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SHORT COMMUNICATION

Cytological evaluation and significance of cell cannibalism in effusions and urine cytology

Farooq AHMED WANI *MD* and Subhash BHARDWAJ* *MD*

*Department of Pathology, Aljouf College of Medicine, Sakaka, Saudi Arabia and *Department of Pathology, Government Medical College, Jammu, India*

Abstract

Cell cannibalism is believed to be an indicator of high-grade aggressive cancers with increased metastatic potential. It denotes both anaplastic grade and invasiveness and is valuable in assessing tumor behavior. The present study was a 2-year retrospective and 1-year prospective study conducted in the Department of Pathology, Government Medical College, Jammu. PAP and MGG stained smears of effusions and urinary cytology were evaluated for cannibalism. Cannibalism was assessed by parameters like cellularity of cannibalism, diameter of cannibalistic cells, chromatin pattern and background of the smears. Of 350 cases evaluated, 260 (74.2%) were benign and 90 (25.8%) were malignant. Cannibalism was absent in all benign cases. Cannibalism was present in 14 ascitic fluids, 7 pleural fluids, 1 pericardial fluid and 3 cases of urine cytology. Comparison of distribution of cannibalism in effusions and urine did not yield statistically significant result ($X^2 = 0.8678$ and $p > 0.05$). Comparison of other parameters between effusions and urine samples also did not yield significant results. We conclude that cytological parameters of cellular cannibalism are better observed in malignant effusions than in urine cytology but did not reach statistical significance. Cannibalism can be assessed morphologically in malignant body fluids and is an indicator of increased tumour growth.

Keywords: cell cannibalism, effusions, urinary cytology

INTRODUCTION

Cell cannibalism was first described by Leyden in 1904 as the ability of one cell to phagocytose another cell. It is also defined as a large cell enclosing a smaller one within its cytoplasm and is known by odd names such as "bird's eye cells" or "signet ring cells".[1] The "bird's eye cells" means smaller tumour cells are found in the cytoplasm of larger tumour cells with crescent shaped nucleus.[2]

Brouwer *et al* proposed the successive steps in the process of cell cannibalism like contact with the cell, engulfment, changes in shape of cannibalistic cell to semi-lunar shape followed by nuclear disintegration and death of the cell.[3] Cell cannibalism is a feature of malignant cells to control the tumour growth. The cannibalistic tumour cell has the unique capacity of phagocytosing not only fellow tumour cells but also other cells like neutrophils, lymphocytes and erythrocytes (Xeno-cannibalism). This suggests that engulfing and killing lymphocytes may help the tumour to escape the immune response.[4]

Brouwer *et al* also proposed that serum factors may be responsible for induction of cannibalism in cells. The main factor regulating the extent of cannibalism would be the hunger of tumour cells and their nutritional deficiencies and imbalances. Acidic conditions are known to increase the phenomenon of cannibalism.[3] Ezrin, an actin binding protein may be involved in promoting cell.[5]

Cell cannibalism has been reported in lung carcinomas,[6] endometrial stromal sarcomas, gall bladder carcinomas, hepatobiliary and pancreatic carcinomas,[7] gastrointestinal malignancies,[8] female genital tract malignancies,[9] malignant melanoma and infiltrative ductal carcinoma of the breast.[10] Cellular cannibalism is also seen in salivary gland carcinomas and lymphomas,[11] high grade transitional cell carcinoma of bladder on urine cytology.[12-14]

Address for correspondence: Dr. Farooq Ahmed Wani, Department of Pathology, Aljouf College of Medicine, Sakaka, Saudi Arabia. P.O. Box: 2014. Tel. no.: 00966593750779. E-mail: fawani@ju.edu.sa

Only few studies on cell cannibalism in effusions like peritoneal, pleural or pericardial fluids are available in the literature.[15,16]

Cannibalism can be assessed by parameters such as cellularity of cannibalism, diameter of cannibalistic cell, chromatin pattern (heterochromatic or euchromatic) and background of the smears (necrosis, isomorphic erythrocytes and dysmorphic erythrocytes).

In view of the paucity of literature on cell cannibalism in effusions and urine cytology, the present work was being designed to describe in detail this entity and study its clinical relevance.

MATERIALS AND METHODS

The study was conducted in the Cytology section of the Postgraduate department of Pathology, Government Medical College, Jammu. It was both a retrospective and prospective study. After obtaining clearance from the Institutional Ethics Committee,all records regarding retrospective study material consisting of all PAP and MGG stained smears of effusions and urinary cytology diagnosed between 01.11.2009 to 31.10.2011 were retrieved from the cytopathology section. Prospective study material included all effusions and urine cytology specimen received in cytology laboratory with effect from 01.11.2011 to 31.10.2012. Standard procedure for diagnostics is carried out in the hospital which includes informed written consent at the time of sample collection.

Urine cytology

Urine for cytological study was obtained 3 to 4 hours after the patient had last urinated. Specimen submitted from the first morning voiding was not received for cytological examination because stagnant cells show degenerative changes. The minimum amount of urine necessary to ensure adequate cellularity was around 25-100ml.

The results of urine cytology were reported using diagnostic categories as "negative" (no malignant cells identified), "atypical" (mildly atypical urothelial cells), "suspicious" (atypical urothelial cells suspicious for malignancy) and "positive" (conclusive for malignancy).

Effusions

Effusions were collected in clean containers. To prevent clotting, fluid was collected in bottles containing 3 units of heparin per milliliter of capacity. In cases of haemorrhagic effusions, 5-6ml of glacial acetic acid was added so that red cells are hemolyzed and malignant cells are clearly visible.[17]

Sample processing

The effusions and urine samples were centrifuged at the rate of 1500 rpm for 10-15 min. The supernatant fluid was discarded and sediment was used for making smear. The fluid was spread on glass slides in two divided parts so that thick and thin smears were made and thereafter staining was done. Two smears were immediately fixed in 95% ethyl alcohol for PAP staining and the rest of the smears were air dried for MGG staining.

Evaluation for cannibalism

The Giemsa and PAP stained smears were examined microscopically for demonstration of cannibalism. The cannibalistic cells were composed of crescent shaped nucleus engulfing another cell with round to oval nucleus (Figure 1). The nucleus of the free cell is unaltered, however, the nucleus of the cannibalistic cell changed to semilunar shape.

Only those portions of smear which were thin and had evenly distributed cells were screened for cannibalism. This was to ensure that overlapping cell clusters do not give a false impression of "cell within a cell" morphology.

The number of cannibalistic cells was counted by two independent observers in 30 random fields at 40X and 100X magnification.

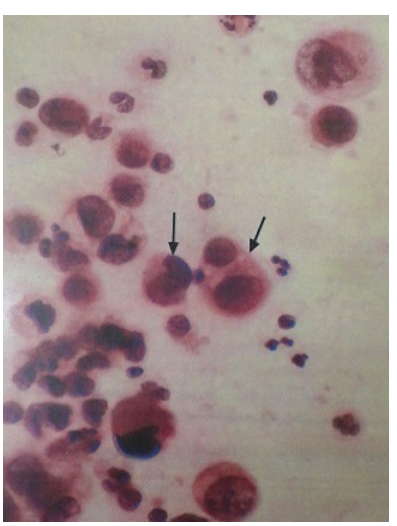

FIG. 1: Photomicrograph from a case of malignant pericardial effusion showing cannibalistic cells (arrows) (MGG 40X)

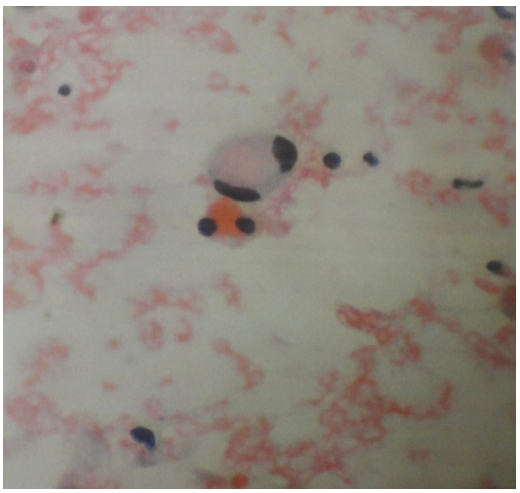

FIG. 2: Photomicrograph from ascitic fluid showing cannibalistic cells having heterochromatin nuclear pattern. Background is dysmorphic erythrocytic (PAP 40 X)

Cannibalism was also assessed by parameters like cellularity of cannibalism, diameter of cannibalistic cell, chromatin pattern (heterochromatic or euchromatic) and background of the smears (necrosis, isomorphic erythrocytes and dysmorphic erythrocytes).

Cellularity of cannibalism was assessed as (1+ low grade) having < 5 cells, (2+ moderate) having 5-20 cells and (3+ high grade) having > 20 cells in each preparation. Diameter of cannibalistic cells was assessed roughly by comparing it with red blood cells and other chronic inflammatory cells like lymphocytes. Chromatin pattern evaluated was heterochromatin (irregularly condensed darkly stained chromatin) or euchromatin (homogenous lightly stained chromatin) pattern and background was either necrotic or isomorphic/dysmorphic erythrocytic (Figure 2).

RESULTS

A total of 350 cases of MGG and PAP effusions and urine cytology smears were included in the study. The retrospective study comprised of 250 cases and the prospective study consisted of 100 cases. Out of the total 350 cases, 260 were benign (74.2%) and 90 (25.8%) were malignant. Our study consisted of 50 cases of malignant ascitic fluids, 25 cases of malignant pleural fluid, 5 cases of malignant pericardial fluid and 10 cases of malignant urine cytology.

Out of 90 malignant cases, 80 (88.8%) cases were in the age group 30-60 years and 10 (11.2%) cases were in the age group 61-80 years. 44 (48.8%) cases were males and 46 (51.2%) cases were females. Cannibalism was absent in all benign effusions and benign urine cytology cases.

Cannibalism was present in 14 ascitic fluids, 7 pleural fluids, 1 pericardial fluid and 3 cases of urine cytology (Table 1). Comparison of distribution of cannibalism in effusions and urine did not yield statistically significant result (X^2 = 0.8678 and p value >0.05) (Table 2). Comparison of different parameters like cellularity, diameter of cannibalistic cell, chromatin pattern and background of the smears between effusions and urine did not yield significant results (Table 2).

TABLE 1: Cytological parameters of cannibalism in all the cases

Cytological parameters		Ascitic fluid		Pleural fluid		Pericardial fluid		Urine cytology	
		No.	%	No.	%	No.	%	No.	%
Cellularity	1+	10	71.4	4	57.2	1	100	1	33.3
	2+	3	21.4	2	28.6			2	66.7
	3+	1	7.2	1	14.2				
Diameter	>RBC	9	64.3	5	71.4	1	100	2	66.7
	=RBC	4	28.5	2	28.6			1	33.3
	<RBC	1	7.2	–	–				
Nuclear Chromatin	Euchromatin	5	35.7	2	28.5	1	100	1	33.3
	Heterochromatin	9	64.3	5	71.5			2	66.7
Background	Necrotic	6	42.8	3	42.8	1	100	1	33.3
	Iso/dysmrophic	8	57.2	4	57.2			2	66.7

TABLE 2: Comparison of malignant effusions and urine cytology

	Malignant effusions cytology	Malignant urine cytology	P value
Positive for cannibalism	22/80 (27.5%)	3/10 (30%)	0.8678# P > 0.05
Cellularity			
1+	15/22 (78.5%)	1/3 (33.3%)	0.5902#
2+ and above	7/22 (21.5%)	2/3 (66.7%)	P > 0.05
Cell diameters			
>RBC	15/22 (78.5%)	2/3 (66.7%)	0.9579#
<RBC	7/22 (21.5%)	1/3 (33.3%)	P > 0.05
Chromatin pattern			
Euchromatin	7/22 (21.5%)	1/3 (33.3%)	P=1.00*
Heterochromatin	15/22 (78.5%)	2/3 (66.7%)	
Background pattern			
Necrotic	10/22 (45.5%)	1/3 (33.3%)	P=1.00*
Isomorphic/ dysmorphic/ erythrocytic	12/22 (54.5%)	2/3 (66.7%)	

Chi-square with Yates correction

* Fisher's exact test

DISCUSSION

Cell cannibalism can be studied morphologically to distinguish a benign lesion from the malignant lesion as it is absent in former. It has been found in carcinoma of the breast, lung, ovary, bladder and other visceral cancers. Krajcovic *et al* proposed that cell cannibalism by entosis, a form of engulfment of live cells, may lead to polyploidy, due to disruption of cytokinesis of engulfing cell hosts by internalized cells. By inducing aneuploidy, this may be a mechanism whereby cannibalistic cell behavior can promote tumour progression. Cannibalism mediated chromosomal instability and aneuploidy may bea means for aggressive behavior of cancers.[18]

Fais had shown in his study that metastatic tumor cells use cannibalism to feed in conditions of low nutrient supply and phagocytic properties offer them a survival advantage.[1] Tumour cell cannibalism is therefore a feature of aggressiveness and advanced tumour stage. Our study was framed to analyze tumour cannibalism in malignant effusions and urine cytology based on morphological criteria.

In our study, the majority of body fluids being 260 (74.2%) cases were benign and 90 cases (25.8%) were malignant. Gupta and Dey in 2002 conducted a study on 40 cases which included 20 benign and 20 malignant cases of effusions.[16] Higher number of cases in our study is attributed to greater sample size as well as the extended duration of three years of study. The age-wise distribution revealed the maximum number of cases in age group 30-60 years and females constituted 46 (51.2%) cases whereas 44 (48.3%) cases were male.

Cannibalism was morphologically identified in 25 (27.7%) cases in our study which included 14 (56%) male and 11 (44%) females with age ranging from 40-50 years. Bansal *et al* reported cannibalism in 6 (54.5%) male and 5 (45.5%) female patients with age ranging from 43-74 years.[15] This is in concordance with our study. Tiwari *et al* found cannibalism in 11% of their cases.[19]

In malignant effusions, we found cannibalism in 14 (63.6%) cases of ascitic fluid, 7 (31.8%) cases of pleural fluids and 1 (4.5) case of pericardial fluid. Bansal *et al* reported cannibalism in 7 (53.9%) cases of ascitic fluid and 6 (46.1%) cases of pleural fluids.[15]

For malignant effusions, 1+ grade was found in 15 (78.5%) cases and 2+/3+ in 7 (21.5%). So the predominant grade was 1+ in our study in contrast to the study done by Bansal *et al* who

reported < 5 cells in 5 cases (45.4%) and > 5 cells in 6 cases (54.6%).[15] In malignant urine cytology, grade 1+ cellularity was observed in one (33.3%) case and grade 2+/3+ in 2 (66.7%) cases. The results are in concordance with the study carried out by Ohsaki *et al* who reported 15 positive cannibalism urine cytology cases and found grade 1+ in 1 (6.7%) case and grade 2+/3+ in 14 (93.3%) cases but the number of cases in our study is too low.[12]

The diameter of the cannibalistic cells was larger than the size of the RBC's in 15 (78.5%) cases and = or <RBC size in 7 (21.5%) cases of malignant effusions. For malignant urine cytology, the diameter of the cannibalistic cells was larger than the red blood cells in 2(66.7%) cases and equal to red blood cell in 1 (33.3%) case. Ohsaki *et al* found maximum diameter of 18.0 - 30.44µ in cannibalistic cells of urothelial carcinoma.[12] Thus our study revealed that cannibalistic cells have significantly larger diameter.

We could not find any study in the literature which used parameters of heterochromatin and background. In our study heterochromatin pattern was observed in 15 (78.5%) cases and euchromatin in 7 (21.5%) cases of malignant fluids. Nuclear chromatin was euchromatic in 1 case and heterochromatic in 2 cases of malignant urine cytology. These findings suggest that heterochromatin pattern (irregular, dark condensed chromatin) is predominant in cannibalistic cells. This could be attributed to lesser degree of maturation and differentiation of nuclear chromatin in malignant cells.

Our study also revealed that isomorphic/ dysmorphic erythrocytic background (54.5%) is of greater significance than that of necrotic background (45.5%) while assessing malignant body fluids positive for cannibalism. Necrotic background was observed in 1 (33.3%) case against 2 cases showing iso/dysmorphic erythrocytic background in malignant urine cases. Kiyomoto *et al* observed necrotic background in 7(46.6%) cases and iso/dysmorphic background in 8 (53.4%) cases out of 15 cases included in their study.[20]

Our study found cannibalistic cells in 27.5% (22/80) of malignant effusions cases as compared to 30% (3/10) malignant urine cytology cases. However, comparison of distribution of cannibalism in effusions and urine did not yield statistically significant result ($X^2 = 0.8678$ and p value > 0.05) (Table 2). Gupta and Dey in 2002 in their study found more cannibalistic cells (3.4/100) in malignant effusions as compared to malignant urine cytology cases (2/100).[16] The lesser number of cannibalistic cells in malignant effusions as compared to the malignant urine cases may be due to less number of urine cytology cases included in our study.

Cannibalistic cell diameter, nuclear chromatin pattern and necrotic background were more pronounced in malignant effusions as compared to malignant urine cytology. However, comparison between various parameters like cellularity, diameter of cannibalistic cell, chromatin pattern and background of the smears between effusions and urine did not yield statistically significant results as the urine cytology cases were less in number (Table 2).

Thus our study showed that morphologic assessment of cannibalism in malignant effusions and urine cytology cases is possible and may be a useful marker for tumour growth.

Conclusion

Cytological parameters of cellular cannibalism are better observed in malignant effusions than in urine cytology but did not reach statistical significance. Diameter of the cannibalistic cells was larger than red blood cells in majority of the cases. A predominant heterochromatin pattern and erythrocytic background was observed in both effusions and urine cytology. We conclude that cannibalism can be assessed morphologically in malignant body fluids. Our study supports the presumption that cannibalism is associated with malignancy and is an indicator of increased tumour growth. Evaluation of this aggressive parameter may have utility as a pharmacological target in the management of metastatic disease.

REFERENCES

1. Fais S. Cannibalism: a way to feed on metastatic tumors. Cancer Lett. 2007; 258(2):155–64.
2. Bauchwitz MA. The bird's eye cell: cannibalism or abnormal division of tumor cells. Acta Cytol.1981; 25: 92.
3. Brouwer M, de Ley L, Feltkamp CA, Elema J, Jongsma AP. Serum-dependent "cannibalism" and autodestruction in cultures of human small cell carcinoma of the lung. Cancer Res. 1984; 44(7): 2947–51.
4. Matarrese P, Ciarlo L, Tinari A, Piacentini M, Malorni W. Xeno-cannibalism as an exacerbation of self-cannibalism: a possible fruitful survival strategy for cancer cells. Curr Pharm Des. 2008; 14(3): 245–52.
5. Hunter KW. Ezrin, a key component in tumor metastasis. Trends Mol Med. 2004; 10(5): 201-4.

6. DeSimone PA, East R, Powell RD Jr. Phagocytic tumour activity in oat cell carcinoma of lung. Hum Pathol. 1980; 11(5 Suppl): 535-9.
7. Khayyata S, Basturk O, Adsay NV. Invasive micropapillary carcinomas of the ampullo-pancreatobiliary region and their association with tumor-infiltrating neutrophils. Mod Pathol. 2005; 18(11): 1504-11.
8. Caruso RA, Muda AO, Bersiga A, Rigoli L, Inferrera C. Morphological evidence of neutrophil-tumor cell phagocytosis (cannibalism) in human gastric adenocarcinomas. Ultrastruct Pathol. 2002; 26(5): 315–21.
9. Chandrasoma P. Polymorph phagocytosis by cancer cells in an endometrial adenoacanthoma. Cancer. 1980; 45(9): 2348–51.
10. Abodief WT, Dey P, Al-Hattab O. Cell cannibalism in ductal carcinoma of breast. Cytopathology. 2006; 17(5): 304-5.
11. Arya P, Khalbuss WE, Monaco SE, Pantanowitz L. Salivary duct carcinoma with striking neutrophil-tumor cell cannibalism. Cytojournal. 2011; 8: 15.
12. Ohsaki H, Haba R, Matsunaga T, Nakamura M, Kiyomoto H, Hirakawa E. 'Cannibalism' (cell phagocytosis) does not differentiate reactive renal tubular cells from urothelial carcinoma cells. Cytopathology. 2009; 20(4): 224-30.
13. Hattori M, Nishino Y, Kakinuma H, Matsumoto K, Ohbu M, Okayasu I. Cell cannibalism and nucleus-fragmented cells in voided urine: useful parameters for cytologic diagnosis of low-grade urothelial carcinoma. Acta Cytol. 2007; 51(4): 547–51.
14. Kojima S, Sekine H, Fukui I, Ohshima H. Clinical significance of "cannibalism" in urine cytology of bladder cancer. Acta Cytol. 1998; 42(6): 1365–9.
15. Bansal C, Tiwari V, Singh U, Srivastava A, Misra J. Cell cannibalism: a cytological study in effusion samples. J Cytol. 2011; 28(2): 57–60.
16. Gupta K, Dey P. Cell cannibalism: diagnostic marker of malignancy. Diagn Cytopathol. 2003; 28(2): 86-7.
17. Miller F. Cytopreparatory methods: Collection, smearing, staining, screening and reporting. In: Keebler CM, Reagan JW, Wied GL, editors. Compendium on cytopreparatory techniques. 4th ed. Chicago Tutorials of Cytology; 1976. p. 59-69.
18. Krajcovic M, Overholtzer M. Mechanisms of ploidy increase in human cancers: a new role for cell cannibalism. Cancer Res. 2012; 72(7): 1596–601.
19. Tiwari N, Gheldof A, Tatari M, Christofori G. EMT as the ultimate survival mechanism of cancer cells. Semin Cancer Biol. 2012; 22(3): 194–207.
20. Ohsaki H, Hirakawa E, Kushida Y, *et al*. Can cytological features differentiate reactive renal tubular cells from low-grade urothelial carcinoma cells? Cytopathology. 2010; 21(5): 326-33.