

Cytokeratin Expression Profile Study in Malignant Ovarian Tumors: A Retrospective Study in Teaching Institution

Jeyanthi Gnanamuthu^{1*}, S. Jenita Christina² Ranjana and P. Kannan¹

¹Department of Pathology, Govt Thoothukudi Medical College, Thoothukudi, Tamilnadu, India

²Department of Pathology, KAVP Medical College, Trichy. India

ABSTRACT

Background: Expression of cytokeratin is seen in varied ovarian tumors including primary surface epithelial tumors, Granulosa cell tumors, Sertoli – Leydig cell tumors, non dysgerminomatous germ cell tumors and metastatic carcinomas. The aim of the study is to demonstrate various patterns of cytokeratin expression in epithelial and non-epithelial malignant ovarian tumors.

Methods: Materials for the present study of 39 cases of malignant ovarian tumors obtained from the patients admitted during the period of two years. For histopathological examination, 10% formalin fixed embedded representative tissue sections were studied with Haematoxylin and Eosin. Detailed microscopic examination was carried out. Application of IHC for cytokeratin expression study was carried by streptavidin – biotin complex method. The details of clinical history and relevant investigations were obtained.

Results: The total number of malignant ovarian tumors studied during two year period was 39 cases. Among that, serous tumors was the most common [25 cases (64.6%)], followed by Sex cord stromal tumors [6 cases (15.3%)], metastatic tumors [4 cases (10.2%)] and Germ cell tumors [4 cases (10.2%)]. Cytokeratin was positive in >50% of serous epithelial cells, followed by krukensberg tumor and showed focal positivity in non-epithelial tumors.

Conclusion: Evaluation for pancytokeratin (AE 1 / AE 3) in the context of ovarian tumors is useful only in specific instances including identification of epithelial differentiation in an apparently undifferentiated neoplasms and distinction of dysgerminoma from non dysgerminomatous germ cell tumors. Non dysgerminomatous germ cell tumors characteristically express cytokeratin diffusely and strongly, whereas in dysgerminoma it shows only focal and weak expression.

Keywords: Malignant, Histopathology, Cytokeratin (CK), Gold Standard

Introduction

Ovarian tumors account for a considerable proportion of clinically important problems in females and they are dangerous due to their silent growth. Cytokeratins(CK) are intermediate filaments and 10-nm in diameter. These ropelike intermediate filament fibres are found predominantly in a polymerized form within the cells. [1] Cytokeratin is a family of water insoluble intracellular fibrous protein present in almost all epithelial cells[2-3]. Keratin represents an excellent marker for epithelial differentiation regardless of whether the tumors are of endodermal, neuroectodermal, mesenchymal or of germ cell origin.[4] It is a useful marker for primary ovarian tumors and various metastatic tumors in the ovary. This study is undertaken to evaluate the usefulness of cytokeratin expression in malignant ovarian tumors.

Materials and Methods

We analysed 39 cases of malignant ovarian neoplasm. This is a retrospective study carried over two years period (01/2015 to 12/2016) in a tertiary care teaching hospital.

The clinico-pathological data was taken from the ward including age, clinical presentation and laterality .We received ovariectomy specimens along with hysterectomy specimens. Gross examination of the specimens was done and representative bits were taken. The histopathological examination was carried on formalin fixed tissues and paraffin embedded blocks. Immunohistochemical staining using the avidin – biotin complex was performed in 5-µm-thick sections. A semi-quantitative grading of cell staining percentage was used to yield quartile scores of 0 to 4 : 0 , (0%-4%) ; 1,(5%-52%) ;2,(25%-49%); 3,(50%-74%) ;4,(75%-100%). Moderate to strong staining of the tumor cells was required for positivity. However, greater than 50% cell staining was chosen as positive in our study.

Result

This retrospective study covered a total number of 39 malignant ovarian neoplasms during the period of two years. In our study, 25 surface epithelial tumor cases (64.6%), four Germ cell tumor cases (10%), six Sex-cord stromal tumor cases (15%), and four metastatic tumor cases (10.4%) were diagnosed (Table1).



Figure 1 Depicts the various types of malignant ovarian tumour encountered in the present study. Out of 25 surface epithelial tumor cases, 17 cases were serous malignant tumors (68%), six cases were mucinous malignant tumors (24%) and two cases were endometrioid tumors (8%).

The antibodies staining pattern in all malignant ovarian tumors were studied. Surface epithelial tumors and metastatic tumors showed >50% positivity with Grade 4 to cytokeratin and non-epithelial tumors showed <50% positivity with Grade 2 to cytokeratin (Table 2).

Table 1: Distribution on Malignant Ovarian Neoplasm

Sl.no	Classification	No.of cases	Total in percentage
1	Surface epithelial tumors	25	64.6%
2	Germ cell tumors	4	10.2%
3	Sex-cord stromal tumors	6	15%
4	Metastatic tumors	4	10.2%

Table 2: The Antibody Staining Pattern – Cytokeratin Positivity in Malignant Ovarian Neoplasms.

Sl.no	Tumor type	Expression pattern	Grading of percentage
1	High grade papillary serous cystadenocarcinoma	Diffuse positive	4 (>75% - 100%)
2	Bilateral mucinous cystadenocarcinoma	Diffuse positive	4 (>75% - 100%)
3	Bilateral Krukenberg tumor	Diffuse positive	4 (>75% - 100%)
4	Mixed Germ cell tumour-Choriocarcinoma	Focal positive	2 (25% - 49%)
5	Mixed Germ cell tumor-Embryonal cell component	Focal positive	2(25% - 49%)
6	Poorly differentiated carcinoma	Focal positive	2(25% - 49%)
7	Dysgerminoma	Focal positive	1(5% - 24%)
8	Granulosa cell tumor	Focal positive	1(5% - 24%)
9	Fibroma with sarcomatoid change	Negative	0(0% - 4%)

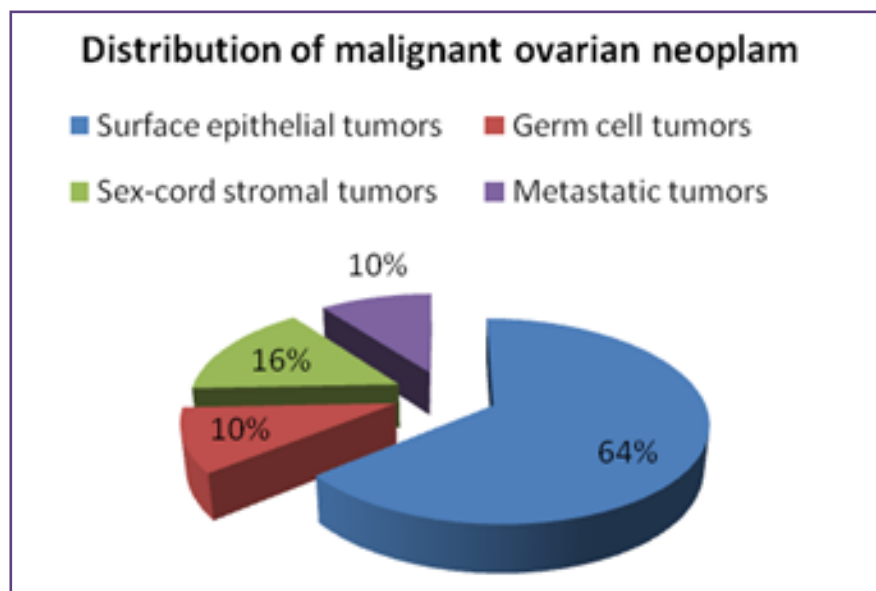


Fig. 1;

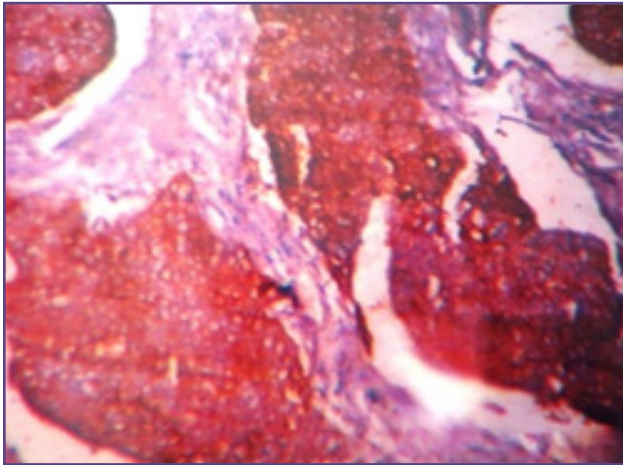


Fig. 2: Serous papillary cystadenocarcinoma. The epithelial lining shows diffuse positivity with CYTOKERATIN, X 100.

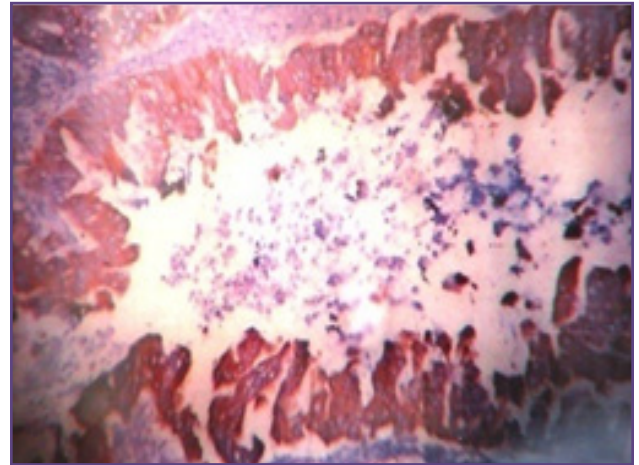


Fig. 3: Mucinous cystadenocarcinoma. The lining tall columnar epithelial cells exhibiting diffuse positivity with CYTOKERATIN, X 100.

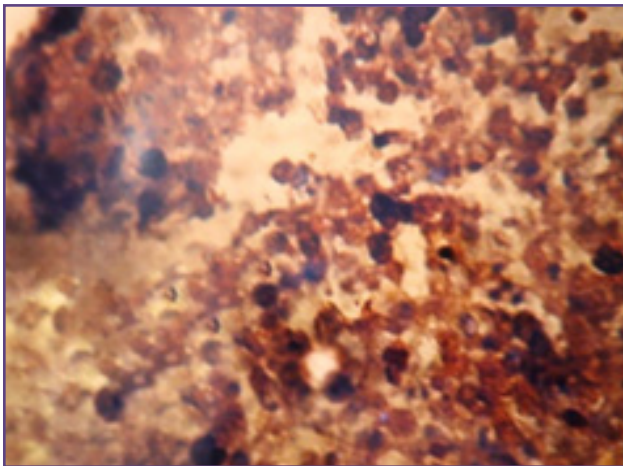


Fig. 4: Poorly differentiated ovarian serous carcinoma showing focal positivity with CYTOKERATIN, X 100.

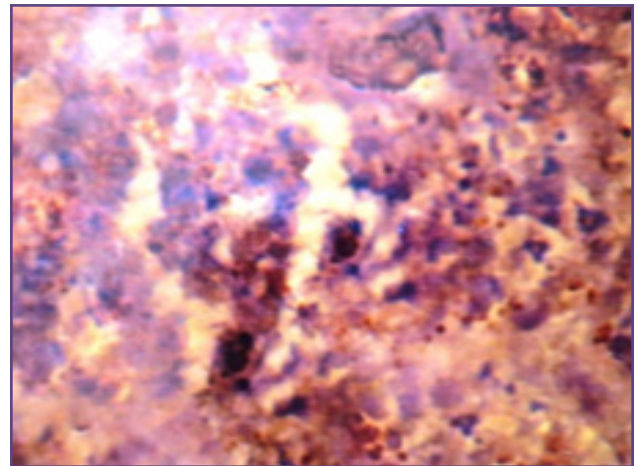


Fig. 5; Granulosa cell tumor. Shows typical punctate CYTOKERATIN expression, x 100.

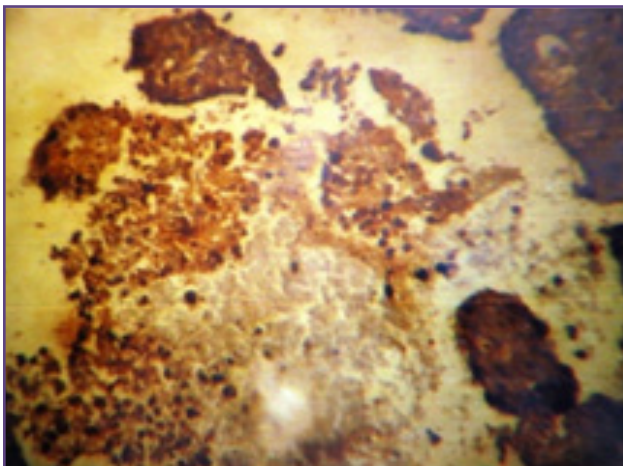


Fig. 6: Mixed germ cell tumor- shows embryonal cell component exhibiting focal positivity with CYTOKERATIN, X 100.

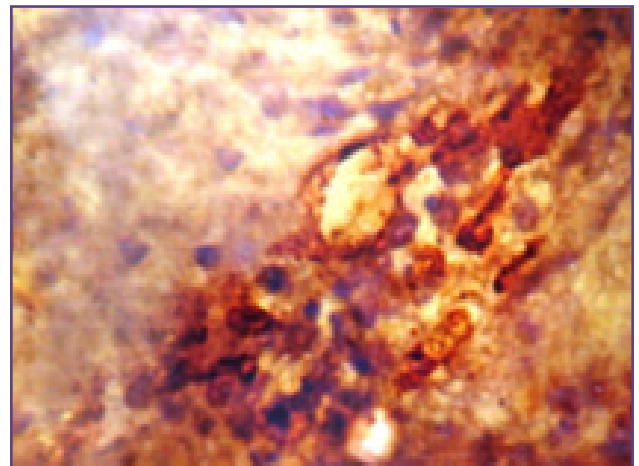


Fig. 7: Mixed germ cell tumor. Choriocarcinoma component shows focal positivity with CYTOKERATIN, X100.

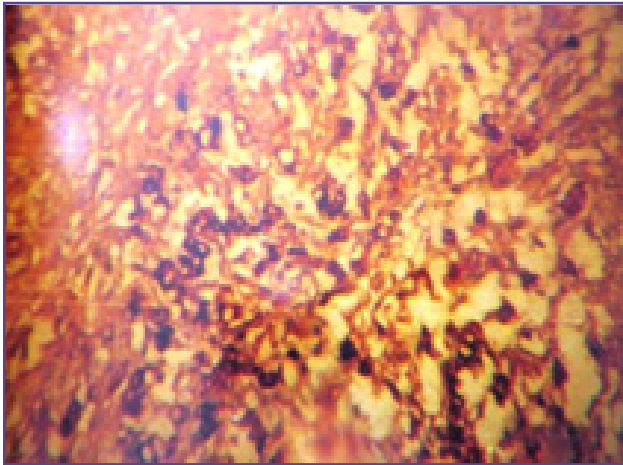


Figure 8- Krukenberg tumor. The Signet ring cells exhibit diffuse positivity with PANKERATIN , X 100

Discussion

Ovarian cancer is the sixth most common cancer and the seventh leading cause of cancer death among women worldwide. In most of the population – based cancer registries in India, ovary is the third leading site of cancer among women, tracing behind cervix and breast. The age adjusted incidence rates of ovarian cancer vary between 5.4 and 8.0/100,000 population in different part of the country.^[4]

The baseline estimated life time risk of developing ovarian cancer is 1.4%.⁽⁴⁾ The greatest known risk factors for the development of ovarian neoplasm is the presence of Germline BRCA 1 & BRCA 2 mutations. Women from Lynch syndrome (HNPCC) kindred are also at an increased risk of ovarian malignant tumors.^[5]

Surface epithelial- stromal tumors are defined by the World Health Organisation (WHO) as being those ovarian tumors that “originate from the ovarian surface epithelium or its derivatives and occur in women of reproductive age and beyond”.^[6] The division of the various epithelial subtypes into benign , borderline, and malignant forms is based on the premise that tumors with architectural and cytological features.^[6]

Cytokeratins are constituents of the intermediate filaments of epithelial cells expressed in various combinations depending on the epithelial type and the degree of differentiation. Cytokeratins have 30 distinct varieties , subdivided into acidic (type I) and neutral/basic (type II) .To maximize cytokeratin detection, proteolysis or microwave – mediated epitope retrieval in citrate buffer is mandatory before application of primary antibodies to rehydrated paraffin sections.^[7]

More recently, the development of antibodies which react to specific cytokeratins (CK) in tissues that has been fixed in formalin and embedded in paraffin has permitted investigations to examine the cytokeratin profile of lesions including benign and malignant tumours.^[8] Serous tumours are immunoreactivity for epithelial markers such as pancytokeratins (Figure 2). Majority of mucinous ovarian tumours show cytokeratin (CK7 and CK20) positivity.^[9] (Figure 3).

Undifferentiated and poorly differentiated serous carcinoma show positive for cytokeratin.^[9-10] In extremely poorly differentiated tumors, as few as 5% of tumour cells express keratin reactivity. In this study, poorly differentiated carcinomas showed focal positivity. (Figure 4)

Granulosa cell tumours may exhibit dot like paranuclear immunoreactivity [punctuate staining] for CK, are negative for EMA, and are usually positive for inhibin.^[9-10] In this study, granulosa cell tumours showed typical punctuate staining. (Figure 5). Juvenile granulosa cell tumor may express cytokeratin focally.^[12]

Dysgerminoma cells are, typically immuno reactive for cytokeratin.^[11] In this study dysgerminoma also showed focal positivity for cytokeratin. The differential diagnosis of dysgerminoma is clear cell carcinoma. Because overlapping H&E similarities include solid growth pattern, lymphocytic infiltrate, clear cytoplasm and prominent nucleoli. Staining with CK7 and EMA may be focally positive in dysgerminoma, but clear cell carcinoma is diffusely and strong positive for these markers.^[12] In yolk sac tumour, the cytoplasm of tumour cells are immuno reactive for cytokeratin. In our study, it did not show positivity. In embryonal cell carcinoma, the cytoplasm of the tumour cells is typically immuno reactive for cytokeratin.^[11-12] In this study, in the mixed germ cell tumour, the embryonal cell component exhibited focal positivity.(Figure 6)

Likewise, in choriocarcinoma, the syncytiotrophoblastic cells are typically immuno reactive with cytokeratin. (Figure 7). A useful panel in the distinction of primary and metastatic adenocarcinoma is cytokeratin.^[5-11] Usually krukenberg tumours are typically diffusely positive for cytokeratin. (Figure 8)

Conclusion

The immunohistochemical marker, cytokeratin is gold standard and useful marker for epithelial tumors and also exhibits focal positivity in non-epithelial tumors like Granulosa cell tumors, mixed germ cell tumors and metastatic tumors.

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***Corresponding author:**

Dr. G. Jeyanthi, Annie Cottage, Twad Colony, Ningth Street, New Natham Road, Madurai, Tamilnadu, India, 625014.

Phone: +91 9344233662

Email: ajmathura@gmail.com

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