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Immunohistochemistry of Germ Cell Tumors at Various Anatomic Sites

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Abstract

Background: The usefulness of IHC markers in the differential diagnosis of germ cell tumors has been recognized for decades. Germ cell neoplasms can show a bewildering array of appearances, and there may be significant morphologic overlap among subtypes. For this reason, Immuno-histochemistry is often performed to assist in accurately assessing the types and extent of germ cell elements present within a tumor. The different IHC markers used in this study included include a) alpha - fetoprotein (AFP), b) the beta subunit of human chorionic gonadotropin c) cytokeratin, d) S-100 protein e) CD30 f) GFAP. g) CD117 h) PLAP. The main objectives of the study were to observe the IHC staining patterns of both gonadal and extragonadal GCTs.

Methods: The study was conducted for a period of 5 years from 2015 to 2019 and was an observational study. The recorded data was compiled and entered in a spreadsheet and then exported to data editor of SPSS Version 20.0. Graphically the data was presented by bar and pie diagrams.

Results: A total of 93 cases were analyzed. IHC was consistent with the histomorphological variants, with YSTs being consistently positive for AFP. Seminoma/Dysgerminoma for CD117/PLAP. Embryonic Carcinomas were seen mostly as mixed component with CD30 cytoplasmic positivity. Mature Cystic Teratomas, the most common histological variant showed frequent GFAP & S100 positivity. Cytokeratin was positive in all cases.

Conclusion: IHC helped in confirming the morphological diagnosis and also showed the extent of a component in a MGCT

Keywords: GCTs • EGCTs • MGCTs

Introduction

Germ cell neoplasms can show a bewildering array of appearances, and there may be significant morphologic overlap among subtypes. For this reason, Immuno-histochemistry is often performed to assist in accurately assessing the types and extent of germ cell elements present within a tumor. The different IHC markers used in this study included include a) alpha - fetoprotein (AFP), b) the beta subunit of human chorionic gonadotropin c) cytokeratin, d) S-100 protein e) CD30 f) GFAP. g) CD117 h) PLAP. The main objectives of the study were to observe the IHC staining patterns of both gonadal and extragonadal GCTs and to show the extent of a GCT in a mixed pattern GCT.

Low molecular weight cytokeratin (CK-lmw) is performed on all of our germ cell tumours. Embryonal carcinoma, yolk sac tumour, and choriocarcinoma are always positive for CK-lmw.

CD30 has been used for some time as a marker of embryonal carcinoma, since it is typically positive in that tumour but negative in other germ cell tumours.

AFP is useful in identifying foci of yolk sac tumour, as it is negative in other tumours.

HCG is well known as a marker of syncytiotrophoblasts in choriocarcinoma, although it will also stain isolated syncytiotrophoblastic giant cells in classical seminoma.

GLYP3[42] is a novel marker expressed in yolk sac tumours and choriocarcinomas.

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Materials and Methods

The study was conducted in the Department of Pathology at Sher-i - Kashmir Institute of Medical sciences (SKIMS) Srinagar, Kashmir. The main objectives of the study were to observe the IHC staining patterns in various GCTs at both gonadal and extragonadal sites which helped in confirming the diagnosis and knowing the extent of tumour in a mixed GCT. Resected specimens and biopsies of patients with germ cell tumours at various sites (gonadal and extragonadal) in different age groups, received in the Department of Pathology were included in the study. Recurrent germ cell tumours were excluded. Gross photographs of the specimens were taken to represent various tumour types. Minimum of four sections from the tumour were taken. 4-5 micrometre thick sections were cut on microtome. Microphotographs of the tumours were taken to represent various histological variants of germ cell tumours. IHC was performed as per the standard protocol. And different markers specific for a particular histological variant were used. For MGCTs a panel of diff markers was chosen based on the histological findings. The final results were confirmed under the microscope

Statistical methods

The recorded data was compiled and entered in a spreadsheet (Microsoft Excel) and then exported to data editor of SPSS Version 20.0 (SPSS Inc., Chicago, Illinois, USA). Graphically the data was presented by bar and pie diagrams.

Results

Max cases were seen in ovaries (45.2%) followed by testis (35.5%) (Figure 1).

Out of ninety three cases, seventy five (80.6%) were in gonads while only eighteen were seen at the extragonadal sites (19.4%) (Figure 2).

The most frequent tumour that was observed in our series was mature cystic teratoma, followed by classical seminoma. Choriocarcinoma, spermatocytic tumour, post pubertal teratoma, Strauma ovari were rare. 13 cases in our series were MGCTs with different components shown in Table 1.

Out of 48 cases of mature cystic teratomas 42 (87.5%) showed strong positivity for GFAP while 30 showed S100 positivity on IHC. 6 cases. Among 18 cases of seminoma which included 4 cases of dysgerminomas in females 100% showed cytoplasmic positivity for CD117 & PLAP. 17 of these were gonadal while only one tumour was seen at extragonadal site.9 gonadal and 1 extragonadal cases of YST showed strong cytoplasmic positivity for AFP (100%) while all but two were strongly positive for Glypican 3 out of the total 10 cases. Single case reported of choriocarcinoma showed strong and intense positivity for βhCG on IHC while spermatocytic tumor showed positivity for CD117/PLAP. Monodermal teratoma strauma ovari showed cytoplasmic positivity for CK (Table 2).

In yolk sac predominant MGCT the IHC showed cytoplasmic positivity for AFP, in all the three cases (100%) while two cases showed positivity for CD117/PLAP and one out of three (33.3%) showed cytoplasmic positivity for pan CK. Among seminoma predominant cases of MGCT all three showed positivity for CD117/PLAP, while one each (33.3%) showed strong and intense positivity for βhCG and GFAP/S100. MGCTs with predominant embryonal component showed cytoplasmic positivity for CK in all three reported cases (100%) while two each showed cytoplasmic positivity for AFP & CD117/PLAP (66.7%) and one showed positivity for βhCG. Only two cases of teratoma predominant MGCTs were reported both of which showed positivity for AFP & GFAP/S100 (100%). Two cases of MGCTs reported had YST and teratoma as dominating component all of which showed cytoplasmic positivity for AFP, GFAP/ S100 and CK (100%) (Table 3).

Mature Cystic teratomas were the most frequent GCTs both at gonadal and extragonadal sites. One each case of Seminoma,YST,MGCT was extragonadal in nature. MGCTs were predominantly testicular in origin (Table 4) (Figures 1-6).

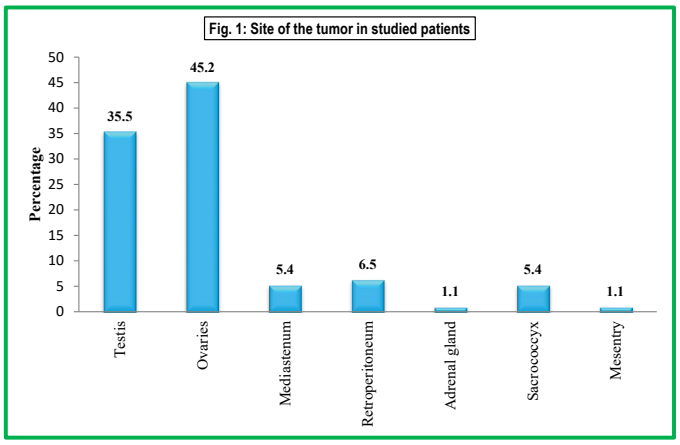

Figure 1. Site of the tumor in studied patients.

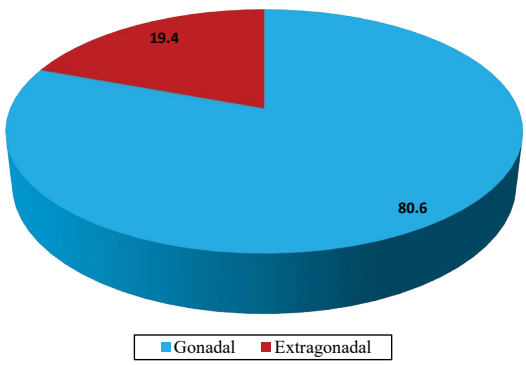

Figure 2. Location of tumor in patients.

Table 1. Histopathological diagnosis of tumor.

Tumor	Number	Percentage
Mature cystic teratoma	48	51.6
Classical seminoma	14	15.1
Yolk sac tumor	10	10.8
Dysgerminoma	4	4.3
Yolk sac predominant MGCT	3	3.2
Seminoma predominant MGCT	3	3.2
Embryonal carcinoma predominant MGCT	3	3.2
Teratoma predominant MGCT	2	2.2
YST and teratoma predominant MGCT	2	2.2
Choriocarcinoma	1	1.1
Choriocarcinoma	1	1.1
Post pubertal teratoma	1	1.1
Spermatocytic tumor	1	1.1
Monodermal teratoma strauma ovari	1	1.1
Total	93	100

Table 2. IHC of germ cell tumors.

IHC Marker	GCT Histology	Gonadal	Extragonadal	Total No.	No Positive	%Age
GFAP	Mature Cystic Teratoma	33	15	48	42	87.5
AFP	Yolk Sac Tumour	9	1	10	10	100
CD117/ PLAP	Seminoma/ Dysgerminoma	09-Apr	1(Seminoma)	14-Apr	14-Apr	100
Lmw-CK	All	75	18	93	93	100
S100	Mature Cystic Teratoma	33	15	48	30	62.5
BhCG	Choriocarcinoma	1	0	1	1	100
Glypican	Yolk Sac Tumor	9	1	10	8	80
NSE	Post Pubertal Teratoma	1	O	1	1	100

Table 3. Immunohistochemistry of mixed germ cell tumors.

Tumor type	AFP	CDII7/ PLAP	β-hCG	GFAP/ S100	pan CK/ CD30
Yolk sac predominant MGCT	3/3	2/3	0/3	0/3	1/3
%age	100	66.7	0	0	33.3
Seminoma predominant MGCT	0/3	3/3	1/3	1/3	0/3
%age	0	100	33.3	33.3	0
Embryonal carcinoma predominant MGCT	2/3	2/3	1/3	0/3	3/3
%age	66.7	66.7	33.3	0	100
Teratoma predominant MGCT	2/2	0/2	0/2	2/2	0/2
%age	100	0	0	100	0
YST and teratoma predominant MGCT	2/2	0/2	0/2	2/2	2/2
%age	100	0	0	100	100

Discussion

The total cases studied in our series were 93. The study was conducted for a 5-year period between 2015-2019 [1]. The most frequent anatomical site for the GCTs was ovaries (42) followed by testis (33). EGCTs although less frequent were reported at mediastinum (5), RPN (6), sacrococcyx (5) adrenal

Table 4. Correlation of tumour diagnosis with primary site of origin.

Tumour	Total No.	Testis	Ovaries	Mediastinum	RPN	Sacrococcyx	Adrenal	Mesentery
Mature cystic teratoma	48	2	31	4	6	4	0	1
Seminoma	14	13	0	0	0	0	1	0
YST	10	6	3	1	0	0	0	0
Dysgerminoma	4	0	4	0	0	0	0	0
Choriocarcinoma	1	0	1	0	0	0	0	0
Post pubertal teratoma	1	1	0	0	0	0	0	0
Spermatocytic tumour	1	1	0	0	0	0	0	0
Monoderma teratoma strauma Ovari	1	0	1	0	0	0	0	0
MGCT	13	10	2	0	0	1	0	0
Total	93	33	42	5	6	5	1	1

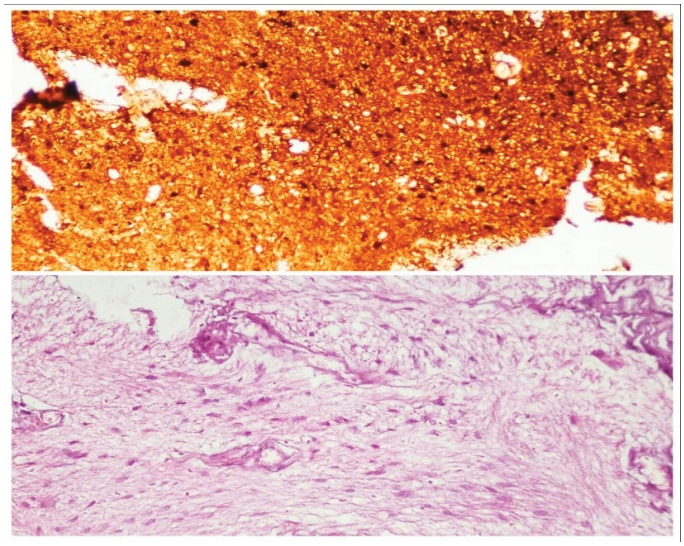

Figure 3. Photomicrograph above shows glial tissue present in a teratoma (H&E X400). Below picture shows strong positivity of the same for GFAP on IHC (DAB X400).

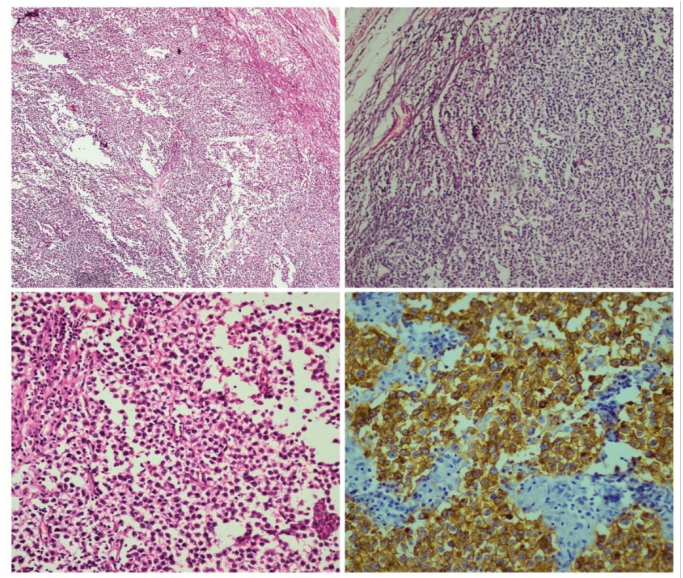

Figure 4. Photomicrographs showing sheets of seminoma tumour cells in an adrenal gland. The tumour had replaced the entire gland with no normal looking adrenal tissue. However, the capsule of the gland could still be appreciated (upper right) which was infiltrated by the tumour cells. (H&E X100). Lower left picture shows lymphoid infiltration admixed with the tumour cells. (H& E X400) Lower right side shows strong and intense staining for PLAP in the cytoplasm of tumour cells. (DAB X400).

gland (1) and mesentery (1) (Figure 1). Our findings were consistent with the study of Arora RS, et al. [2] which also showed that GCTs were more common

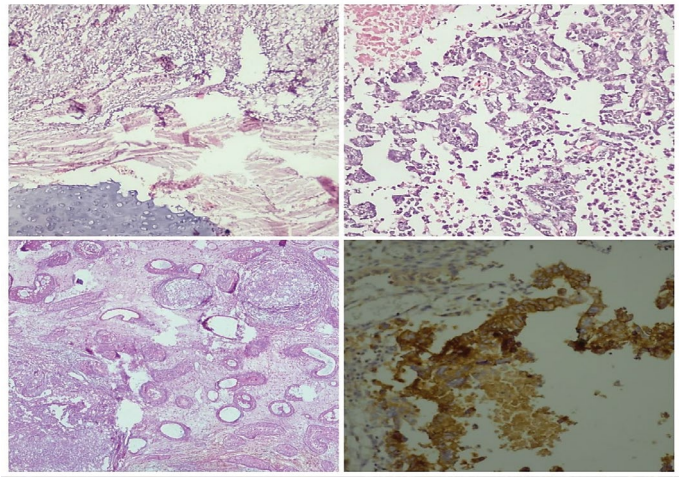

Figure 5. Photomicrographs showing different components in a mixed germ cell tumour. Upper left picture shows presence of seminomatous tumour cells along with mature cartilage (H&E X100). The upper right picture shows embryonal component which was present in the same tumour (H&E X400). Lower left side is showing presence of yolk sac elements & seminomatous elements along with ITGN (H&E X100). Picture on the lower right corner is showing CD30 cytoplasmic staining in embryonal tumour cells (DAB X400).

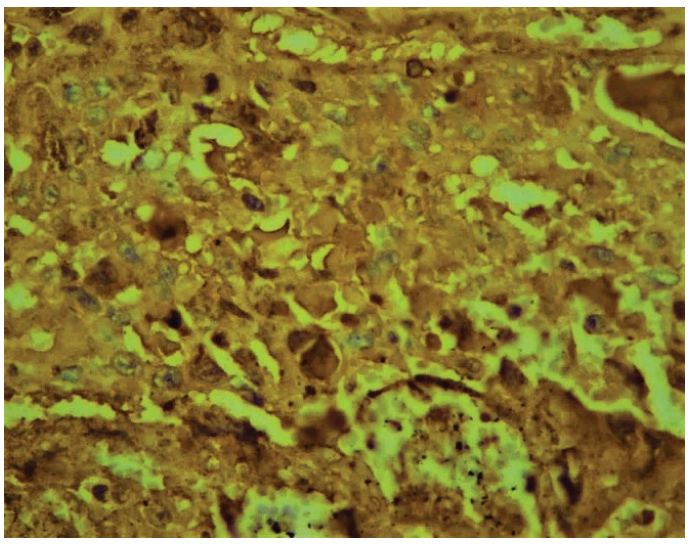

Figure 6. Photomicrograph showing intense βhcg staining in the cytoplasm of choriocarcinoma tumour cells (DAB X400) .

than extragonadal ones. Out of 93 cases, in our series, gonadal GCTs (75) outnumbered the extragonadal ones. Only 18 cases of the latter were seen (Figure 2).

In our study RPN was the most frequent extragonadal site for GCTs. We

reported one case of adrenal gland GCT in our series (Table 4). The patient was a 44 year old male who presented with pain and swelling in the right flank and histopathology revealed a classical seminoma subtype which was later on confirmed on IHC. Literature on the incidence of germ cell tumours arising at the adrenal gland is limited. Although cases have been reported of other GCTs at this site like Malik A, et al. [3] reported the occurrence of adrenal gland MGCT in a 77-year-old male which showed components of YST, immature teratoma and squamous cell carcinoma. Similarly, adrenal gland teratoma has been reported in studies like Shrestha MK, et al. [4], Lam KY, et al. [5] and Hui, JPK et al. [6]. In our study we also reported a case of mesenteric cyst teratoma in a 15 month old female patient, (Table 4) a rare site for EGCT. The cyst showed numerous areas of glial tissue having strong cytoplasmic positivity for GFAP (Figure 3). The same has been reported earlier by Al-Arfaj, AA et al. [7] who in his review article reported a teratoma within a mesenteric cyst in two children-5 month old Saudi girl and a 4 month old Saudi boy.

The commonest histopathological variant was mature cystic teratoma (51.6%). Classical seminoma was next in line comprising of 15.1% of the total cases. YST was less frequent (10.8%) comprising of only 10 cases. MGCTs comprised of only 13 cases in our series (Table 1).

Among extragonadal sites the most frequent site for these tumors was retroperitoneum (Figure 1). which reported 6 cases of mature cystic teratoma followed by mediastinum (4) and sacrococcyx (4). This observation was comparable to Arora, RS et al. [2] In their study the frequency of teratomas at these sites was equal.1 out of the 10 cases of yolk sac tumor was seen at the mediastinum. Takeda S, et al. [8] and his colleagues studied GCTs at the mediastinum and also found it to be a rare site for non seminomatous GCTSs. Knapp, R et al. [9] reported 3 YSTs at this site in 32 patients of NSGCT. In our study only one MGCT was seen at the extragonadal site (sacrococcyx) while rest of the MGCTs were mostly testicular in origin (Table 4).

87.5% of mature cystic teratomas at gonadal and extragonadal site and one case of post pubertal teratoma (Table 2) in our study were strongly positivity on IHC for neural tissue markers like glial fibrillary acidic protein (GFAP) and S100. These markers were also positive in 5 cases of mixed GCTs (Table 3). The expression of these tumour markers were studied by Gu S, et al. [10]. A strong expression of GFAP often suggests that tumour cells are mature and well differentiated. In Neihans GA, et al. [11] study S100 was positive in two cases of teratomas.

All cases of seminomas and dysgerminomas in our study showed strong and diffuse cytoplasmic positivity for PLAP /CD117 on IHC. This included tumours at both gonadal and extragonadal site (Tables 2). PLAP was also positive for 7 MGCTs and 1 case of spermatocytic tumour (Tables 2 and 3). Neihans GA, et al. [11] study found 61 seminomas/ dysgerminomas to be positive for PLAP on IHC. In their study PLAP expression was found in other GCTs as well, like YST and embryonal tumours, however it was patchy.

Pure YSTs and 9 MGCTs in our series showed cytoplasmic positivity for AFP (Tables 2 and 3) both at the gonadal and extragonadal site. All but 2 YSTs were positive for Glypican 3, a novel marker highly sensitive in detecting yolk sac components. Neihans GA, et al. [11] found this marker to be positive in 74% of YSTs and 33% of embryonal carcinomas. Embryonal components in mixed germ cell tumours was strongly positive for CD30 (Tables 3). All GCTs including strauma ovari showed CK positivity (Table 2). Beta-hCG showed strong and intense cytoplasmic positivity on IHC for choriocarcinoma and 2 cases of MGCTs in our series (Tables 2 and 3) consistent with Neihans GA et al study [11]. Hans Post Pubertal teratoma lone case had primitive neuroepithelial component showing strong cytoplasmic staining for NSE (Table 2). 6 cases of mixed non seminomatous GCTs were positive on IHC for pan CK/CD 30. In these tumours embryonal and yolk sac components were a frequent (Table 3). This was consistent with the study of Neihans GA, et al. [11] where non seminomatous germ cell tumours were more likely to express this marker.

Conclusion

There are well characterized readily available IHC markers that can resolve diagnostic difficulties in cases showing morphological overlap and in assessing the extent of a tumour in a mixed GCT. Teratomas showed diffuse and strong positivity for GFAP/S100. YSTs showed strong cytoplasmic positivity for AFP and Glypican-3. Choriocarcinoma showed strong and intense cytoplasmic positivity for βhCG. CD117/PLAP expression was found in all seminomas dysgerminomas and spermatocytic tumor. CD 30 expressions was noted only in embryonal component containing tumours. Ck expression was shown by all GCTs irrespective of the histological type

Limitations

It was a single center study and the sample size was small. Markers like OCT ¾, SALL4 were not available in our institute

Declaration

Funding

None.

Conflict of interest

None declared.

Ethical approval

Not required.

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