
ORIGINAL ARTICLE**Homotypic Cell Cannibalism and Cannibalism Index-A Four Year Study in Infiltrating Ductal Carcinoma Breast***Alok Mohan^{1*}, Pradeep Kr. Sharma¹, R.K. Thakral¹**¹Department of Pathology, Muzaffarnagar Medical College and Hospital, Muzaffarnagar-251203 (Uttar Pradesh) India***Abstract :**

Background: Homotypic cell cannibalism is a hallmark of malignant lesions. It serves as an emerging indicator of both the anaplastic grade and invasiveness. *Aim and Objectives:* The present study was conducted to correlate tumour cytological grade with the incidences of cell cannibalism. *Material and Methods:* Three stained smears per case were submitted for cytological grading. The smears were then submitted for visually counting the number of examples of cell cannibalism and the cannibalism index was calculated. *Results:* Seventy cytological diagnosed cases of breast malignancies were assessed for the presence of cell cannibalism. The results were correlated with the cytological grade of carcinoma. The study revealed that cell cannibalism was encountered in 68.57% cases of breast carcinoma. 44.4% of grade I, 71.4% of grade II and 100% of grade III tumours grade. It was observed that grade I tumours had mean cannibalism per smear of 1.62/1000 tumour cells smear, grade II mean cannibalism per smear of 2.50/ 1000 tumour cells and grade III mean cannibalism per smear 4.90/1000 tumour cells. Tumour diathesis and metastasis were also found more in cannibalism positive cases. *Conclusion:* The phenomenon of cell cannibalism was encountered more in high grade tumours. Thus it can be designated as an emerging marker of anaplasia and aggressive tumour behaviour.

Keywords: Cytology, Carcinoma, Cannibalism Index, Breast, Malignancy

Introduction:

“Cell-in-cell” phenomenon refers to formation of “cell-in-cell” structures due to the entering of one or more living cells into another cell. This is an old biological phenomenon which was traced back about one hundred years ago. Recently, “cell-in-cell” has attracted attention of pathologists because of its close relationship in understanding clinical pathogenesis [1]. “Cell-in-cell” structures occur *in vitro* or *in vivo*, either homotypically or heterotypically, thus representing unique intercellular interactions of different cells [2]. Mostly homotypic type of “cell-in-cell” interactions occur between sibling tumour cells, whereas heterotypic type of “cell-in-cell” structures are found in-between the immune cells and tumour or various tissue cells [3].

Cannibalism is described to be a process in which metastatic tumour cells undergo starvation and exhibit their ability to actively take or eat other homotypic or heterotypic live or dead cells. Degradation of effector cells inside cannibalistic cells relies on the acidic digestive machinery in cavesomes that requires scaffolding proteins like caveolin-1 or ezrin as well as activation of proteolytic enzymes. This “cell-in-cell” death supplies nutrients in starvation. Moreover, this mechanism saves the tumour cells from immune attack [4-6].

Cellular cannibalism was first described by Leyden in 1904. He used the name “bird's eye cells” for cannibalistic cells [7]. It is defined as the ability of a cell to phagocytose another cell. The ingested cell is contained in a vacuole that pushes the nucleus of the cannibal cell to the periphery of the cell. This appearance gave rise to the names such as “bird's eye cells” or “signet-ring cells” [8].

The feature of cell cannibalism has been identified in various tumours *in vivo* as well as *in vitro* [9]. In metastatic melanoma, tumour cannibalism was observed *in vivo*, and confirmed *in vitro* as a feature of metastatic lesions. However, it was not found in primary tumour site [5].

Many workers have recognized this morphological phenomenon in several tumour types e.g.; carcinoma breast, giant cell carcinoma of lung, small cell carcinoma of lung, endometrial stromal sarcoma, but they have referred to this phenomenon by different names such as cellular phagocytosis of tumour cell by another tumour cell, cell-in cell appearance or bird's eye cells [10-13].

Cannibalism index refers to the number of examples of cell-cannibalism per 1000 tumour cells [14]. This is calculated for the quantitative assessment of cell cannibalism phenomenon. Cell cannibalism positive cytological smears of malignant lesions are subjected to microscopic examination. This parameter is introduced to maintain uniformity in counting and to decrease the observer-bias. Cannibalism index of high grade tumours tends to be high.

Hetero-cannibalism means eating up of non-self cells, mostly leucocytes. Many past studies have described the features of hetero-cannibalism [15, 16]. In 2008, few studies have suggested that

hetero-cannibalism can be explained as an exacerbation of self cannibalism and may be a survival strategy for cancer cells [17]. The ability for phagocytosis could be used as a marker for the malignant potential of the tumour cells [16].

With these considerations, the present study was undertaken to evaluate the presence of cell cannibalism in cytologically diagnosed breast malignancies and to correlate the occurrence of cell cannibalism with cytological grading of malignant tumour.

Material and Methods:

The present study was conducted on the cytology samples collected from the cytology laboratory in department of Pathology in Muzaffarnagar Medical College and Hospital, Muzaffarnagar. Meerut from August 2011 to July 2015.

The cases studied were of Fine Needle Aspiration Cytology (FNAC) and nipple discharge cytology, with a cytologically diagnosed breast malignancy.

1. Three smears per case, stained with the MGG were submitted for cytological grading with the reference to cytological criteria laid down by Robinson et al.
2. The smears were firstly submitted for visually counting the number of examples of cell cannibalism in 20 high power fields (X40) objective. The counting was done by two pathologists individually (AM and PS). Then the mean per smear was calculated. This reduced observer bias. Smears showing cell cannibalism were labeled as cannibalism positive.
3. Cell cannibalism positive smears were then expressed as number of examples of cannibalism per 1000 tumour cells (i.e. cannibalism index).

4. Finally, the index was correlated with the cytological grading of malignancy, in order to explore the relation between cell cannibalism, grading and aggressive tumour behaviour.
5. Presence of metastasis in lymph nodes, body fluids and other organs along with tumour diathesis was studied to establish the aggressiveness of malignancy in order to assess the behaviour of malignancy.

Results:

A total of seventy cases of breast tumours were studied, including 68 females and 02 males. The mean age of the cases studied was 52 years. Invasive ductal carcinoma, Not Otherwise Specified (NOS) formed the major group with 65 cases (92.85%). In 61 cases FNAC was done from the palpable mass, while in 04 cases discharge from nipple was taken for smear preparation as no well defined mass was observed on palpation. There were two cases of medullary carcinoma breast (2.86%), and one case each of tubular carcinoma breast, invasive papillary carcinoma and metaplastic carcinoma of breast (each 1.43 %).(Table 1) According to Robinson's grading [18], 18 were grade I (25.71%), 42 were grade II

(60%) and 10 were grade III tumours (14.28%). Cell cannibalism was demonstrated in 48 (68.57%) cases out of 70 cases of infiltrating ductal carcinoma of breast not otherwise specified. It was positive in 44.4% of grade I, 71.4% of grade II (Fig.1) and 100% of grade III carcinomas (Fig.2). On comparison of tumour grade and cannibalism index, it was observed that grade I tumours had a mean of 1.62/1000 tumour cells, grade II (Fig. 1) with a mean of 2.50/1000 tumour cells and grade III (Fig.2) with a mean of 4.90/1000 tumour cells (Table 2). It was also observed that tumour diathesis and metastasis were commonly encountered in cannibalism positive cases.

Table 1: Distribution of 70 Cases of Carcinoma Breast

Types of Carcinoma	Number of Cases	Percent
Infiltrating Ductal Carcinoma (NOS)	65	92.85
Medullary Carcinoma	02	2.86
Papillary Carcinoma	01	1.43
Tubular Carcinoma	01	1.43
Metaplastic Carcinoma	01	1.43

Table 2: Grade wise Cannibalism Positivity

Robinson's grade	Cases		Cannibalism Positivity		Total Example of Cell Cannibalism per 1000 Malignant Cells among Positives	Mean Cannibalism Index
	Numbers	Percent	Numbers	Percent		
Grade I	18	25.70	08	44.4	13	1.62
Grade II	42	60.00	30	71.4	75	2.50
Grade III	10	14.30	10	100.0	49	4.90

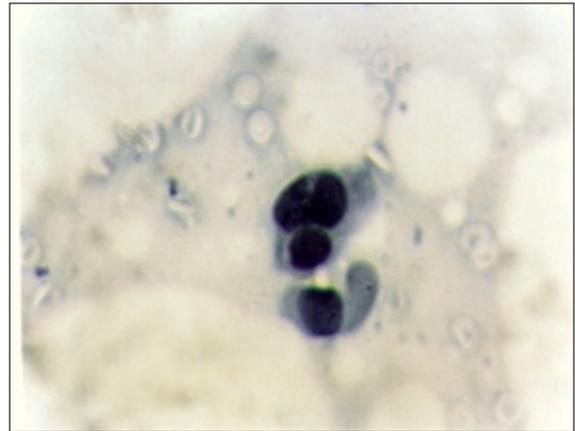
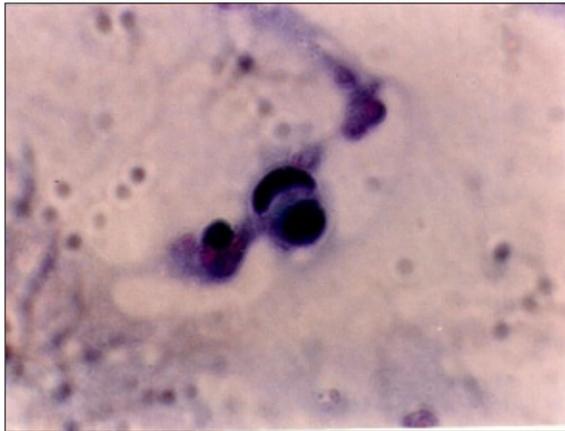


Fig. 1 & 2: Photomicrograph of Aspiration Smear Showing Cell Cannibalism in Grade II Breast Carcinoma (PAPX400)

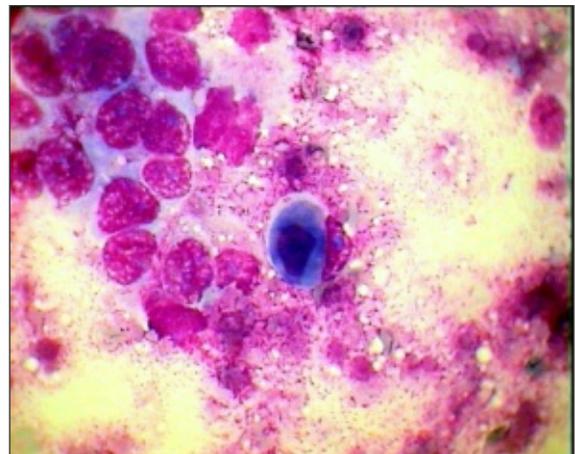
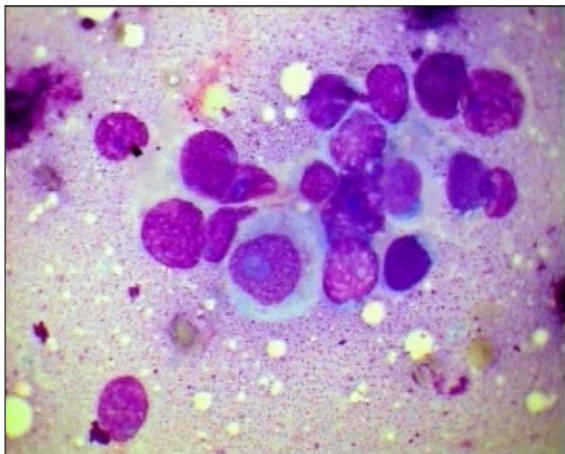


Fig.3 & 4: Photomicrograph of Aspiration Smear Showing Cell Cannibalism in Grade III Breast Carcinoma (GIEMSA X400)

Discussion:

Cellular cannibalism is an important feature to distinguish benign from malignant lesions. The presence of cannibalism besides being an indicator of malignancy is also related with the aggressiveness of the malignancy.

It is mainly in the last two decades that the role of cell cannibalism is recognized and researched in

relation to tumour behaviour. It is regarded as an exclusive characteristic of malignant tumour cells, and this phagocytic behaviour is correlated with the degree of malignancy [19].

Formation of endosomes seems to be the driving structure of cannibalism. The endosomes contain constitutively activated and highly efficient acidic digestive system. This allows the tumour cells to

endocytose and digests other cells without the need for specific receptor-ligand interactions. An intact membrane-to- cytosol framework, including a dynamic link between caveolin-1, ezrin, and actin seem to be necessary for the formation of “cannibalistic vacuole”. Down-regulation of any of these leads to substantial inhibition of cannibalistic activity [4].

Cannibalism by tumour cells can contribute to their survival and proliferation. Phagocytosis of other tumour cells resemble autophagic digestion of cell organelles under starvation thus fulfilling feeding requirements under adverse conditions while ingestion of immune cells (neutrophils and lymphocytes) leads to escape from immune system mechanisms. Cannibalistic cells are resistant to acidic environment, while macrophages or other tumour cells die in that environment [19].

Several steps have been proposed to describe the process of cell cannibalism. At first, the cannibalistic cell engulfs the free cell with change in shape of the nucleus from circular to semilunar. The nuclear shape of the interiorized cell remains unchanged. In the course of time, the interiorized cell is completely encircled and dies off. The death of the interiorized cell is apparently due to lack of nutrition, rather than due to effect of lysosomal enzymes. This was concluded as electron microscopy showed that lysosomal structures were sparsely present in cannibalistic cells as compared to the interiorized cell.

Cell cannibalism is different from the phenomenon of phagocytosis. In phagocytosis, there are usually abundant lysosomes in the outer cell and this leads to destruction of the interiorized cell.

In the present study a total of seventy cases of breast carcinomas, including 68 females and two males, were studied. The mean age of the cases studied was 52 years. Oiler Kjellgren [20] studied 253 cases of palpable breast masses with a bloody discharge and mean age of 48 years.

Many studies on breast cytology have been done so far and many cytologists have encountered this phenomenon of cannibalism. They gave due importance to it and mentioned it in their studies. Oiler Kjellgren [20] observed 13 cases of invasive ductal carcinoma showing active cell cannibalism along with other features of cytological atypia. Similarly, Uei *et al* [12] have reported cytophagocytosis in 47% cases presenting with small clusters and in 65% cases with large clusters. This is comparable with our study. In our study we got 68.57% (48 out of 70 cases) of breast carcinoma positive for cell cannibalism during initial screening. Ng & Hong [21] demonstrated “cell in cell” appearance in all 5 cases of metaplastic carcinoma, of which 4 were diagnosed as well differentiated squamous cell carcinoma and 1 invasive ductal carcinoma NOS.

In the present study cell cannibalism was observed in 68.57% cases (48 out of 70) cases of infiltrating ductal carcinoma of breast, 44.4% of grade I, 71.4% of grade II and 100% of grade III carcinomas according to Robinson's grading system.

Comparative study of malignancy grade and cannibalism index, showed that grade I carcinomas had a mean of 1.62/1000 tumour cells, grade II (Fig.1) with a mean of 2.50/ 1000 tumour cells and grade III (Fig.2) with a mean of 4.90/

1000 tumour cells. These findings are well in concordance with study done by W.T. Abodie et al (2006) [14]. They studied 50 cases of ductal carcinoma breast. They observed cell cannibalism/1000 tumour cells grade I showing a mean of 1.07/1000 tumour cells, grade II showing a mean of 2.67/1000 tumour cells and grade III showing a mean of 4.89 /1000 tumour cells. In our study sample size is bigger and the results are slightly higher in case of grade I cases and comparable in grade II and grade III cases.

The relationship between cell cannibalism and distant metastasis should be explored in further

studies, as it may prove to be a criterion of malignancy [22]. In the present study, there had been a good correlation between tumour grade and cannibalism index in the cases of carcinoma breast. The phenomenon of cannibalism thus, predicts both the anaplastic grade and invasiveness, although its value as an independent prognostic marker has yet to be established. Hence, a conclusion can be drawn that a correlation between cell cannibalism and aggressive tumour behaviour do exists. But still a further extensive study regarding cell cannibalism in relation to tumour behaviour is required.

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