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GRANULOSA CELL TUMOR: A CLINICOPATHOLOGICAL STUDY IN A TERTIARY HOSPITAL IN SOUTH INDIA

Pathology

Dr. Jeyalakshmidevi Namasivayam*

M.D Path., DGO., Associate Professor of Pathology, Kilpauk Medical College, The Tamil Nadu Dr. MGR. Medical University, CHENNAI 37 *Corresponding Author

Dr. A.V.Shanti

M.D Path., Retd. Director, Institute of Pathology, Madras Medical College. Dr. MGR. Medical University

ABSTRACT

Ovarian malignancies are the second most common malignancies(5-6%) of the female genital tract .and Granulosa cell tumors (GCT) are the most common Sex cord stromal tumors.

The aim is based on clinico-pathological correlations of GCTcases with the Stage of the disease as per FIGO classification, to analyse various histo-morphological patterns and to highlight the utilitarian value of Immuno histochemistry.

There were 475 ovarian Neoplasms and 165 were malignant. 30 of them were GCTs (18.8 %). 23 patients (76.6%) had stage I, (56.6% had stage Ia, 6.6% had stage Ib , 13.3% had stage Ic)and 23.3%had stage IIIc . Patients with early stages had well differentiated GCT and late stages were poorly differentiated. Patients with Stage I had a mean survival of 11.2 months and Stage III had a mean survival of 8.2 months.

KEYWORDS

GCT Ovary, FIGO Staging

Introduction

Ovarian malignancies constitute 5 to 6% of all the cancers in women and the leading cause of death .

Sex cord stromal tumors account for approximately 5% and GCT are slow growing, of low malignant potential with a well-known predilection to spread beyond the ovary with recurrence or metastasis after complete removal .

The Adult GCTs account for 1 to 2% of all ovarian tumors and 95% of all GCTs occurring often in menopausal and post-menopausal women with peak age incidence between 50–55 years .

Surgical intervention is the most accepted modality of treatment . Radiotherapy could play some role in advanced or recurrent disease with minimal residual tumour after operative debulking. Chemotherapy is advisable in recurrences or metastases.

The aim of the present study is based on Clinico-pathological correlations of GCT ovary cases with the Stage of the disease using the FIGO (International Federation of Gynaecology and Obstetrics, 1976);to analyse the various histo-morphological patterns with the stage of the disease and to highlight the utilitarian value of Immuno histochemistry in Sex cord stromal tumors.

Materials and Methods :

A prospective descriptive study was done on 30 GCT Ovary cases from 475 patients with ovarian tumors. The hospital case files of the Departments of Pathology, Institute of Obstetrics and Gynaecology and Kasthurba Gandhi Hospital from January 2003 to June 2006 were analyzed. Samples included total abdominal hysterectomy with bilateral salpingo oophorectomy, debulking, vaginal hysterectomy with bilateral oophorectomy.13 patients were followed up with the data and the remaining 17 patients had lost follow-up despite strenuous efforts.

Surgical specimens of these tumors were subjected to meticulous gross and microscopic examinations. The specimens were fixed in 10% neutral buffered formaldehyde. Extensive sampling was done , processing and paraffin blocks (number of blocks depending on the size of the tumor) were made. Uterus with cervix, other ovary, lymphnodes and omental tissues were also studied.

Histologic Sections (5 to 6 µm) were stained with Hematoxylin and Eosin, special stains like PAS (periodic acid - Schiff), Reticulin by Gomori's method were done. (Figure 23) Additional sections were made for Immuno histochemistry Panel in a poorly differentiated GCT.

Procedure

I. Hematoxylin and Eosin

1 Dewax Sections. Hydrate through graded alcohols to water.
2 Stain in Harris hematoxylin for 5 minutes.
3 Wash well in running tap water.
4 Differentiate in 1% acid alcohol.
5 Wash well in tap water until sections are again blue for 10-15 minutes.
6 Stain in 1% eosin for 1 to 2 minutes.
7 Wash in running tap water for 1 to 5 minutes.
8 Dehydrate through alcohols, clear in xylol and mount is DPX.

II. PAS Technique (Mc Manus)

1 Dewax sections and bring to distilled water.
2 Treat with periodic acid for 5 minutes.
3 Wash well with several changes of distilled water.
4 Cover with Schiffs solution for 15 minutes.
5 Wash in running tap water for 5 – 10 minutes.
6 Stain nuclei with Harris hematoxylin differentiating as appropriate in acid-alcohol and blueing as usual.
7 Wash in water.
8 Rinse in absolute alcohol.
9 Clear in xylene and mount.

Results: Call Exner bodies showed a magenta pink stained material in well-differentiated GCTs.

III. Gomori's method for reticular fibers

1 After dewaxing, bring sections to water.
2 Treat with 1% potassium permanganate solution for 2 minutes.
3 Rinse in tap water.
4 Bleach in 2% potassium meta bisulfate solution .
5 Rinse in tap water.
6 Treat with 2% iron alum 2 minutes.
7 Wash in several changes of distilled water.
8 Place in Coplin jar of silver solution 1 minute.
9 Wash in several changes of distilled water.
10 Reduce in 4% aqueous formalin solution, 3 minutes.
11 Rinse in tap water.
12 Tone in 0.2% gold chloride solution, 10 minutes.
13 Rinse in tap water.
14 Treat with 2% potassium metabisulfite solution 1 minute.
15 Rinse in tap water.
16 Treat with 2% sodium thio sulfate solution, 1 minute.
17 Rinse in tap water.
18 Counterstain with Von Gieson stain.
19 Dehydrate through alcohols.
20 Clear in xylene and mount.

Inference: Reticulin stained black which invests the aggregates of granulosa cells.

Ethical clearance for conducting this study was obtained from the Institutional ethics committee. Patients were staged according to the FIGO staging of ovarian tumours. Microsoft excel was used for the statistical analysis.

Results

Out of 475 ovarian neoplasms, 165 were malignant(34.7%) and remaining 310 were benign. GCTs constitute 30 cases (18.8%) of ovarian malignancies. Out of 165 ovarian malignancies, Surface epithelial tumors were 102 cases (62%), Germ cell tumors contributed 15 cases (9%), Sex cord stromal tumors comprised 32 cases (19%) and secondary (metastatic) tumors were 16 cases (10%). There were 54 cases of GCT out of 839 malignancies (6.4%) in a study of Himanshu et al and 47 GCTs out of 952 ovarian malignancies during a 19-year study of Kazim Uygun et al.

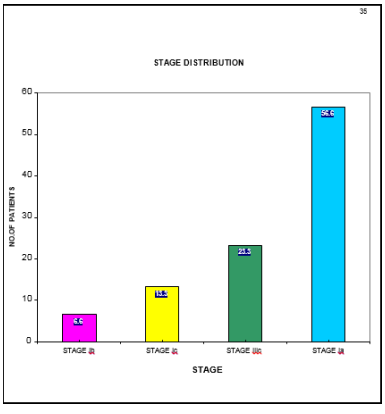
Majority (66.7%) were > 40 years of age and 96.6% were adults and 1 case (3.3%) being juvenile GCT. Among the adults, 3 patients had bilateral ovarian involvement. Reproductive status revealed 53% post-menopausal, 13.3% pre-menopausal and 30% were of reproductive age group and this is comparable to 56% of post-menopausal women by H. Fox et al in their study and 51.4 % by Himanshu et al. The parity status was available for 57% cases and 76% were multiparous .

The predominant symptom in the postmenopausal women was vaginal bleeding (40%) and, menorrhagia (33.3%) or irregular bleeding in pre-menopausal patients whereas Arthur Evans et al. and Cronje et al reported 60% and 63% respectively. 50% presented with abdominal pain and distension . The mean duration of symptoms was 17.8 months in the present study and a study by Fox et al reported 9 months. 76.6% of the patients were in stage I in our study which is comparable with 62.1% of the patients in a study by Himanshu et al. Ascites was reported in 9 cases (30%) and torsion in 2 cases (6.7%).

Table 1: Profile of the study participants

Clinical Parameters	Number of patients (%)
Age in years	
≤ 40 Years	10 (33%)
> 40 Years	20 (66.7%)
Menopause	
Pre	14 (46.7%)
Post	16 (53.3%)
Stage	
I - II	23 (76.7%)
III-IV	7 (23.3%)
Residual Disease	
Present	4 (13.3%)
Absent	26 (86.7%)
Size	
≤15 cm	25 (83.3%)
>15 cm	4 (16.7%)

Regarding Tumor stage as per FIGO classification., 23 patients (76.6%) had stage I, (17 patients (56.6%) had stage Ia, two patients (6.6%) had stage Ib and 4 patients (13.3%) had stage Ic)(13.3%). The remaining 7 patients had stage IIIc (23.3%).



Surgical extirpation namely the Total abdominal hysterectomy with bilateral Salpingo oopherectomy was performed in 24 cases (80%) with stage I, one had total abdominal hysterectomy with right oopherectomy and an other case ended with vaginal hysterectomy with bilateral oopherectomy. Debulking was performed in 4 cases (13.3%) with Stage III.

The median diameter of tumor was 10 cm (range, 4–28 cm) with no evidence of tumour rupture. On gross appearance, 12 cases (41%) were solid and cystic (Fig. 1), 10 cases (34%) were predominantly cystic with haemorrhage (Fig. 2) and 7 cases (24.13%) were solid (Fig. 3). GCTs were most often cystic (92%). Associated mucinous cystadenoma was present in the contralateral ovary in one case (3.3%).

On meticulous histopathological examination , micro follicular pattern was the predominant pattern in 18 cases (60%) (Fig. 4), macro follicular in Juvenile GCT (3.3%), (Fig 9) insular (Fig. 7) and trabecular in 3 cases (6.6%) (Fig. 6) and diffuse pattern was seen in 8 cases (26.6%) (Fig 8). Thus, well differentiated tumors accounted for 63.3%, moderately differentiated constitute 6.6% and poorly differentiated tumors were 26.6%. Call-Exner bodies were present in 15 cases (50%) with micro follicular pattern, two with trabecular pattern and an occasional finding in a diffuse type (Fig 5A & 5B).

Tumors were graded based on nuclear atypia or mitotic activity. Nuclear atypia was 2+ in 9 cases(30%), 3+ in a case with diffuse pattern. All the remaining 20 (66.7%) cases had mild (1+) nuclear atypia (Fig 11). In 9 cases with diffuse sarcomatoid pattern, mitotic count was more than 6 per 10 high power fields (3+). A total of 21 (70%) cases (19 micro follicular and 1 trabecular) had occasional mitoses less than 1 to 2 per 10 high power fields (1+). Luteinization was present in three cases (10%) with microfollicular pattern and one case of juvenile GCT with macrofollicular pattern. Omental deposits were present in 7 cases with diffuse sarcomatoid pattern (23%) and two cases showed metastatic deposits in the para aortic nodes (Fig 12).

Capsular invasion was present in 10 out of 30 cases (33.3%), predominantly in 8 cases with diffuse pattern (poorly differentiated), (26.7%) , in one case of juvenile GCT (well differentiated) and one case with insular / trabecular pattern (intermediate) (Fig 13). Lympho vascular invasion was seen 4 cases with diffuse pattern (13.3%) (Fig 14).

The diagnosis of poorly differentiated GCT was made morphologically based on the nuclear features, uniform, pale nuclei and often grooved with focal areas having mild to moderate nuclear atypia. Mitotic figures were less than 6/10 HPF. But no atypical mitotic figures were seen which are frequent in poorly differentiated carcinoma. Most of them had focal Call Exner bodies. One case of JGCT had moderate to severe nuclear atypia with absence of grooving but diagnosed with the characteristic macro follicular pattern (Fig. 16)

Regarding the laterality, bilaterality was seen in three (10%) out of 30 GCTs (10%) whereas it is 25% in poorly differentiated carcinoma cases. The misinterpretation of a poorly differentiated carcinoma as an AGCT with diffuse pattern is one of the most frequent errors in ovarian malignancies. Call Exner bodies simulating glandular structures (Fig. 15A & 15B) and occasional papillary structures can give rise to this misinterpretation (Fig. 15C).

Of the 8 cases with diffuse pattern, only one case (HP: 814/06) had varied morphology for which immuno histochemistry was done. The panel of markers included Inhibin, Calretinin, CD99, Cytokeratin 7 and 20, EMA, Vimentin, CA 125, Calcitonin and Cytokeratin. Inhibin was positive in a case of well differentiated GCT with micro follicular pattern (Fig. 17) but negative in the problematic case (HP: 814/06). Inhibin is a distinctive marker for GCT and it is negative in 20% of GCTs.

The immunopanel showed negativity for an epithelial neoplasm and the only marker positive was the vimentin. (Fig 20) Hence based on the histomorphological features particularly the nuclear morphology (Fig. 22) with IHC, a diagnosis of poorly differentiated carcinoma was excluded. GCT diagnosis is entertained despite the absence of alpha-inhibin but with positivity for vimentin. This particular case has been highlighted to elaborate the importance of IHC to distinguish between poorly differentiated carcinoma and GCT.

Associated uterine findings:
In GCTs, endometrial hyperplasia is due to an effect of prolonged unopposed estrogen production. In the present study, simple hyperplasia was present in 14 out of 30 cases (46.6%) (Fig 21) while it was 31.5% and 21% of cases in the studies by Fox et al and Himanshu et al respectively

.Adenomyosis was present in nine cases and leiomyoma in two out of 30 cases.

Findings from the follow-up:
Out of 30 Patients, follow up records were available for 13 cases. The duration of follow up ranged from 2 to 24 months. 8 cases were above 40 years. The tumor size varied from 5 cm to 28 cm. Bilaterality was observed in 2 cases. 8 patients with stage I had better survival (mean duration 11-25 months) of which six cases presented with micro follicular pattern and two with diffuse pattern. Five patients (three diffuse, one trabecular and one macrofollicular pattern) presented with Stage III with the mean survival period as 8.2 months . Two patients had post-operative chemotherapy and one patient expired of intestinal obstruction after 12 months of follow up. Of the 13 patients who had a follow up, omental deposits and capsular invasion were present in six cases (46%). Lympho vascular invasion was present in four cases (31%).

Table 2: Clinical and pathological findings of thirteen patients on follow up.

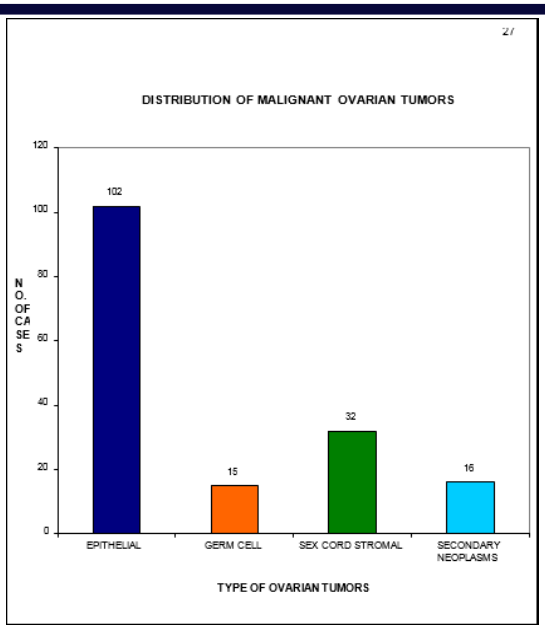
Case No	Age in years	Tumor size	Tumor pattern	FIGO stage	Call-Exner	LV invasion	Mitotic Count	Luteinization	Nuclear Atypia	Omental Deposit	Capsular Invasion	Follow up
1	36	5cm	Micro follicular	Ia	+	-	1+	Nil	1+	-	-	24 months
2	45	11 cm	Micro follicular	Ia	+	-	1+	Nil	1+	-	-	3 months Ascites, Icterus
3	50	R-12cm L-10cm	Diffuse	IIIc	-	+	2+	Nil	2+	+	+	2 months
4	30	14cm	Diffuse	IIIc	-	+	2+	Nil	2+	+	+	9 months CT given
5	58	7cm	Trabecular	IIIc	-	-	2+	Nil	2+	+	+	12 months patient expired
6	48	7cm	Microfollicular	Ia	+	-	1+	+	1+	-	-	18 months
7	38	20cm	Micro & macrofollicular	Ia	+	-	+	focal	1+	-	-	20 months
8	55	R-25cm L-28cm	Microfollicular	Ia	+	-	1+	+	1+	-	-	6 months
9	25	16 cm	Microfollicular	IIIc	-	-	1+	+	2+	+	+	8 months
10	41	14 cm	Diffuse	Ic	occasional	-	1+	-	1+	+	+	10 months
11	63	8	Diffuse	IIIc	-	+	2+	-	3+	+	+	10months CT given
12	40	11	Diffuse	Ia	-	+	2+	-	2+	-	-	9 months
13	43	8cm	Microfollicular	Ia	-	-	1+	-	1+	-	-	10 months

The mean age of patients was 46.16 years (range, 25–65 years). All the 13 followed up patients were post-menopausal and parous and six were nulliparous; in 11 women, no parity details were available. The mean duration of symptoms in all patients is 8 months. Despite abnormal bleeding, four premenopausal women presented with secondary amenorrhoea (13.8%). Abdominal distension was present in nine cases (30%) and abdominal pain in six cases (20%).

Conclusion
In conclusion, GCTs of the ovary are sex cord stromal tumors of low malignant potential with a well-known predilection to recur or metastasize after complete removal. Tumor staging (FIGO) is an important prognostic factor on which clinical treatment modality is based, thereby preventing recurrences. In the present study, we have analysed staging for all GCTs and compared them with their clinical presentation and varied histomorphological patterns.

Menstrual irregularities were the presenting symptoms in early stages and mass abdomen in late stages. Patients with early stages had a well differentiated GCT and late stages were diagnosed histologically as poorly differentiated GCTs. Follow up records for 13 patients showed patients with Stage I had a mean survival of 11.2 months than patients with Stage III who had a mean survival of 8.2 months.

The misinterpretation of poorly differentiated carcinoma was avoided by strictly adhering to the morphological criteria, the most important criterion being the nuclear details (Fig. 24) . One case was problematic with varied appearance for which panel of immuno histochemical markers, Inhibin, calretinin, CD99, Cytokeratin 7 and 20, EMA Vimentin, CA 125, Calcitonin and Cytokeratin were done. All immuno markers were negative except for vimentin. This application is best used as an adjunct to tissue morphology and a negative marker does not detract the diagnosis as no single antibody is specific or sensitive for a given neoplasm.



Figures

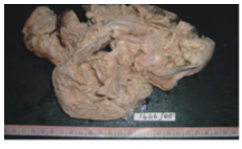


FIG - 1 : GROSS - SOLID & CYSTIC TYPE. JUVENILE GRANULOSA CELL TUMOUR

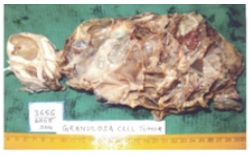


FIG - 2 : GCT - GROSS - CYSTIC WITH HAEMORRHAGE


FIG - 3 : GCT - GROSS - PREDOMINANTLY SOLID

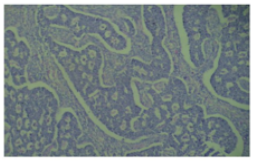


FIG - 4 : GCT - MICRO FOLLICULAR H & E 10 X 10

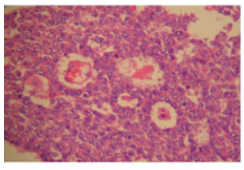


FIG - 5 A : GCT - CALL EXNER BODIES H & E 10 X 10

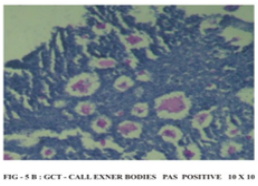


FIG - 5 B : GCT - CALL EXNER BODIES PAS POSITIVE 10 X 10


FIG - 6 : GCT - TRABECULAR PATTERN H& E 10 X 10

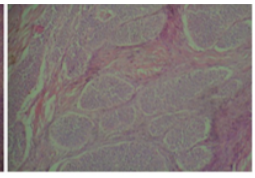


FIG - 7 : GCT - INSULAR PATTERN H & E 10 X 10

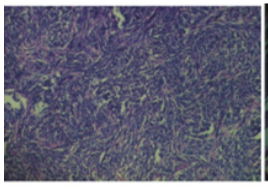


FIG - 8 : GCT - DIFFUSE SARCOMATOID H&E - 10 X 10

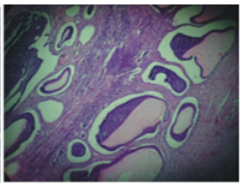


FIG - 9 : GCT - MACRO FOLLICULAR H & E 10 X 10

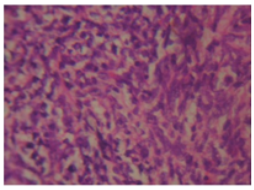
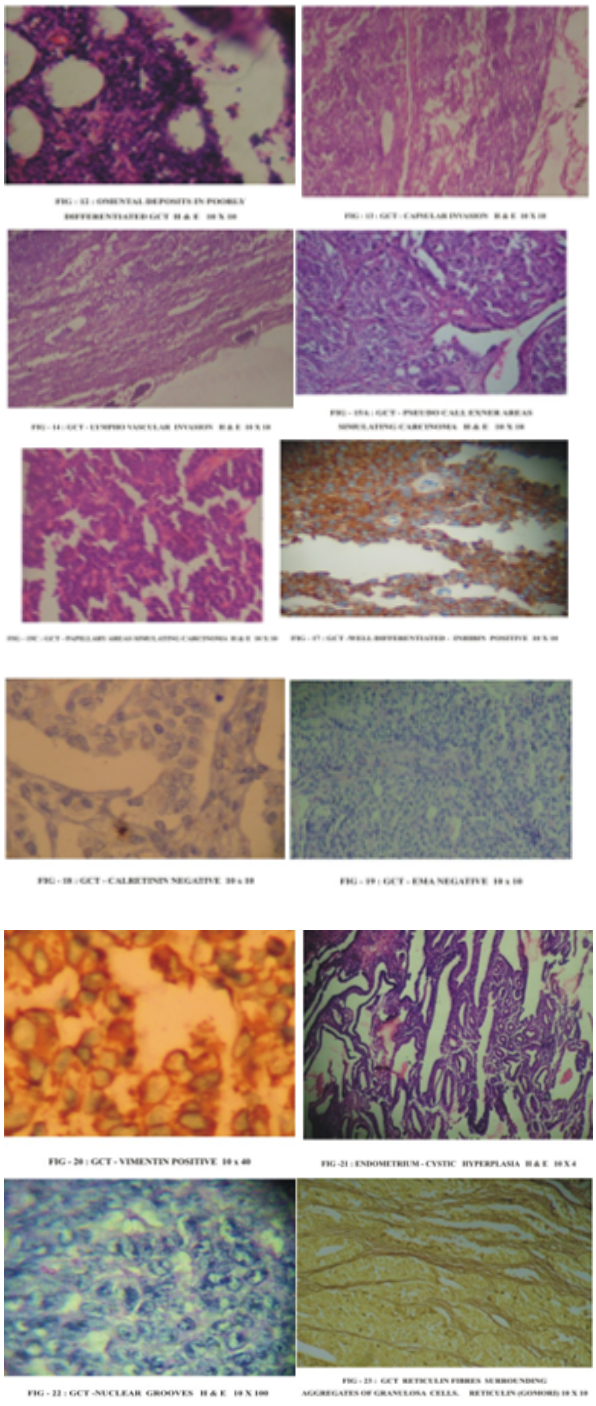


FIG - 11 : GCT - NUCLEAR ATYPIA H & E 10 X 40



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