MICROPHOTOMETRIC DNA ANALYSIS IN CONDYLOMATOUS AND DYSPLASTIC LESIONS OF THE UTERINE CERVIX

V. KASHYAP • P. BHATNAGAR • U. K. LUTHRA

SUMMARY

The DNA ploidy pattern of cells from cervical condylomatous lesions (10 cases), dysplastic lesions (10 cases) and in Co-existence of both condylomatous lesions and dysplastic lesions (10 cases) were studied by microphotometry on paraffin sections of cervical tissue. Five normal cervical epithelium were also subjected for DNA analysis and taken as control. The condyloma cell nuclei either/or in co-existence with dysplastic lesions revealed diploid DNA value in 18(90%) cases and polyploid DNA value in 2(20%) cases only. No DNA aneuploidy was observed in condyloma cell nuclei, however, dysplastic cell nuclei irrespective of association of condylomatous lesion revealed diploid DNA value in 3(15%) cases, polyploid DNA value in 8(40%) cases and aneuploid in 9(45%) cases. Thus, it is evident from the study that by DNA cytometry it is possible to distinguish condylomatous lesion from the dysplastic lesion and DNA ploidy pattern can be used as an additional diagnostic parameter with the histopathology.

Introduction

In recent years human papilloma virus has been recognised as a likely etiologic agent for cervical carcinoma. The cytologic similarities of Condyloma to cervical dysplasias of the uterine cervix and their frequent co-existence have led a number of investigators to the conclusion that the papova virus causing condylomas may be a factor in the cervical carcinogenesis (Meisels et al 1979). Cervical condyloma produce a characteristic pattern in cells exfoliated from the surface of the lesion. A large percentage of these lesion exfoliate superficial epithelial cells with a large perinuclear halo. These cells were named as "koilocytes" by Koss and Durfee (1956). The presence of koilocytes in cervical biopsies and smears is considered to be pathognomic of HPV infection (Boon 1981, Das et al. 1987, Luthra et al 1989).

During 1950s microphotometry had become of diagnostic interest with the discovery that tumor cells exhibit increased

Department of Cytology and Preventive Oncology (ICMR), Maulana Azad Medical College, New Delhi.

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amount of DNA (Mellor et al 1952, Atkin and Richard 1956). Now it is well known that DNA distribution in a tumor cell can be considered as a prognostic factor - (Fu et al 1981; Goppinger et al 1986; Dudzinski et al 1987). In 1974, Jagella and Stenger studied the DNA distribution in 50 condylomas and found aneuploid DNA values in several such lesions and suggested that certain condylomas have a malignant potential. From the literature it is evident that little work has been done regarding the DNA content of condylomatous lesion of the uterine cervix (Shevchuk and Richart 1982; Evans et al 1983; Reid et al 1983 and Hughes et al 1987). Such type of study has not performed in Indian context. The present attempt is made to measure the nuclear DNA content of condylomatous cells, their comparision with the DNA value of dysplastic cells and to find out the differences in DNA value of condylomatous cell nuclei either alone or in association with dysplastic lesion as well as on the use of DNA cytometry to distinguish condylomatous lesion from the dysplastic lesions.

Materials and Methods

Ten cases of condylomatous lesion were selected as the main subject for the study (Group A) In addition to it, ten cases of various grades of dysplasia associated with condylomatous lesion (Group B) and five cases of moderate dysplasia (Group C) were also taken for comparision. Five cases of normal cervical epithelium were taken as control (Group D). All the cases were histopathological diagnosed by Cytopathologists. Haematoxylin and eosin stained section of all the cases were reviewed to localise the area of the condylomatous lesion and dysplastic lesion separately. For DNA microphotometry five micron thick sections from the paraffin blocks of the above 30 cases were cut and processed for Feulgen reaction (Koss 1979). Leitz microscope photometer with standard outfits for absorption measurements with automatic data analysis system was used to determine the nuclear DNA content. 40 nuclei were measured in each case and thirty small lymphocytes in each section were used as control and their mean DNA value was considered as diploid and denoted by 2 N in the histogram, thus 4 N would be tetraploid and so on. The different ploidy pattern described by Fu et al (1979) was adopted in this study.

Results

All the 10 cases histopathological diagnosed as "changes suggestive of condylomatous lesions only" (Group A) had the finding as koilocytotic changes, nuclear pyknosis thickening of cytoplasmic margins (Fig. 1) and revealed diploid DNA distribution with main DNA peak in 2 N region (Fig. 2). The individual cell DNA value ranged upto 4 N region. These cases

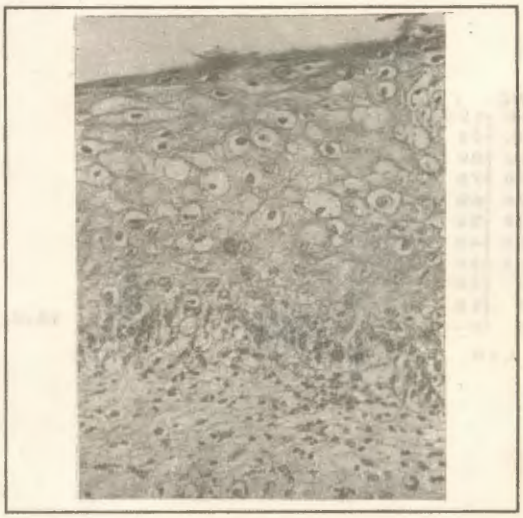

Fig. 1 Histopathological section showing condylomatous change (koilocytes) in middle and upper third of the epithelium (H & E x 400).

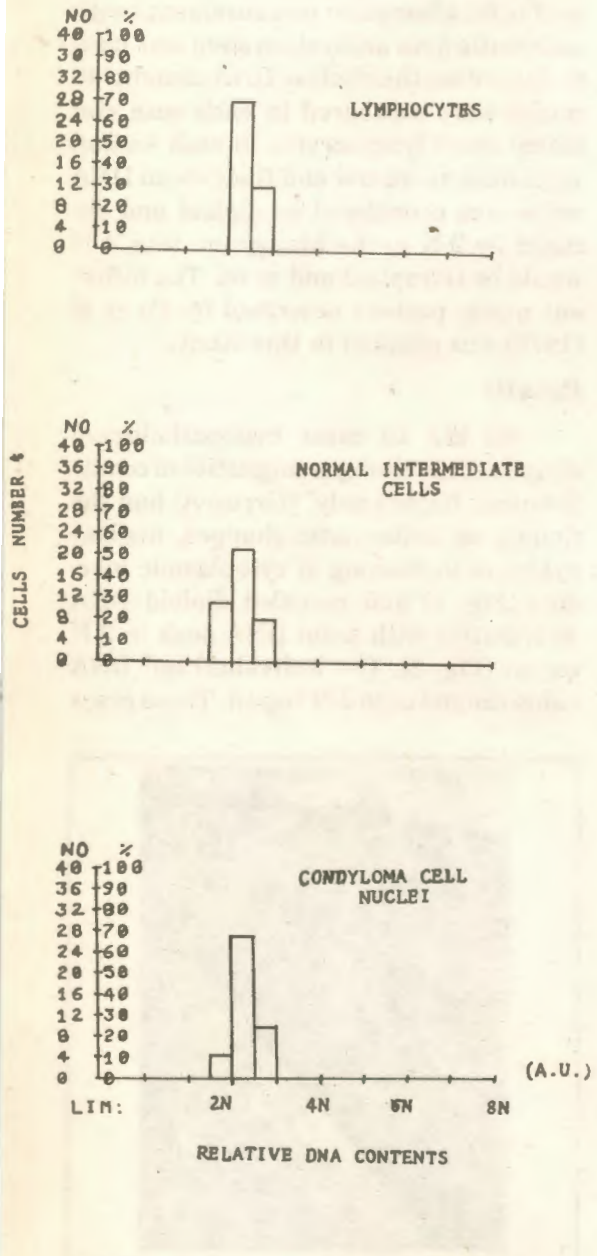

Fig. 2 DNA distribution pattern in cells histopathologically diagnosed as condylomatous lesions (Koilcytes) Group A.

cannot be distinguished from the normal cervical epithelium (Group D) as they also revealed diploid DNA value (Fig. 3). Group B consists of ten cases of dysplasias (4 mild, 4 moderate and 2 severe) which were associated with the condylomatous lesions. In these cases the DNA measurements were performed on dysplastic cell nuclei and condylomatous cell nuclei separately from the two different areas of the same section. The condylomatous cell nuclei showed diploid/hyperdiploid DNA value in eight cases (4 mild and 3 moderate and 1 severe) polyploid DNA value in two cases (1 moderate and 1 severe). In contrast the dysplastic cell nuclei from the same group (Group B) showed diploid DNA value in three mild dysplasia, polyploid DNA value in one mild and two moderate dysplasia and aneuploid DNA value in remaining four cases (2 moderate and 2 severe). DNA ploidy pattern of condylomatous cell nuclei and severe dysplastic cell nuclei of one case is deplicted in Fig. 4. The group C consists of five cases of moderate dysplasia, of which 3 cases revealed polyploid DNA value and 2 showed aneuploid DNA value (Fig. 5).

Discussion

Many investigators have shown that more malignant the tumor, the higher and more widely scattered DNA content of tumor cell nuclei (Atkin and Richard 1962, Sandritter et al 1966, Nasiell et al 1979, Bohm and Sandritter 1975, and Kashyap et al 1988). It is evident from the literature that only a few studies have been carried out on nuclear DNA content of condylomatous lesion and polyploid and aneuploid DNA pattern was reported by DNA microphotometry and flow cytometry (Shevchuk and Richart 1982 and Hughes et al. 1987) respectively. In the present study the DNA

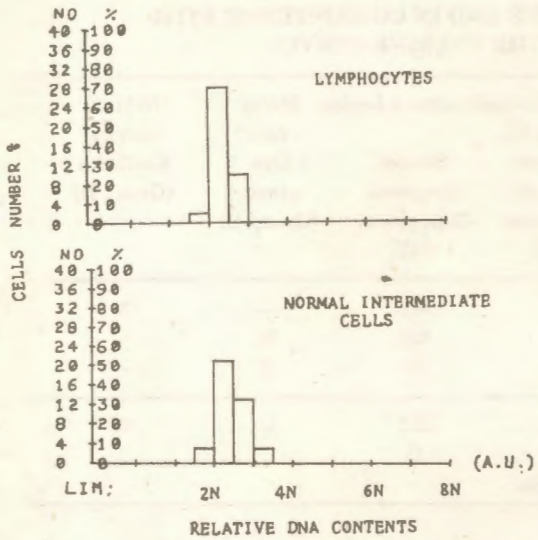

Fig. 3 DNA distribution pattern of normal cervical epithelium showing diploid value (Group D)

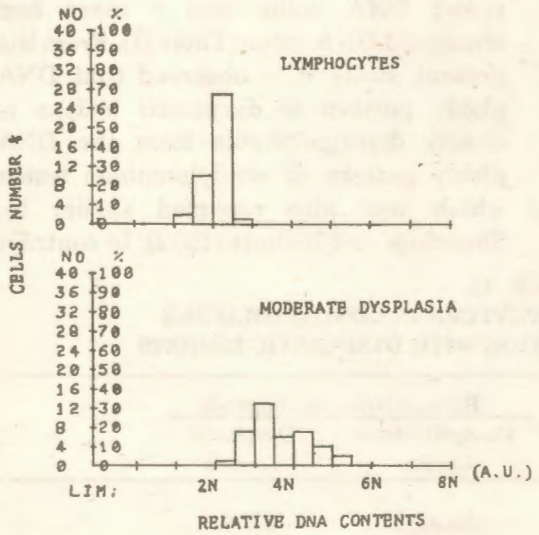

Fig. 5 DNA distribution pattern of a case of a moderate dysplasia (Group C) showing aneuploid DNA value.

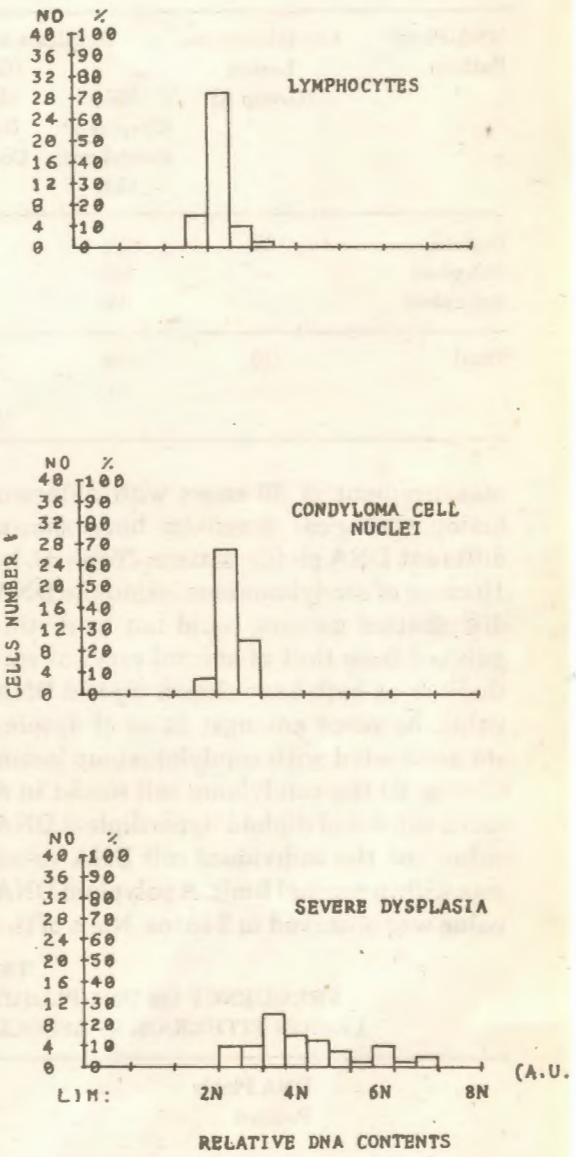

Fig. 4 DNA distribution pattern of condyloma cell nuclei showing diploid DNA values and simultaneous dysplastic cell nuclei revealed aneuploid DNA value (Group B)

TABLE - I
FREQUENCY OF DNA PLOIDY PATTERN IN HISTOLOGICAL SPECIMEN OF CONDYLOMATOUS LESION ALONE AND IN CO-EXISTENCE WITH DYSPLASTIC LESION OF THE UTERINE CERVIX

DNA Ploidy Pattern	Condylomatous Lesion (Group A)	Dysplasia with Condylomatous Lesion (Group B)			Moderate Dysplasia (Group C)	Normal Cervical Epitheliu (Group D)
		Mild Dysplasia/ Condyloma Cell	Moderate Dysplasia Condyloma Cell	Severe Dysplasia Condyloma Cell		
Diploid	10	3/4	0/3	0/1	—	5
Polyploid	—	1/0	2/1	0/1	3	—
Aneuploid	—	0/0	2/0	2/0	2	—
Total	10	4/4	4/4	2/2	5	5
		()		()		
		10 cases				

measurement of 30 cases with different histopathological diagnosis have shown different DNA ploidy pattern (Table I). In 10 cases of condylomatous lesion the DNA distribution pattern could not be distinguished from that of normal cervical epithelium as both have shown diploid DNA value, however amongst cases of dysplasia associated with condylomatous lesion (Group B) the condyloma cell nuclei in 8 cases exhibited diploid/hyperdiploid DNA value but the individual cell DNA value was within normal limit. A polyploid DNA value was observed in 2 cases. None of the cases showed aneuploid DNA pattern. Regarding the DNA value of dysplastic lesions (mild, moderate and severe dysplasia) altogether 20 cases were studied (Group B and C) amongst them 3 cases had diploid DNA value, 8 cases had polyploid DNA value and 9 cases had aneuploid DNA value (Table II). From the present study it is observed that DNA ploidy pattern of dysplastic lesions is clearly distinguishable from the DNA ploidy pattern of condylomatous lesion which was also reported earlier by Shevchuk and Richart (1982). In contrast

TABLE - II
FREQUENCY OF DNA PLOIDY PATTERN IN CONDYLOMATOUS LESION EITHER/OR IN ASSOCIATION WITH DYSPLASTIC LESIONS

DNA Ploidy Pattern	Histopathological Diagnosis	
	Condylomatous Lesions	Dysplastic Lesions
Diploid	18(90%)	3(15%)
Polyploid	2(10%)	9(45%)
Aneuploid	0(00%)	8(40%)
Total	20(100%)	20(100%)

Jagella ans Stenger (1974) found aneuploid DNA value in several condylomatous lesion. It is further evident from the present results that moderate dysplasia had slightly increased and scattered DNA value in comparision to mild dysplasia which was also observed earlier by others (Fu et al 1981, Bibbo et al 1985. Nasiell et al 1979 and Kashyap et al 1988). Due to small sample size of diagnostic group it is rather difficult to distinguish normal cell from the condylomatous cell by DNA quantitation but it seems possible to make out the differences between condylomatous lesion and dysplastic lesions of the uterine cervix. Hence DNA microphotometry can be used as an adjunct to histopathological diagnosis.

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