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Numerical Chromosomal Variations in Ascitic Effusions of Predominant Female Cancers

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ABSTRACT

Cancerous effusions, especially pleural and ascitic are mostly due to the extensive disease or metastasis. Due to the high proliferative activity of cancerous cells in the effusion, mitotic cells are available for conventional karyotyping. Several reports indicate that chromosomes of cancer effusion usually differ from the normal diploid cells. Numerical alterations in chromosomes are extensively studied in various predominant female solid tumours but there is a paucity of information regarding cancerous effusions. In the present study, an attempt was made to study malignant effusions of breast, ovarian and cervical cancers with special emphasis on variations in chromosome number and its significance, if any. Results revealed remarkable variation characterizing the model chromosome numbers of these tumours, ranging from marked hypo diploidy to super hyper diploidy.

Key Words*: Malignant effusion, human chromosomes, breast cancer, cervical cancer, ovarian cancer, numerical variations, female cancers, hypodiploid, hyper diploid, metaphase plates.*

INTRODUCTION

Effusion fluid examination on cytology is an easy and quick method for the diagnosis of primary and secondary malignancy [1]. The information provided by body fluid analysis serves several functions, first, it assists the clinician in formulating, in order of priority, a list of differential diagnoses, second it allows one to follow the result of therapy [2]. Cytogenetic abnormalities are a characteristic attribute of cancer cells. Many of these aberrations have emerged as prognostic and predictive markers in hematologic cancers and certain types of solid tumors [3]. Especially, numerical abnormalities can throw some light on disease progression.

This study is an attempt to evaluate numerical variations in effusion chromosomes and its association with cancer.

MATERIALS AND METHODS

Direct chromosome preparation

Approximately 15 ml of Ascitic effusion (after Informed consent) was taken from female patients of breast, ovarian and cervical cancer, who had not undergone any chemotherapeutic treatment. Samples were collected in a simple sterile vial from Lions Cancer Detection Center and Bharat Cancer Hospital, Surat respectively. The collected effusion was centrifuged at 2000 rpm for 10 minutes and supernatant was discarded. The cells were incubated at 37° C for 25 minutes in 10 ml of 0.075 M KCl containing colchicine (20 g/ml). Thereafter, the cells were fixed in chilled fixative (3:1, methanol: acetic acid) for 30 minutes. This step was repeated twice. Slides were prepared by conventional air drying method [6] and stained with 3% Giemsa and were examined for chromosomal rearrangements.

Experimental protocol

Total 7 samples each of ascitic effusion of breast, ovarian and cervical cancer patients were studied. From each effusion sample about 20-80 well spread metaphase plates were examined for chromosomal abnormalities. The effusion samples were considered malignant when minimum 10 percentages of metaphase plates were hyperdiploid.

RESULT

Numerical chromosomal alterations were studied in 21 ascitic effusion samples collected from of breast, ovarian and cervical cancer patients in unbanded chromosomes by direct preparations. The chromosome counts revealed a great variability in the number of chromosomes per cell of each tumor. The range being from 24 to more than 200 in all cases studied (Figure 2). Marker chromosomes were found to be present in most cases. Remarkable variation characterized the modal chromosome numbers of these tumors, ranging from marked hypodiploidy to super hyper diploidy. The ploidy designations are as follows.

Hypo diploidy: Less than 46 chromosomes / cell.
Pseudo diploidy: 46 chromosomes / cell with abnormal karyotype.
Hyper diploidy: 46-59 chromosomes / cell.
Tri ploidy: 60-80 chromosomes / cell.
Tetra ploidy: 80-120 chromosomes / cell.
Super hyper diploidy: More than 120 chromosomes /cell.

Total of 441 metaphase plates were scored from breast effusions and 539 and 504 were that of ovarian and cervical cancer respectively (Table 1).

Table 1: Frequency of ploidy in different cancers

Ploidy designations	Breast Cancer	Ovarian Cancer	Cervical Cancer
Hypo diploidy	245 (55.55 %)	191 (35.43 %)	103(20.43%)
Pseudo diploidy	001 (0.22 %)	001 (00.18 %)	000(00.00%)
Hyper diploidy	129 (29.25 %)	161 (29.87 %)	177(35.11%)
Tri ploidy	039 (8.84%)	120 (22.26 %)	164(32.53 %)
Tetra ploidy	023 (5.21%)	064 (11.87 %)	049 (09.72 %)
Super hyper diploidy	004 (0.90%)	002 (00.37 %)	011(02.18%)
Total metaphase plates scored	**441**	**539**	**504**

Breast cancer effusions showed approximately half of the metaphase plates as hypodiploid (55.55 %), which is highest among all 3 study types. Where as in case of cervical cancer the percentages were as low as 20.43 and were 35.43 in ovarian cancer. Further, it was interesting to note that only one plate with 46 chromosomes with abnormal Karyotype was observed in both breast and ovarian cancer each. Whereas, we didn't found any pseudo diploid plate in cervical cancer (Figure 1).

In all study types, the percentages of hyper diploid plates were of nearer value of 29.25, 29.87, and 35.11 in breast, ovarian and cervical cancer respectively.

We have found few plates with tri diploid karyotype in breast cancer (8.84 %), whereas this value was found to be high in case of ovarian cancer (22.26 %) and even higher in cervical cancer (32.53 %). Many metaphase plates were found to have chromosome number variations between 80-120 (tetra ploidy). The order of frequency was as follows.

Ovarian (11.87 %) > Cervical (9.72 %) > Breast cancer (5.21%).

Interestingly we observed metaphase plates with abnormal karyotype along with very high chromosome number/cell. We found 2 (0.37 %) such plates in ovarian, 4 (0.9 %) in breast and 11(2.18 %) in cervical cancers.

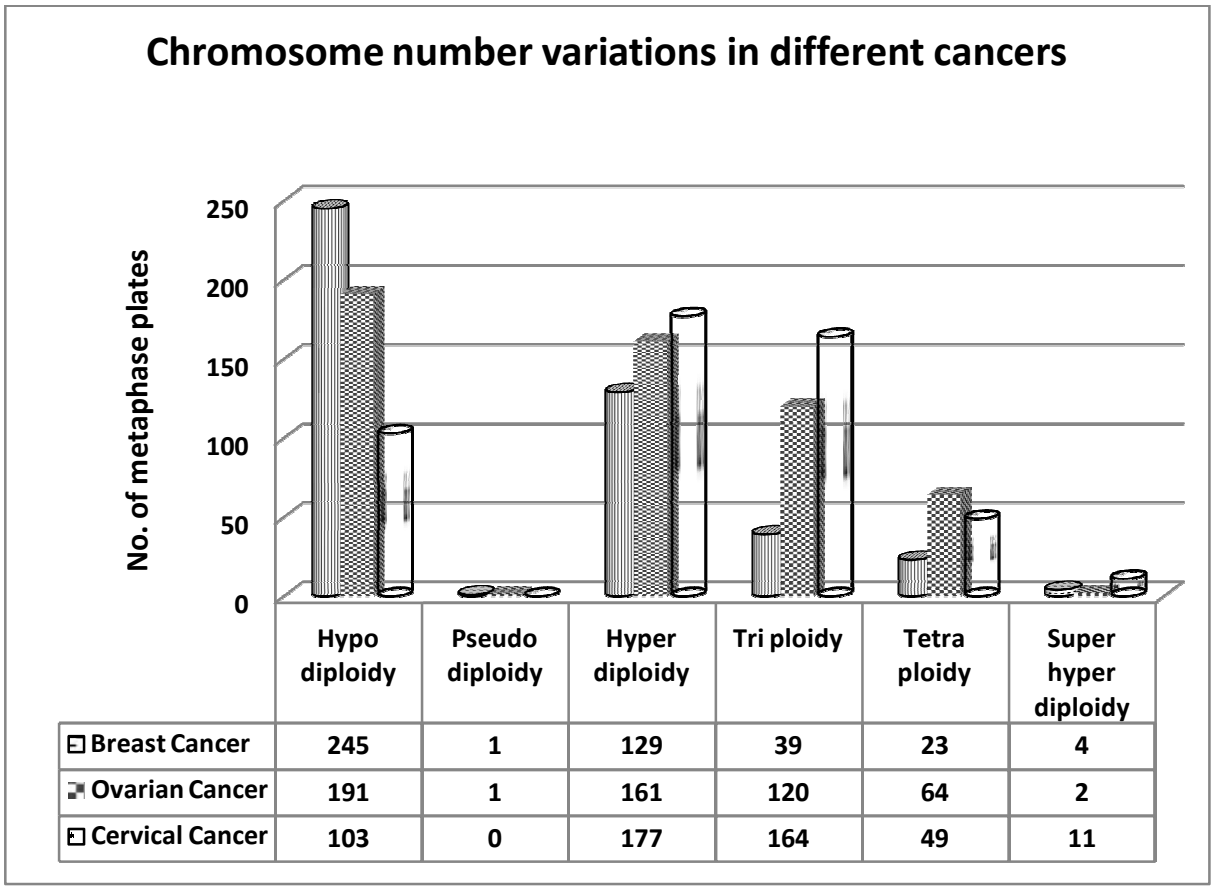

Figure 1: A chart showing chromosome number variations in different cancers

DISCUSSION

The relative ease of pleural fluid aspiration, analysis and cytological examination has kept alive the search for a test to differentiate the various causes of effusion. The cytological examination of body effusion is a complete diagnostic modality which aims at pointing out the etiology of effusions [4, 5].

In the present work, we studied numerical chromosomal aberrations in 21 ascitic effusion samples collected from of breast, ovarian and cervical cancer patients by direct preparations.

Several cytogenetic studies of cancer cells exfoliated into effusions have revealed that cancer cells usually have a different chromosome number and constitution from normal diploid cells [6, 7, 8, 9]. Ishihara and Sandberg have pointed out that certain tumors may have a pseudodiploid chromosome constitution with a total of 46 chromosomes but differ remarkably in the distribution of chromosomes from the normal diploid karyotype. We have also found pseudo diploid plates in case of breast and ovarian cancer. Thus karyotype analysis has been proposed to be of value in the diagnosis of cancer effusions, especially when the karyotype is abnormal [10]. The presence of abnormal marker chromosomes and abnormal karyotypes in pseudodiploid cells are features that strongly support the diagnosis of malignancy.

Cytologic diagnosis of pleural effusions is often complicated by both the neoplastic appearance of benign reactive mesothelial cells and by the lack of a sufficient number of malignant cells for examination.

Misawa *et al*., in their study of gastric cancer effusion found hyper diploid, hyper triploid and hypo tetraploid plates. Our results are in good agreement with them.

Ascitic effusion indicated diverse numerical abnormalities in all study groups. We may conclude that this type of study can be used to characterize malignant effusions and may also unravel other unknown aspects of cancer.

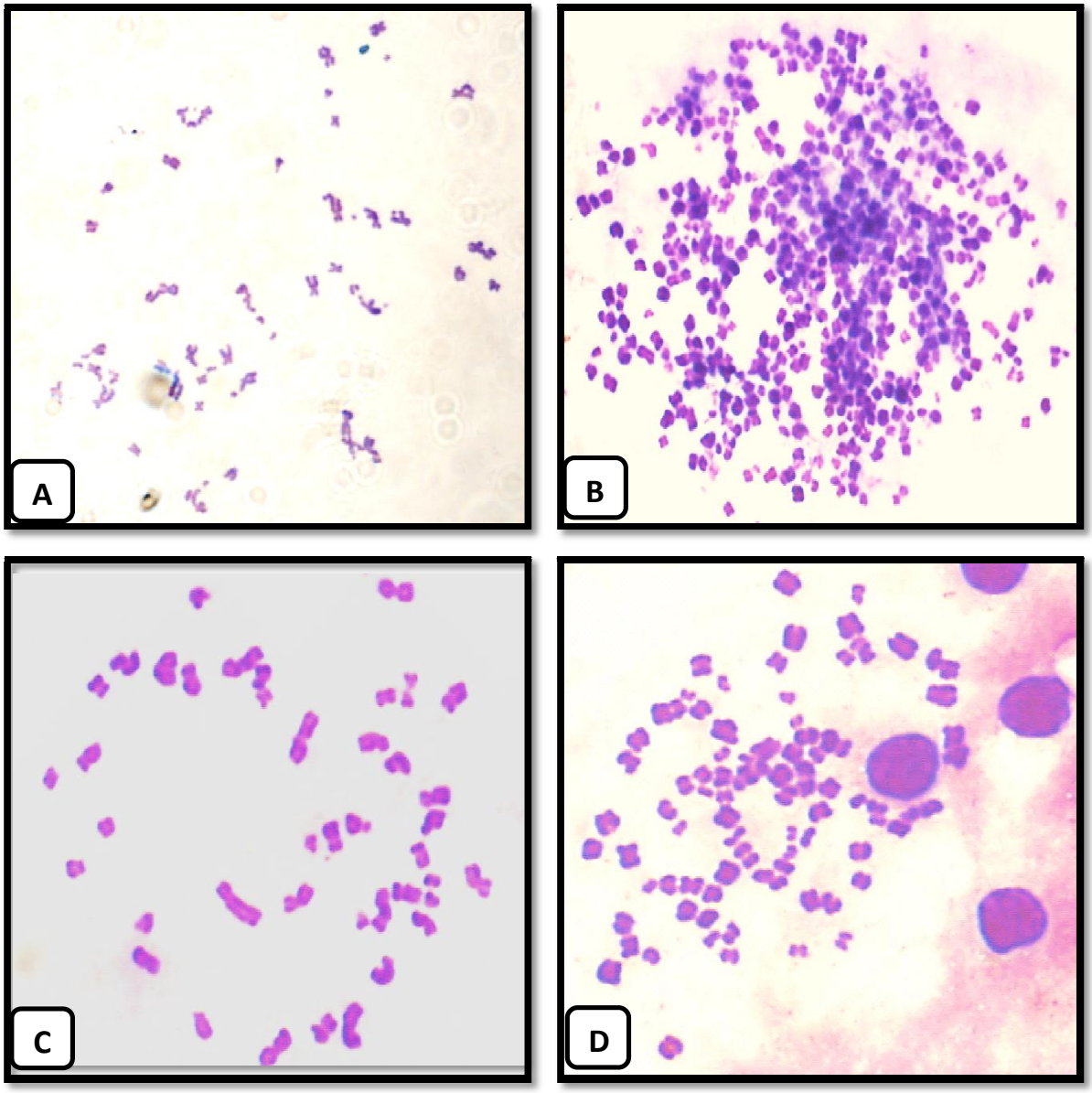

Figure 2 : Different types of chromosome number variations in effusioins.

A. Pseudodiploid metaphase.
B. Super Hyperdiploid metaphase.
C. Hypodiploid metaphase.
D. Triploid metaphase.

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