotic with indistinct chromatin, (Craibe, 1971). Radiotherapy or chemotherapy may produce atypical nuclear changes which must be differentiated from primary dysplasia (Cowen, 1975; Tweeddale, 1977). Historical data are necessary to make this distinction.

The cytoplasmic changes in dysplasia are both qualitative & quantitative. The former is evident by abnormal differentiation and the latter by reduction in the size of cytoplasm. Planimetric studies of cytoplasmic and nuclear areas in normal, dysplastic and malignant transitional cells were reported by Kern (1975). In dysplastic cells, the cytoplasmic area is smaller and the nuclear area is larger than normal cells, hence the N/C ratio is helpful in differentiating between them. Conversely, the size changes in nuclei and cytoplasm in dysplasia do not differ much from those observed in malignant cells hence the N/C ratio is of little help to distinguish dysplastic from malignant cells. The previously mentioned nuclear cytomorphologic features are more helpful to make this distinction. Qualitative cytoplasmic changes may also be observed in dysplastic cells, particularly in transitional cells which undergo squamous metaplasia, a rather common finding in chronic bilharzial cystitis. Abnormal differentiation of cytoplasm of squamous cells is known as dyskeratosis (Takahashi, 1981). Cytoplasmic overmaturation is characterized by intense orangeophilia and appearance of keratohyaline granules. In our experience, dyskeratotic cells in urine are often associated with dysplastic changes in the nuclei as well.

Dysplastic cells in urine are classified according to the degree of severity as well as according to the cell type. In the present study, dysplasia was classified into mild, moderate and marked. The diagnostic criteria were previously reported by the authors (Tawfik et al, 1981). The cell type in urinary dysplasia may be transitional type, squamous type or columnar type. Atypical squamous cells may be exfoliated in cases of chronic bilharzial cystitis or urinary calculi. Dysplastic mucus secreting glandular cells are shed in case of glandular cystitis (Umiker et al, 1962). However, atypical transitional cells may show cytoplasmic vacuoles with mucin inclusions (Dorfman and Monis, 1964). All the above mentioned dysplastic cells are shed from atypical transitional epithelium of corresponding nature. The histopathology of these bladder changes encountered in the bilharzial bladder was previously reported (El-Bolkainy et al, 1981).

Few reports are available on the incidence of urinary dysplasia in well-defined populations. Two reports are available for studies done on Egyptian material. In the screening project done by the authors on a rural community infested with schistosomiasis, urinary dysplasia was encountered in 909 individuals (10.4%) of the total 8744 subjects screened (El-Bolkainy and Chu, 1981; El-Bolkainy et al, 1982). Mild dysplasia was observed in 9%, moderate dysplasia in 1.2% and marked dysplasia in 0.2% of the total screened subjects. Moderate and marked dysplasia were more common in males (66.4%) than females (33.6%). There was also a marked increase in the frequency of dysplasia with age.

In another study reported by El-Hawary (1981) screening by urine cytology was done for the workers of a textile factory including 422 workers exposed to aromatic amines and 220 unexposed workers. In this material, 70.6% gave a history of bilharziasis and 59.5% were smokers. Simple squamous metaplasia in urine smears was found in 6.4% of the exposed group and 5% of the control, and this change was probably related to schistosomal infestation rather than chemical exposure. A total of 106 cases of urinary dysplasia was detected in the 642 subjects studied, an overall frequency of 16.5%. Cellular atypia was more frequent in the exposed group (20.9%) than in the unexposed control, and this difference was statistically significant. Atypia was mild in 71 cases (80.7%), 12 were moderate (13.6%) and 5 were marked (5.7%). The frequency of cellular atypia increased proportionately with the duration of exposure to aromatic amines. Thus, in the exposed group, the frequency of atypia was significantly higher among cases with past-history of bilharziasis, but the smoking habit did not add any statistically significant risk. The fact that no tumors were detected in that screening in spite of long occupational exposure indicates minimal carcinogenic activity of the chemicals used in that factory.

The natural history of urinary dysplasia is still unknown in view of the lack of prospective follow up studies. The present investigation is an attempt in this direction, with a median follow up of about two years. Subsequent cytologic study of the 120 subjects with mild dysplasia revealed that 66.7% reverted to normal, 31.7% remained unchanged, only 1.7% progressed

to moderate dysplasia and no tumor developed in this group. In the 100 cases with advanced dysplasia (including 85 moderate and 15 cases of marked dysplasia), 23 cases reverted to normal, 37 regressed to a lower degree of dysplasia, 30 remained stationary 8 progressed to a higher degree of dysplasia and 2 cases developed carcinoma. Thus, advanced dysplasia has more tendency to progression and less tendency to regression as compared to mild dysplasia. Thus mild dysplasia appears to be a stable change and its premalignant potential is minimal and may be disregarded. Whereas, the malignant potential of advanced dysplasia is more serious hence it is important to follow up these cases by cytology for early detection of bladder cancer.

Our findings are supported by recent studies of Koss and associates (1977). In a computerized study of urothelial cells they demonstrated that dysplastic cells form a distinct category with features between benign and malignant cells. They also showed that the atypical cells fall into two subsets, one closely resembling benign cells and the other closely resembling malignant cells. These atypical subsets were referred to as atypical I, and atypical II and this classification appears to be of diagnostic and prognostic benefit.

The progression rate of urothelial dysplasia appears in our study to be lower than that reported in dysplasia of the uterine cervix. However, it is noteworthy that the period of follow up in the present study is rather short. In a series of 278 patients (Fox, 1967) in whom the diagnosis of cervical dysplasia was made by cytology and who had no therapy, follow up

over an eleven-year period revealed that the condition regressed in 86 (31.2%), persisted unchanged in 25 (9.1%), and progressed to either severe dysplasia and/or cancer in 167 (60.1%). In another prospective study on 206 patients with cervical dysplasia (Hall and Walton, 1968), 13.4% of mild dysplasia progressed to a higher grade of dysplasia, 6.2% progressed to carcinoma in situ and 62.2% regressed. In severe dysplasia, 29.1% developed carcinoma and only 19.1% regressed. The rate of malignant transformation increases with time. Thus, in the study of Petersen (1956) on 127 patients with untreated cervical dysplasia, invasive carcinoma developed in 11% at the end of 3 years, in 22% at the end of 5 years and in 33% at the end of nine years.

Cytogenetic studies also support our findings and offer a tool for the fine delineation of the extent of dysplasia and help to predict its progression risk. Cytogenetic studies revealed that most dysplasias seem to be diploid, though, however, heteroploidy have been occasionally reported (Katayama and Jones, 1972; Kirkland, 1966). Conversely, carcinoma in situ exhibits a higher degree of heteroploidy with predominant diploid and tetraploid ranges. Invasive carcinoma is highly heteroploid with wide scatter of Karyotypes usually in diploid and triploid modes. Thus the onset of invasion in carcinoma in situ is usually associated with reduction in the ploidy values. Similar findings are reported from quantitative DNA cytophotometric measurements on Feulgen stained cells (Wied et al 1966; Bohm and Sandritter, 1975). The DNA values of dysplasia cells are generally intermediate between those

of normal and malignant cells. The first step in the direction of malignancy is the transformation of the euploid-polyploid DNA distribution of dysplasia into a heterogeneous aneuploid one. Thus, quantitative cytophotometric DNA analysis provides an objective and reproducible way to classify dysplastic lesions and identify particular subgroups which are more liable to malignant change.

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