DOI: 10.14260/jemds/2014/2212

ORIGINAL ARTICLE

ROLE OF IMMUNOHISTOCHEMISTRY IN OVARIAN TUMORS

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HOW TO CITE THIS ARTICLE:
Lubna Khan, Amita Arora, Asha Agarwal, Chayanika Pantola, Sanjay Kala, Rahul K. Rathi, Kiran Pandey. "Role of Immunohistochemistry in Ovarian Tumors". Journal of Evolution of Medical and Dental Sciences 2014; Vol. 3, Issue 11, March 17; Page: 2814-2820, DOI: 10.14260/jemds/2014/2212

ABSTRACT: BACKGROUND: Ovarian tumors are characterized by marked heterogeneity in their clinical presentation, so an accurate histopathological diagnosis is needed. Immunohistochemistry is helpful in vast number of cases where the morphology and clinical data alone do not allow definite diagnosis of tumor present in tissue sections. **AIMS:** 1. To evaluate the role of immune-histochemistry in classification and histogenesis of ovarian tumours and in resolving diagnostic dilemma in closely mimicking and poorly differentiated tumours. 2. To evaluate the role of immunohistochemistry in ovarian tumours and to differentiate primary from metastatic tumours. **MATERIALS AND METHODS:** 80 operated cases of ovarian tumours over a period of one and half year (January 2008 to September 2010) were studied. Paraffin blocks of various ovarian tumours for relevant immunostains were subjected for automated immunostaining. Total 52 immunostains were included in the study. **RESULTS:** Out of the 80 cases of ovarian tumours, benign ovarian lesions were more common (75%) than malignant lesions (25%). Serous cystadenoma was the commonest benign tumor (45%). Overall surface epithelial carcinomas were responsible for 70% of all malignant lesions among which serous cyst adenocarcinoma was most common (45%). 88.8% cases of serous carcinomas showed diffuse positivity for CK7, 60% showed positivity for CA125 and 100% were negative for CK20. 100% cases of mucinous carcinoma showed positivity for CK7, 66.66% showed positivity for CEA and 100% were negative for CA125. ER and PR showed nuclear positivity in both cases (100%) of endometrioid carcinoma. Dysgerminoma showed positivity for placental alkaline phosphatase (PLAP). Yolk sac tumor was positive for alpha-feto protein (AFP). Embryonal carcinoma was positive for CD30. Granulosa cell tumor was positive for Calretinin and Inhibin and negative for AFP. **CONCLUSION:** Thus IHC is helpful in confirming the histological diagnosis, to know the histogenesis of ovarian tumours. It is particularly helpful in resolving the diagnostic dilemma in closely mimicking and poorly differentiated ovarian tumours. IHC is also helpful in differentiating primary ovarian tumours from metastatic tumours. This differentiation is important for both therapeutic and prognostic reasons.

KEYWORDS: Ovarian tumours, immunohistochemistry, immunostains, histogenesis, diagnostic dilemma.

INTRODUCTION: An accurate histopathological diagnosis of ovarian tumours is vital for their management and to predict the outcome of therapy. In view of large number of differential diagnosis, immunohistochemistry is helpful in vast number of cases to resolve the diagnostic dilemma. Immunohistochemistry enables the surgical pathologist to extract additional information from fixed, deparaffinised tissue specimens and to provide data critical to optimal clinical management of the patients.

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MATERIALS AND METHODS: 80cases with the diagnosis of ovarian tumours over a period of one and half year (January 2008 to September 2010) were included in the study. The specimens were collected in 10% formalin/ buffered formalin. Each specimen was inspected grossly and all relevant details were noted. Following this specimens were routinely processed and sections were stained by Hematoxylin and eosin. For immunohistochemistry, paraffin blocks were subjected for relevant immunostain in automated immunostainer (Biogenex i6000) and the detection system was Biogenex Super Sensitive Streptavidin Biotin Kit. Total 52 immunostains were included in the study and following primary antibodies were used- CK7, CK20, CA125, WT1, CEA, ER, PR, PLAP, AFP, CD30, S-100.

RESULTS: Out of the 80 cases, 75% (60/80) were benign ovarian lesions and 25% (20/80) were malignant ovarian lesions.

According to histogenesis, most common were the surface epithelial tumours constituting 63.75% (51/80) cases. Histologically Serous cyst adenoma was most common benign tumor constituting 45% (27/60) cases. (Table 1).

Surface epithelial carcinomas were responsible for 70% of all malignant lesions; serous cyst adenocarcinoma was most common malignant tumor constituting 45% (9/20) cases. (Table 2).

71.66% (43/60) of benign lesions were observed in below the age of 40 years. Age incidence was variable with majority (75%) of cases presenting between 3rd to 6th decades. On IHC 88.8% (8/9) cases of serous carcinomas showed diffuse positivity for CK7 (Figure 1&2), 60% (3/5) showed positivity for CA125 and 100% (9/9) were negative for CK20. 100% (3/3) cases of mucinous carcinoma showed positivity for CK7, 66.66% (2/3) showed positivity for CEA and 100% (2/2) were negative for CA125. ER and PR showed nuclear positivity in both cases (100%) of endometrioid carcinoma (Figure 3 & 4).

Dysgerminoma showed positivity for placental alkaline phosphatase (PLAP). Yolk sac tumor was positive for alpha-feto protein (AFP) (Figure 5). Embryonal carcinoma was positive for CD30 (Figure 6), negative for AFP. Calretinin and Inhibin were positive, EMA was negative in both adult and juvenile granulosa cell tumor. Immature teratoma showed S-100 protein positivity in neuroectodermal element. (Table 3, 4 & 5).

DISCUSSION: Ovarian cancer is a cancerous growth arising from the ovary. Ovarian cancer is the sixth most common cancer diagnosed in women but is the most common gynecological cancer causing death.[1] Signs and symptoms of ovarian cancer are frequently absent early on and when they exist they may be subtle.[2] In most cases, the symptoms persist for several months before being recognized and diagnosed. Most typical symptoms include: bloating, abdominal or pelvic pain, difficulty eating, and possibly urinary symptoms.[3] Recent onset of symptoms and their occurrence more than 12 times per month may give a clue to the diagnosis of ovarian neoplasm.[3]

Other findings include an abdominal mass, back pain, constipation, tiredness and a range of other non-specific symptoms, as well as more specific symptoms such as abnormal vaginal bleeding or involuntary weight loss.[4]

Patients of ovarian neoplasms are assessed by physical examination, a blood test for various tumor markers (e.g. CA-125) and transvaginal ultrasonography followed by biopsy and cytological examination of abdominal fluid.

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Treatment protocols either by surgery or chemotherapy or both depend upon type, stage and grade of cancer, thereby necessitating the need for definitive diagnosis.

Most (85-95%) ovarian cancers are classified as "epithelial" and are believed to arise from the surface (epithelium) of the ovary, 5 to 8 percent from stromal cells, and 3 to 5 percent from germ cells.[5] 5-30% of ovarian cancers are due to metastases while the rest are primary. Among the secondary/ metastatic ovarian tumours, common primary sites are breast, colon and stomach.[6] Epithelial ovarian cancer (EOC) is identified as a heterogeneous malignancy with various histological subtypes. It is now well known that these different histological subtypes show differences in terms of presentation, response to treatment, immunohistochemical (IHC) reactivity, molecular profiling and thus prognosis.[7]

Immunohistochemistry (IHC) aids in the evaluation of tumours which show similar patterns on routine microscopic examination. The distinction between a sex cord tumor and an endometrioid carcinoma with sex-cord-like patterns may be greatly aided by the triad of epithelial membrane antigen (EMA), inhibin, and calretinin, the latter two being typically positive and EMA negative in sex cord tumours, the converse being typical of endometrioid carcinoma.[8] There is significant overlap between metastatic colorectal adenocarcinoma and those of primary epithelial ovarian neoplasms especially endometrioid and mucinous adenocarcinomas.[9-11] Bilaterality, high-stage disease, multinodularity, surface implants, infiltrative pattern of invasion, invasion of hilar structures and vascular invasion are strong markers for metastatic ovarian tumours.[9] Prominent intraluminal dirty necrosis with a garland and cribriform pattern is characteristic of metastatic colorectal carcinomas. Features favoring primary ovarian endometrioid or mucinous neoplasm include; unilateral involvement, large size, an expansile pattern of invasion, complex papillary pattern and presence of Mullerian features.[9,11]

Tumours with a pseudoendometrioid histologic pattern are most readily identified by immunophenotyping. However when the tumor is of mucinous type, immunostains are less useful. A panel comprising of CDX2, β-Catenin and P504S is helpful in distinguishing primary mucinous or endometrioid adenocarcinoma from colorectal metastasis to the ovary in the majority of cases and is a useful adjunct to the already established role of differential staining with CK 7/CK 20, CA 125, CEA in this differential diagnosis.[10] Dysgerminoma shows positivity for placental alkaline phosphatase (PLAP).[12] Yolk sac tumor is positive for alpha-feto protein (AFP).[13] Embryonal carcinoma is positive for CD30, negative for AFP.[14] Immature teratoma show S-100 protein positivity in neuroectodermal element (Table 6).

Thus various ovarian tumours have distinctive immunohistochemical features that can be used to suggest or confirm a diagnosis. IHC is often useful to differentiate between primary ovarian adenocarcinoma and metastatic adenocarcinomas specially those of colorectal origin. This differentiation is important for both therapeutic and prognostic reasons.

Ovarian cancer usually has a relatively poor prognosis. It is disproportionately deadly because it lacks any clear early detection or screening test, meaning that most cases are not diagnosed until they have reached advanced stages. More than 60% of women presenting with this cancer have stage III or stage IV cancer, when it has already spread beyond the ovaries. The five-year survival rate for all stages of ovarian cancer is 47%.

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Lesions	No. of cases	Percent
Surface epithelial tumor	51	63.75
Germ cell tumor	14	17.5
Sex cord stromal tumor	4	5
Tumor like lesion	11	13.75
Total	**80**	**100**

Table 1: Distribution of lesions according to histogenesis

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Category	Histological type	No. of cases	Percent
Surface epithelial tumor	Serous adenocarcinoma	9	45
	Mucinous adenocarcinoma	3	15
	Endometrioid carcinoma	2	10
Germ cell tumor	Dysgerminoma	1	5
	Embryonal carcinoma	1	5
	Yolk sac tumor	1	5
	Immature teratoma	1	5
Sex cord stromal tumor	Adult granulosa cell tumor		
	Juvenile granulosa cell tumor	1	5
Total		**20**	**100**

Table 2: Distribution of malignant lesion according to histological types

Differential Diagnosis	Immunopanel WT1	ER	Final Diagnosis
High grade serous carcinoma/ High grade endometrioid carcinoma	+	–	High grade serous carcinoma
High grade serous carcinoma/ High grade endometrioid carcinoma	–	+	High grade endometrioid carcinoma

Table 3: Immunohistochemistry in resolving diagnostic dilemma between high grade serous carcinoma and high grade endometrioid carcinoma

Differential Diagnosis	Immunopanel AFP	CD30	Final Diagnosis
Embryonal carcinoma/ Yolk sac tumor	–	**+**	Embryonal carcinoma

Table 4: Immunohistochemistry in resolving diagnostic dilemma Between embryonal carcinoma and yolk sac tumour

Differential Diagnosis	Immunopanel CK7	CK20	Final Diagnosis
Primary ovarian carcinoma/ Metastatic colonic carcinoma	+++	+	Primary ovarian carcinoma

Table 5: Immunohistochemistry in resolving diagnostic dilemma between primary ovarian carcinoma and metastatic colonic carcinoma

Tumours	Markers
Epithelial	
Serous- High grade Low grade	(+) p53, WT1, p16, ER (+) WT1, ER
Mucinous	(+) CK7, CK 20 (weak, focal), CDX2 (weak, focal), SMAD4, DPC4 (-) ER, WT-1 (not diffusely positive), beta-catenin-1, p16

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Endometrioid	
Clear cell	(+) HNFI-β, beta-catenin-1 (-) ER, WT-1, typically lack p53 unless high grade
Transitional cell	(+) WT-1, ER, p53 (-) p53, typically lack p53 unless high grade
Granulosa/ Sertoli	(+) inhibin, WT-1, calretinin
Germ cell	(+) CA 125, inhibin, AFP, βhCG

Table 6: List of various IHC markers in different ovarian tumours

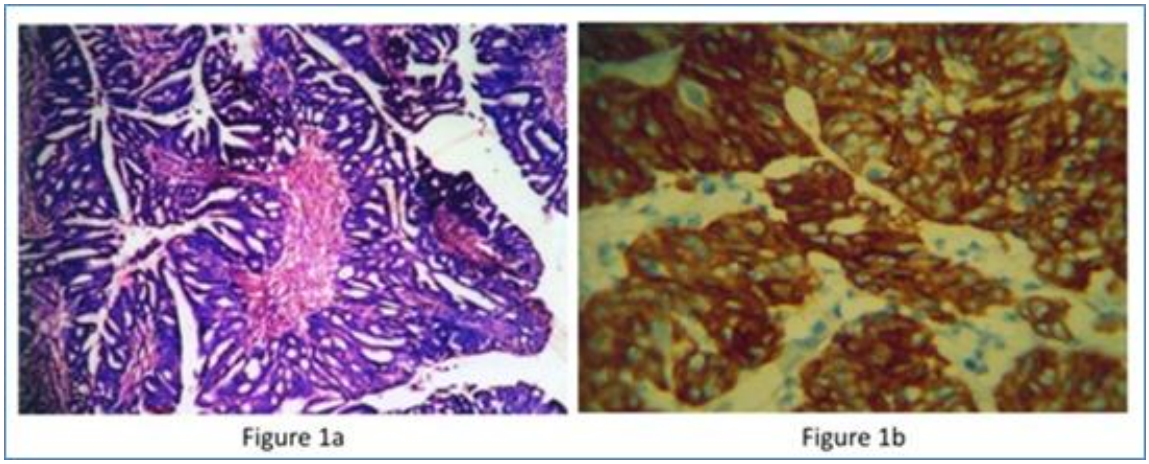

Figure 1a: Serous adenocarcinoma showing complex papillary architecture (H&E, X200)
Figure 1b: Serous carcinoma showing diffuse cytoplasmic CK7 positivity (IHC for CK7, X400)

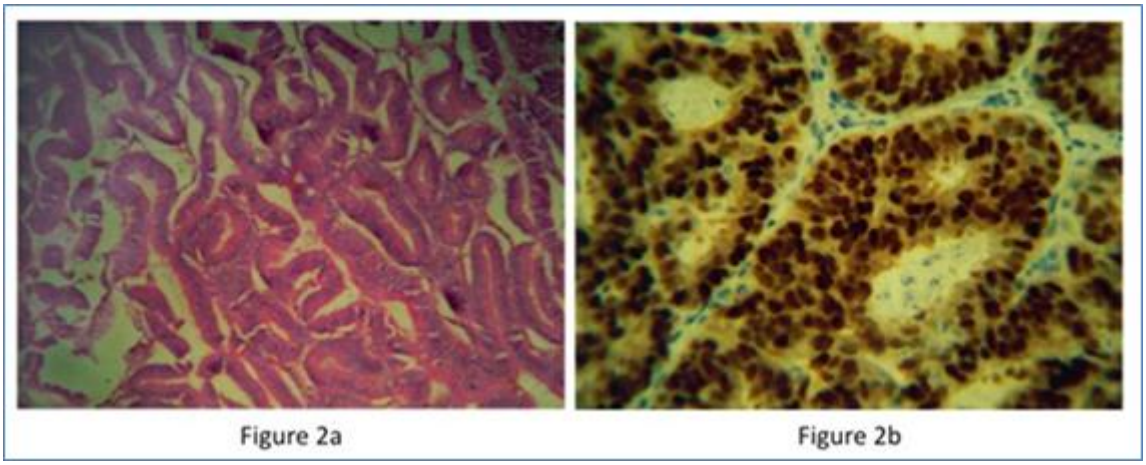

Figure 2a: Well differentiated endometrioid carcinoma of ovary (H&E, X400)
Figure 2b: Endometrioid carcinoma showing strong nuclear positivity for ER (IHC for ER, X400)

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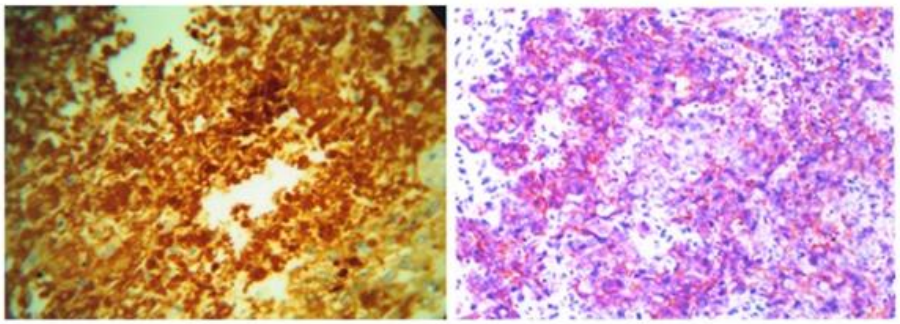

Figure 3 Figure 4

Figure 3: Yolk sac tumor showing diffuse cytoplasmic AFP positivity (IHC for AFP, X400)
Figure 4: Embryonal carcinoma showing strong and diffuse membranous CD30 positivity (IHC for CD30, X400)

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Date of Submission: 15/02/2014.
Date of Peer Review: 17/02/2014.
Date of Acceptance: 26/02/2014.
Date of Publishing: 12/03/2014.