ORIGINAL ARTICLE

HISTOMORPHOLOGICAL SPECTRUM OF OVARIAN TUMORS WITH IMMUNOHISTOCHEMICAL ANALYSIS OF POORLY OR UNDIFFERENTIATED MALIGNANCIES

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ABSTRACT

Background: Ovarian cancer has the worst prognosis and highest case fatality rate among all gynecological malignancies. Differential diagnosis remains a major problem in histopathology of ovarian tumors especially for poorly differentiated or undifferentiated malignancies. This study was conducted to determine the frequency of morphologic spectrum of ovarian tumors and the role of immunohistochemistry in definite histogenesis of poorly differentiated or undifferentiated ovarian malignancies.

Material & Methods: This descriptive study was conducted at Histopathology section of Dow Diagnostic Reference and Research Laboratory at Dow University of Health Sciences, during a period of 2 years from 2008 to 2010. The study material included 123 consecutive samples of ovarian tumors. Inclusion criteria were pretreatment cases with sufficient tumor material. In addition we also included 20 cases/blocks of poorly or undifferentiated malignancies submitted for immunohistochemistry.

Results: The mean age of study group was 33.7±13 years (range 5-72 years). Out of 123 cases, 93(75.6%) were benign, 4(3.3%) borderline and 26(21.1%) malignant. Amongst benign tumors, serous cystadenoma was the most frequent subtype, while for the malignant tumors serous cystadenocarcinoma was the commonest. Regarding the analysis of 20 undifferentiated or poorly differentiated tumors, sex cord tumors with 7 cases were found as the commonest malignancy.

Conclusion: Malignant germ cell tumors appear to be more common in our population. Immunohistochemical analysis is helpful in the proper diagnosis of undifferentiated or poorly differentiated tumors.

Key words: Ovarian tumors, Immunohistochemistry, Undifferentiated malignancies.

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INTRODUCTION

Ovarian cancer has often been called the "silent killer" because of non-specific symptoms and a lack of trustworthy screening for the early detection, so majority of cases are diagnosed in an advanced stage which contributes not only to diagnostic delay and a poor prognosis but is also responsible for increase burden of the disease. It accounts 3% of all cancers among women in US. In year 2010, an estimated 21,880 new cases and 13,850 deaths occurred in the US due to ovarian cancer.[1] It has the worst prognosis and highest case fatality rate among all gynecological malignancies.[2-4]

Among Asian countries, Pakistan has one of the highest rates of ovarian cancers and among the South Asian countries including Srilanka, India, Bhutan and Bangladesh it is relatively frequent in Pakistan.[5,6] According to Karachi Cancer Registry, ovarian cancer is the third most common malignancy among Pakistani women.[7] The exact reasons for the relatively high rates of cancers of the ovary in Pakistan are not known. However, the substantial pro-portion of ovarian cancers in Pakistani women is due to germ-line mutations in the BRCA1 and BRCA2 genes.[5,8]

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Differential diagnosis remains a major problem in histopathology of ovarian tumors especially when the tumor is poorly differentiated or undifferentiated. On conventional histomorphology a small number of cases usually remain unclassified. Here the morphological assessment can be supplemented by IHC. Accurate and precise classification is a good predictor of tumor response to chemotherapy and can also suggest chemotherapeutic agents to be used.[9]

The aim of the present study was to observe histomorphological pattern of ovarian tumors in our setup and to categorize the poorly or undifferentiated ovarian malignancies by IHC.

MATERIAL AND METHODS

This descriptive study was conducted at Histopathology section of Dow Diagnostic Reference and Research Laboratory at Dow University of Health Sciences, during a period of 2 years from 2008 to 2010. The study material included 123 consecutive samples of ovarian tumors. Inclusion criteria were pretreatment cases with sufficient tumor material. In addition we also included 20 cases/blocks of poorly or undifferentiated malignancies submitted for immunohistochemistry

All the specimens of ovarian tumors were fixed in 10% neutral buffered formalin over night. The specimens were grossed according to standard protocols (10) to take optimal number of representative sections. Automated tissue processor was used to obtain the paraffin embedded tissue blocks. 3-5 micron meter sections were cut and stained with Hematoxylin and Eosin (H&E). Special stains PAS, PAS-D, reticulin and trichrome were used as adjuncts in the diagnosis. The cases were diagnosed and classified according to WHO classification of ovarian tumors.[11]

Poorly or undifferentiated malignancies were subjected to IHC for exact histogenesis. IHC was done on 4μm thick sections of representative tumor area, on 20 poorly or undifferentiated malignancies. Histological slides were deparaffinized in xylene followed by target retrieval of histological sections in water bath at 95ºC for 40 minutes. Background quenching in all specimens was performed by 3% H_2O_2 for 10 minutes. The antibodies used were CK7, CK20, CKAE1/AE3, EMA, CEA, WT1, Inhibin, vimentin, calretinin, CD117, CD99, PLAP, CD30, OCT4, AFP, KI67, CD20, CD79a, CD3, CD5, LCA, CKHMW, CKLMW, Desmin, Synaptophysin and chromogranin, from (DAKO, Denmark). The panel of primary antibody was decided according to the histomorphology. Primary antibody was incubated for 1 hour in optimized dilution at room temperature. Immunohistochemical detection was performed using Envision + system (Dako, Denmark). Positive and negative control with omission of primary antibody was used with each batch of immunostained sections. Slides were examined for the presence of nuclear/ cytoplasmic/ membranous staining (depending on the location of the positivity) in the control and within the tumor itself. Each case for IHC was evaluated by 3 histopathologists separately. Disagreement was resolved by joint review on multi-head microscope and a final consensus was established in each case.

The data was analyzed using SPSS version 16. Age was computed in terms of mean and standard deviation. The different histopathologic types of ovarian tumors were described in terms of frequencies. To find out the correlation between size of the tumor and histological type, we applied one-way analysis of variance (ANOVA). P value d" 0.05 was considered as significant.

RESULTS

The mean age of study group was 33.7±13 years (range: 5-72 years). The mean age of patients with benign surface epithelial tumors was 32.24±12 years while that of malignant surface epithelial tumors was 41.5±13 years. Mean age for benign germ cell tumors was 31.1±9 years and malignant germ cell tumors 22.75±15.2 years. However, mean age for benign sex cord stromal tumors was 45±10 years while it was 35.33±21 years in malignant sex cord stromal tumors. The age distribution of the patients and the histological type of ovarian tumors are summarized in Table 1.

Ovarian neoplasms ranged in size from 3 to 30 cm in this series. We analyzed the correlation between histological type and size of the neoplasms by using one-way analysis of variance (ANOVA). The size of the tumor was significantly different among benign (n=93) tumors [12.06±5.5 cm], malignant (n=26) tumors [14.85±5.77 cm] and low malignant potential (n=4) tumors [24.25±6.2 cm] ($p< 0.0001$). (Table 2)

The histopathological typing of 123 cases of ovarian tumors showed a markedly predominant frequency of surface epithelial tumors with 79 cases (64.2%) followed by germ cell tumors having 33 cases (26.8%) and 6 cases (4.9%) of sex cord origin. Of the benign neoplasms, serous cystadenomas was the most common neoplasm, 40 cases (43%). While dermoid cyst, the second most common neoplasm comprised of 28 cases (30%), followed by mucinous cystadenomas 22 cases (23%), and 3 cases of fibromas (3%). Among the neoplasms of low malignant potential (LMP), four mucinous borderline tumors were encountered in our series. The commonest malignant tumors in our series were serous cystadenocarcinoma; 5 cases (19.2%), mucinous cystadenocarcinoma; 4

Table 1: Distribution of ovarian neoplasms by age and type.

Histopathological Types		Age in years								Total
		1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	(Per cent age)
Benign tumors n=93 (75.1%)	Serous cystadenoma	0	4	23	6	3	2	2	0	40 (32)
	Dermoid cyst	1	3	13	7	4	0	0	0	28 (22)
	Mucinous cystadenoma	0	3	10	5	3	1	0	0	22 (17)
	Fibroma	0	0	0	1	1	1	0	0	3 (2)
Borderline malignant n=4 (3.3%)	Mucinous tumor	0	1	1	0	0	1	0	1	4 (3.3)
Malignant tumors n=26 (21.1%)	Serious cystadeno-carcinoma	0	0	0	1	2	1	1	0	5 (4)
	Mucinous cystadeno-carcinoma	0	0	2	2	0	0	0	0	4 (3)
	Endometrioid Adenocar-cinoma	0	0	1	2	0	0	0	0	3 (2)
	Clear cell Adenocar-cinoma	0	0	0	0	0	1	0	0	1
	Dysger-minoma	0	2	1	0	0	0	0	0	3 (2)
	Granulosa cell Tumor	0	1	1	0	0	1	0	0	3 (2)
	Undifferen-tiated or Poorly differentiated tumors	0	1	1	0	2	1	0	0	5 (4)
	Yolk sac	0	1	1	0	0	0	0	0	2 (1)
Total		1	16	54	24	15	9	3	1	123

cases (15.3%), endometrioid carcinomas; 3 cases (11.5%), granulosa cell tumor; 3 cases (11.5%), dysgerminoma; 3 cases (11.5%), Yolk sac tumor; 2 cases (7%), and clear cell carcinoma; 1case (3.8%). There were 5 cases (4% of all ovarian tumors and 19.2% of all malignant tumors) of poorly or undifferentiated tumors in the series of consecutive cases.

Immunohistochemical analysis of 20 poorly or undifferentiated malignancies showed 7 cases of sex cord origin, 5 of germ cell origin, 2 from surface epithelial tumors, 2 metastatic adenocarcinomas, 2 lymphomas and 2 tumors with neuroendocrine differentiation. (Table-3)

DISCUSSION

Ovarian cancer is often the cause of diverse, diagnostically challenging issue for pathologists especially when the tumor is poorly differentiated or undifferentiated. The mean age of epithelial ovarian cancers in our study was significantly younger than reported in western literature[11] but similar to other Pakistani series.[12] This early age incidence can be attributed to different genetic makeup and exposure to different environmental factors as compared to the western population.

Our study showed significant difference among the histologic type and size of the tumors. As the size of the tumor increases it is more likely that it would be malignant. The difference between benign, malignant, and borderline tumors has also been described in the western studies.[13]

The frequency of benign and malignant ovarian tumors in our study matches with the published international and national data[13-15] with some variations at national level.[16,17] These differences could be related to referral biases.

The surface epithelial tumors in similarity with western data[11] and other Asian studies[18-20] comprised the major (64.2% of all ovarian tumors) histologic type in our series. Whereas malignant epithelial ovarian cancers comprised of 50% of all malignant tumors concurrent with most of the local studies[19,20] but in contrast to western figures i.e.

Table 2: Relationship between size of the tumor and histological type.

Tumor types	Histological type	Mean Size (cm) ±SD	Size of tumor (cm)				Total (Percentage)
			<4	5-9	10-19	>20	
Benign tumors (12±6cm)	Mucinous cystadenoma	14.7±7.2	1	5	10	6	22 (17)
	Serous cystadenoma	10.5±4.1	1	17	20	2	40 (32)
	Dermoid	12±5.2	1	9	14	4	28 (22)
	Fibroma	13±3.6	0	1	2	0	3 (2)
Malignant tumors (14.9±6cm)	Serous cystadenocarcinoma	15±6.6	0	1	4	0	5 (4)
	Mucinous cystadenocarcinoma	19.±1.8	0	0	2	2	4 (3)
	Endometrioid adenocarcinoma	11±3.6	0	1	2	0	3 (2)
	Clear cell adenocarcinoma	17	0	0	1	0	1
	Dysgerminoma	17±7	0	0	2	1	3 (2)
	Yolk sac tumor	16.5±6.3	0	0	1	1	2 (1)
	Granulosa cell tumor	12.3±8.3	0	2	0	1	3 (2)
	Undifferentiated/ poorly differentiated tumor	12.6±6.2	0	2	2	1	5 (4)
Borderline tumors (24.3±6cm)	Mucinous tumors with borderline malignant potential	24.2±5.6	0	0	1	3	4 (3)
Total		13.0±6.2	3	38	61	21	123

Table 3: Immunohistochemical analysis of poorly or undifferentiated malignancies.

S. No.	Provisional histological diagnosis	Immunomarkers used	Positive Immunomarkers	Final diagnosis
1	Undifferentiated tumor	LCA, CKAE1/3, CD20, CD79a, Ki 67, CD3, CD5	LCA, CD20, CD79a, Ki67	Burkitt lymphoma
2	Undifferentiated tumor	Synaptophysin, LCA, CD20, CD79a, Ki 67, CD3, CD5, Inhibin, PLAP, CK7, CK20	LCA, CD79a, CD20, KI 67	Non-Hodgkin lymphoma of B cell phenotype
3	Poorly differentiated tumor	Vimentin, inhibin, CKAE1/AE3, EMA, PLAP	Inhibin, Vimentin	Adult granulosa cell tumor
4	Mixed germ cell - sex cord stromal tumor	PLAP, CD117, CKAE1/3, Inhibin, CD30, OCT4	PLAP, OCT4	Dysgerminoma
5	Poorly differentiated malignant neoplasm	Vimentin, Inhibin, Calretinin, EMA, LCA, CKLMW, CKHMW, Synaptophysin	Vimentin, Calretinin	Adult granulosa cell tumor
6	Poorly differentiated tumor	CKAE1/AE3, CD30, PLAP, CD117 and OCT4	PLAP, OCT4, CD117	Dysgerminoma
7	Poorly differentiated malignant neoplasm	PLAP, AFP, CD30, CD117, CKAE1/3	PLAP, AFP	Yolk sac tumor
8	Neoplastic lesion	PLAP,CKAE1/AE3, CD117, OCT4, CD30	PLAP, OCT4, CD117	Dysgerminoma
9	Poorly differentiated tumor	Inhibin, Calretinin, CD99, CKAE1/3, Synaptophysin.	Inhibin, Calretinin, CD99	Adult granulosa cell tumor
10	Undifferentiated tumor	CK7, CK20, CEA	CK7, CK 20	Metastatic Adenocarcinoma
11	Poorly differentiated tumor	CK7, CK20, CEA	CK20, CEA	Colonic Adenocarcinoma
12	Embryonal carcinoma	PLAP, AFP, CD30, OCT4	PLAP, AFP	Yolk sac tumor

13	Granulosa cell tumor	Inhibin, Vimentin, Calretinin, EMA, Synaptophysin, Chromogranin, CD99	Vimentin , Calretinin tumor	Sertoli leydig cell
14	Spindle cell lesion	Vimentin, Desmin SMA, CK7	Vimentin, Desmin, SMA	Sclerosing stromal tumor
15	Granulosa cell tumor	Synaptophysin, Chromogranin, LCA, PLAP, Inhibin, CD99, CK7	Synaptophysin, Chromogranin	Neuroendocrine tumor
16	Cystadenocarcinoma	CK7, CK20, EMA, Vimentin, p53 and WT1.	CK7, EMA, P53 and WT1	Serous cystadenocarcinoma
17	Papillary Cystadenocarcinoma	CK7, CK20, EMA, Vimentin, P53	CK7, EMA, P53	Clear cell carcinoma
18	Dysgerminoma	CKAE1/3, CK7, CK20, Inhibin, Synaptophysin, Chromogranin, PLAP	CKAE1/3, Synaptophysin, Chromogranin	Neuroendocrine tumor
19	Stromal tumor	CKAE1/AE3, Inhibin, Calretinin, Vimentin, EMA, ASMA, Desmin.	Inhibin, calretinin, vimentin	Adult granulosa cell tumor
20	Ovarian carcinoma	Ck7, Ck20, Inhibin, Calretinin, PLAP, Synaptophysin, Chromogranin.	Inhibin, Calretinin	Sex cord tumor with annular tubule

Abbreviation: CK7: Cytokeratin 7, CK20: cytokeratin 20, CKLMW: Cytokeratin Low molecular weight, CKHMW: Cytokeratin High molecular weight, CKAE1/3: Cytokeratin AE1/3, PLAP: Placental Alkaline Phosphatase, ASMA: Anti smooth muscle Actin, AFP: Alpha fetoprotein, EMA: Epithelial membrane Antigen.

90%.[11] Although the most common carcinoma observed in this series was serous cystadenocarcinoma consistent with most of the national and international studies.[13,17,19] However mucinous cystadenocarcinomas ranked as the second commonest tumor, while the international data displays it as the fourth common malignant neoplasm.[21]

Malignant germ cell tumors are more common being 19.2% of all primary malignancies in our series, with difference to other local series as depicting 11%, 12% and 14.2%.[15-17] However, in western population the cumulative data represents 3% of all ovarian cancers,[11] which is definitely not in conformity with ours.

Frequency of Sex cord stromal cell tumors in our series was 4.9%, however variable frequency is quoted in different Pakistani series.[14,16,20]

Poorly differentiated or undifferentiated tumors contributed to 19.2% of all malignant tumors in our series, in accordance with other studies which reported undifferentiated carcinomas as 16.3% and poorly differentiated cystadenocarcinoma as 19.6%.[19] Most of the previous studies only described the frequency of undifferentiated or poorly differentiated tumors. They did not exactly characterize these tumors in order to get definite diagnosis because of lack of IHC facility. Patients with these undifferentiated malignancies suffer most in terms of diagnostic delay, opting treatment modalities, and prognostic factors, because of the incorrect diagnosis.

CONCLUSION

Malignant germ cell tumors appear to be more common in our population. Immunohistochemical

analysis is helpful in the proper diagnosis of undifferentiated or poorly differentiated tumors.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
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