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HISTOPATHOLOGICAL SPECTRUM OF OVARIAN TUMORS WITH SPECIAL REFERENCE TO C-KIT EXPRESSION IN GERM CELL TUMOURS WITH EMPHASIS ON DYSGERMINOMA

Medical Science

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ABSTRACT

Introduction: Ovarian cancer is a common malignancy in Indian women and it ranks third in women globally.Germ cell tumours are a group of ovarian tumours affecting young age that respond very well to chemo and radiotherapy.Tumors that are positive for CD117 marker have treatment in the form of imatinib. Present study was done to look at the rate of positivity of CD117 in malignant germ cell tumors and especially dysgerminomas.

Aim of the study: To study the histo-morphological features of ovarian tumors with emphasis on germ cell tumours including tumour size, macroscopic appearance, histological type, grade, stage and to study the immunohistochemical expression of CD117 in germ celltumours of ovary, particularly in dysgerminomas.

Materials and methods: This was a prospective study done in the Institute of Pathology and Electron Microscopy,Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai,over a five year nine months period.The ovarian tumors were studied for light microscopy. Immunohistochemistry for CD117 was done in malignant germ cell tumors and it was correlated for patient age, histologic type, grade and stage of tumor.

Results: Surface epithelial tumors (75.5%) were most common followed by germ cell tumors(GCT) (16.5%).In the malignant GCT, 11 (36.6%) were dysgerminomas.The mean age for malignant GCT was 22.4 yearsand the average size was 15.7 cm.The immunohistochemicalexpression of C-kit was more in pure dysgerminoma (90.9%) than other malignant GCT but the correlation was not statistically significant.

KEYWORDS

C-kit expression, Ovariantumors, Dysgerminomas

INTRODUCTION

Ovarian and cervical cancers are the most common gynecological cancers affecting women worldwide and also in India. Ovaries are subjected to monthly endocrine and traumatic insult and prime site of ovarian carcinogenesis.The primary and secondary carcinomas of ovary are frequent with variety of histological patterns, and are seen in all age and ethnic groups.[1,2]Almost 50% of ovarian tumours are benign tumours and of the malignant tumours, 90% are epithelial tumours.

Germ cell tumours are encountered at all ages from infancy to old age, but are seen most frequently from first to sixth decades of life. In children and adolescents, more than 60% of ovarian neoplasms are of germ cell origin and one third of these are malignant.

C-KIT: The proto-oncogene C-kit encodes for a 145–160 kDa, type III transmembrane tyrosine kinase receptor known as C-kit or CD117[3] which belongs to the same family of receptors as platelet-derived growth factor (PDGFR) and colony-stimulating factor-1(CSF-1)

Aberrant expression of this C-kit has been implicated in the development of a number of human cancers, including malignancies of the germ cell tumors and other ovarian tumours[4, 5] lung[6], breast[7], skin, uterus, endometrium,[8, 9] urinary bladder[10] as well as in certain types of leukemia[11], gastrointestinal stromal tumors (GISTs)[12, 13] and Ewing's sarcoma.[14]The advent of therapies targeted to C-kit has proven highly effective in treating some of the cancers that over express this receptor, such as CML[15]and GISTs.

Apart from c-kit expression by IHC, there are many studies which evaluated c-kit mutations. In a study by Liang Cheng et al, dysgerminoma cells from 22 patients were analysed for kit mutations at exon17 codon 816. Kit amplification and chromosome 12p anomalies were investigated. [16]They reported kit exon 17 codon 816 mutations and kit amplification were each detected in 6 cases of dysgerminoma (27%).They also showed that kit mutation was associated with advanced pathological stage ($p < .05$); kit amplification was associated with elevated kit protein expression ($p < .05$).Chromosome 12p anomalies were found in 82% of the dysgerminomas and did not correlate with kit abnormalities.[16]

AIM OF THE STUDY

1. To study the histo-morphological features of ovarian tumors with special emphasis on germ cell tumours.
2. To study theimmunohistochemical expression of CD117 in germ celltumours of ovary and to determine the correlation of CD117 expression with known prognostic factors such as tumor size, histological type, grade, stage and also with the outcome of the disease.

MATERIALS AND METHODS

This was a prospective descriptive study conducted in the Institute of Pathology and Electron Microscopy, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai, over a five year nine months period from January 2007 to October 2012.

Detailed history of cases including age, presenting complaints, duration of complaints, USG and CT findings, any hormonal elevation (AFP, β-HCG, LDH), were noted.

In gross, types of specimen, size and appearance of the tumor were noted. The patients were followed up in medical oncology and details of chemotherapy, number of cycles given and outcome of the patient in terms of months of symptom free interval, recurrence or death were noted. Follow up period was of minimum six months and maximum two years.

The representative tissue bits were processed and stained with hematoxylin and eosin stains for light microscopy. The type of tumor, if mixed germ cell tumor (MGCT), components of MGCT, omental infiltration by tumor, malignant cells in ascitic fluid, and peritoneal washings, all were studied.

Representative formalin fixed paraffin embedded tissue samples of all the 30 cases were subjected to immunohistochemistry (Cd117). Immunohistohemical staining with a polyclonal anti-CD117 antibody was doneusing Super-sensitive30 polymer HRP system based on non-biotin polymeric technology. Staining was graded in a semiquantitative manner as follows:

No staining - Negative
1-10% staining - 1+
10-29% staining - 2+
30-50% staining - 3+
>50% staining - 4+

The statistical analysis was performed using statistical package for socialscience software version 11.5.

INCLUSION CRITERIA:

1. Malignant germ cell tumors of ovary

EXCLUSION CRITERIA:

1. Benign germ cell tumors of ovary
2. Surface epithelialtumors,sex cord stromaltumors and metastatic tumors

OBSERVATIONS AND RESULTS

In our institute, we received a total of 725 ovarian neoplasms, 487 cases (67.13%) were benign, 34 cases(4.7%) were borderline and 204 cases(28%) were malignant.

TABLE 1 Broad histopathological group of ovarian tumors

Tumor type	No of cases	Percent (%)
Surface epithelial tumors	547	75.5%
Germ cell tumors	120	16.5%
Sex cord stromal tumors	52	7.2%
Secondaries	6	0.8%
Total	725	100%

Surface epithelial tumors(75.5%) were most common followed by germ cell tumors(16.5%). In surface epithelial tumors, commonest was benign serous cystadenoma. In malignant tumors, papillary serous cystadenocarcinoma was very common (90cases).6 cases of clear cell carcinoma were also reported.

In Germ cell tumors 88 cases(73.33%) were mature cystic teratomas, 30 cases(25%) were malignant germ cell tumours and 2 cases(1.67%) of strumaovarii were recorded.In the 30 malignant germ cell tumors, dysgerminoma accounted for 11 cases.

In sex cord stromal tumors, granulosa cell tumours were common which constituted 27 cases out of which 5 cases were frankly malignant.Rare cases like steroid cell tumor (2 cases) and one case ofsclerosing stromal tumorwere also reported.

In the malignant germ cell tumors, 11 were dysgerminoma(36.66%), mixed germ cell tumour were 10(33.34%), yolk sac tumour were 4 (13.34%) and immature teratoma were 5cases(16.66%).(Figures 1, 2 and3)

Age of the patients of malignant germ cell tumors: The patient age ranged from 13 to 40years,with mean age of occurrence was 22.4years. The maximum number of patients were seen in second decade (50%) followed by third decade (40%).

Distribution of malignant germ cell tumors according to laterality: Of the 30 cases that were studied,60% (18 cases) were right sided, 36.6% (11 cases) were left sided and 3.3% (1 case) was bilateral.

Distribution of malignant germ cell tumors according to size: There were 16.6% (5 cases) of 5-10 cm size, 43.3% (13 cases) of 11-15 cm size, 13.3% (4 cases) of 16-20 cm size, 20% (6 cases) of 21-25 cm size and 6.6% (2 cases) of 26-30 cm size.The average size of tumourswasabout 15.7 cm. Most of the tumors (60%) were in a range of 5-15cm.

Distribution of malignant germ cell tumors according to the USG findings: In ultra sound findings, out of the 30 cases, 26.7% (8 cases) showed solid lesions, 6.7% (2 cases) showed purely cystic shadows and 66.6% (20 cases) were reported as mixed lesions.From our observations dysgerminoma exhibited solid morphology in most cases. Mixed germ cell tumours and immature teratoma presented with mixed or variegated morphology.

TABLE 2 C-kit positivity in different malignant germ cell tumors

Cd 117	Dysgerminoma	Yolk sac tumor	ImmatureTeratoma	Mixed germ cell tumor	Total	Pearson chi square test.
0	1(9.0%)	2(50%)	4(80%)	3(30%)	10(33.3%)	P=0.367
1	1(9.0%)	1(25%)	0(%)	2(20%)	4(13.33%)	
2	3(27.3%)	0(%)	0(%)	1(10%)	4(13.33%)	
3	3(27.3%)	1(25%)	1(20%)	2(20%)	7(23.33%)	
4	3(27.3%)	0(%)	0(%)	2(20%)	5(16.67%)	
Total	11(100%)	4(100%)	5(100%)	10(100%)	30(100%)	

The percentage of positivity in histological subtypes of germ cell tumors was compared. C –kit positivity was more (90.9%) in cases of pure dysgerminoma compared to other tumors.

The percentage of C-kit positivity in mixed germ tumours with and without dysgerminoma was compared. The positivity rate was more in the group with dysgerminoma component about 100%(6/6 cases showed positivity) but only 1 case out of 4 cases showed positivity in mixed germ cell tumors without dysgerminoma component(25%).

Comparison of C-kit positivity in mixed germ cell tumors with and without dysgerminoma component: There were 6 cases ofmixed germ cell tumors with dysgerminoma and all (100%) showed C-kit positivity. There were 4 casesof mixed germ cell tumors without dysgerminoma of which only 1 case (25%) showed C-kit positivity. The positivity rate was more in the group with dysgerminoma component. (Figure 4,5)

TABLE 3 Correlation of expression of CD117 with histopathological diagnosis

Tumor type	No of cases	Cases showing C-kit positivity	Percent (%)
Dysgerminoma	11	10	90.9%
Yolk sac tumor	4	2	50%
Immature teratoma	5	1	20%
Mixed germ cell tumor	10	7	70%
Total	30	20	66.6%

Cd117 expression was more common in dysgerminoma than other germ cell tumor subtypes, but the correlation was not statistically significant. Out of 20 cases showing positivity,7 cases showed3+ positivity. Out of the 5 cases showing strong (4+) C-kit positivity 3 were dysgerminoma.

Correlation of age with CD117 expression: Patients in age group> 20years exhibited more CD117 (70%) positivity than patients under age <20(65%), but the correlation was not statistically significant.

TABLE 4 Correlation of size and stage of tumor with CD117 expression

CD 117	Size<20cm	Size>20cm	Total	Stage I	Stage II	Stage III	Total
0	8 (36.3%)	2(25%)	10(33.3%)	3 (27.3%)	1 (50%)	6(35.3%)	10(33.3%)
1+	2(9.1%)	2(25%)	4(13.3%)	1(9.1 %)	0(0.0%)	3(17.6%)	4 (13.3%)
2+	3(13.6%)	1(12.5%)	4(13.3%)	2(18.2%)	0(0.0 %)	2 (11.8%)	4(13.3 %)
3+	5(22.7%)	2(25%)	7(23.3%)	4(36.4%)	0(0.0 %)	3(17.6%)	7(23.3 %)
4+	4(18.2%)	1(12.5%)	5(16.7%)	1(9.1%)	1(50.0%)	3(17.6 %)	5(16.7%)
Total	22(100%)	8(100%)	30(100%)	11(100%)	2(100%)	17(100%)	30(100%)

The Pearson chi square test was P=0.631 and P=0.792 for correlation of CD117 expression with size of tumor and stage of tumor respectively.

Tumors with size more than 20 cm showed more positivity (75%) than tumors with size less than 20 cm(63.6%) but the correlation was not statistically significant.Stage III tumors showed more positivity than stage I tumors, but the correlation was not statistically significant.

TABLE 5 Correlation Of Expression Of Cd117 With Outcome Of The Disease

CD 117	Sympto m free	Symptoms persisting	Expired	Total	Pearson chi square test
0	4(23.5%)	2(100%)	1(100%)	7(35%)	P=0.585
1	2(11.8%)	0(0%)	0(0%)	2(10%)	
2	2(11.8%)	0(0%)	0(0%)	2(10%)	
3	3(35.3%)	0(0%)	0(0%)	3(30%)	
4	6(17.6%)	0(0%)	0(0%)	6(15%)	
Total	17	2	1	20	

All the tumors showing CD117 positivity were symptom free.

Correlation of chemotherapy with outcome of the disease: The disease outcome was studied in 20 patients who were available for follow up. The minimum and maximum follow up periods were 6months and two years respectively. In this group 16 patients received platinum based chemotherapy of which 13 patients were symptom free, in 2

patients the symptoms persisted and 1 patient expired. Chemotherapy was not given in 4 cases and all 4 patients were symptom free in the follow up period. The Pearson chi square test was P=0.643.

Disease recurrence and death occurred in patients who were given chemotherapy,whereas, all patients who did not receive chemotherapy were symptom free but the correlation was not statistically significant.

DISCUSSION

Ovarian tumors are important gynecologicaldiseases and have variable presentation. They tend to affect all age groups. Different histological types of tumors occur in different age groups.

Germ cell malignancies generally affect younger women.In our study, clinical and histopathological features of malignant germ cell tumors were studied and their immunohistochemical expression of C-kit was studied.

In our study, we received a total of 725 ovarian neoplasms during a period of 70 months

We encountered 67.1% benign tumors, 4.7% of borderline tumors and 28.1% of malignant tumors. (Table 1).Ahmed et al,[17] Pilli et al [18] and Gupta et al[19] in their studies observed 59.1%, 75.2% and 72.9% benign tumors, 0.2%, 2.8% and 3.3% borderline tumors and 40.8%, 21.8% and 22.9% malignant tumors respectively. Our findings are comparable to these studies.

TABLE 6 Comparison Of Histopathological Types Of Tumors With Other Studies

Tumour type	Jha et al[20]	Pradhan et al[21]	Swamy et al[22]	Present study
Surface epithelial tumors	52.2%	46.9%	61.6%	75.5%
Germ cell tumors	42.2%	45.7%	21.7%	16.5%
Sex cord stromal tumors	3.1%	3.6%	11.7%	7.2%
Metastasis	2.4%	3.6%	5.0%	0.8%

The different histological types of ovarian tumors were compared with different studies by Jha et al,[20] Pradhan et al,[21] Swamy et al.[22] Present study incidences are more correlating with study of Swamy et al.[22]

TABLE 7 Comparison Of Malignant Germ Cell Tumors With Other Studies

Tumor type	Stangjitgamol et al (n-130)[23]	Chow et al (n-50)[24]	Present study (n-30)
Dysgerminoma	37.7%	26%	36.66%
Yolk sac tumor	26.2%	30%	13.34%
Immature teratoma	23.1%	26%	16.66%
Mixed germ cell tumor	13%	16%	33.34%
Total	100%	100%	100%

In germ cell tumors distribution of different histological subtypes was compared with studies by Stangjitmol[23]and Chow et al.[24]Compared to both these studies, incidence of yolk sac tumor was less in current study and incidence of mixed germ cell tumorswas more.

The mean age of patients in present study group was 22.4 years which is almost similar to studies by Stangjitmol et al[23] (21 years), Chow et al (21.5 years)[24]and Lai et al(23 years). [25]

The mean size of tumorsin our study was 15.7cm which is similar to study by Chow et al[24]who reported it as 16 cm.

In our study, (Table 2 and 3) percentage of C-kit positivity in germ cell tumors subtypes was reported as 90.9% in dysgerminomas, 50% in yolk sac tumors and 20% in immature teratomas respectively. In a similar study byTrihn et al[26]they observed 100% positivity of C-kit in both dysgerminoma and yolk sac tumor and a positivity of 29% in cases of immature teratoma.

Since current study emphasizes more on dysgerminomas, the percentage of C-kit positivity in various studies was compared. In our study 90.9% dysgerminomas showed C-kit positivity.

Sever et al,[27] Tsuura et al,[28] Sakuma et al[29] reported similar values of 87%, 75% and 100% respectively. Our observations compare well with these studies.

Dysgerminoma commonly affects the second and third decades. Since they are affecting the reproductive age group people, preserving the fertility with cure of the patient is a big challenge. At present Bevacizumab is used for standard chemotherapy resistant ovarian cancers. Targeted therapy against C-kit is available for diseases like chronic myeloid leukemia, and it can be of use in germ cell tumours too.

But even in a tertiary care hospital like ours, the incidence of germ cell tumours is only 30 cases for a period of 70 months period.Currently there are no clinical trials for targeted therapy in Dysgerminoma.

CONCLUSION

Malignant germ cell tumours are the second common group of ovarian malignancy after surface epithelial malignancies. Dysgerminoma commonly affectsthe second and third decades. Dysgerminomas show a high percent positivity for C-kit.

Hence, trials involvinga large group of people with germ cell tumours should be done and administration of anti-C kit compounds like imatinib should be explored in these patients so as to provide an effective alternative for conventional platinum based regimens.

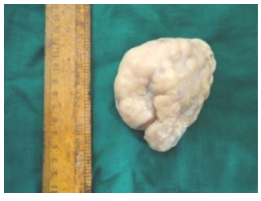

FIG 1: 145/11- Gross specimen of Dysgerminoma

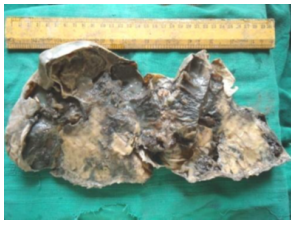

FIG 2: 4280/07- Gross specimen of yolk sac tumour

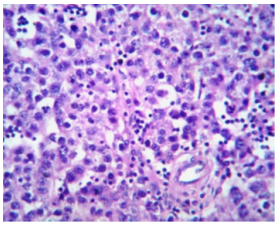

FIG 3 Microscopy of Dysgerminoma (Hematoxylin and eosin staining 400X)

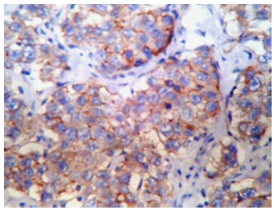

FIG 4 C-kit positivity in dysgerminoma, 4+ staining, 400X

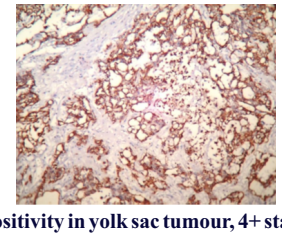

FIG 5: C-kit positivity in yolk sac tumour, 4+ staining, 100X

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