ejpmr, 2023,10(5), 248-254

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

25 YEARS EXPERIENCE OF CLINICOPATHOLOGICAL PROFILE OF OVARIAN GERM CELL TUMORS IN INDIAN WOMEN AND THE UTILITY OF SALL4 AND OCT3/4 AS A DIAGNOSTIC IMMUNOHISTOCHEMICAL MARKER

Deepshikha Arora[1#], Ruchi Rathore[1#] M. D., Sonal Sharma[1]* M. D. and Sarla Agarwal [1] M.D.**

*Professor and Head **Ex Professor, #Ex Senior Resident.
Pathology, University College of Medical Sciences and Guru Teg BahadurHospital.

***Corresponding Author: Dr. Ruchi Rathore**
Ex Senior Resident Pathology, University College of Medical Sciences and Guru Teg Bahadur Hospital.

Article Received on 09/03/2023 **Article Revised on 29/03/2023** **Article Accepted on 19/04/2023**

ABSTRACT

Introduction: Ovarian germ cell tumors (GCT) differ from other ovarian tumors in their histology, age of presentation, prognosis and treatment. Complete knowledge of the Clinicopathological profile of these tumors and proper diagnosis of these neoplasms is essential for appropriate treatment and maintenance of fertility wherever possible. **Material and Methods:** This is a retrospective study of clinicopathologic profile of all the GCT of ovary received over a period of 25 years. The immunostaining characteristics of two immunohistochemical markers OCT3/4 and SALL4 were studied on 32 cases of malignant GCTs and their sensitivity and specificity for malignant GCTs were compared. Results: 25.7% (284/1102) of all the ovarian tumors were GCTs. Mature cystic teratoma was the most common tumor in our study forming 20.2% of the total ovarian neoplasms. Oct 3/4 displayed 100 percent positivity in dysgerminomas and mixed tumors with dysgerminomatous component but was largely negative in yolk sac tumors and was only focally positive in immature teratomas. SALL4 immunostain results were similar to OCT3/4 in dygerminomas, mixed GCTs and immature teratomas but SALL4 was found to be a far better marker for yolk sac tumors displaying 100 percent positivity with strong intensity. **Conclusion:** 25.7% (284/1102) of all the ovarian tumors were GCTs in our study. The present study indicates that SALL4 is a better marker than OCT3/4 for diagnosis of malignant GCTs and may fulfill the need for a pan germ cell marker.

KEYWORDS: Germ cell tumor, OCT3/4, SALL4, dysgerminoma, ovary, immature teratoma, yolk sac tumor.

INTRODUCTION

Ovarian cancer is the sixth most common malignancy in females worldwide.[1] The germcell tumors (GCT) of the ovary account for 20% of all the ovarian tumors, with benign cystic teratoma being the single most common ovarian tumor. Malignant GCTs on the other hand are rare tumors accounting for 2.6% of ovarian tumors, frequently occurring in women less than 30 years of age.[2] Recent improvements in combination chemotherapy have now made it possible to achieve cure in patients with malignant ovarian GCTs.[3] Therefore proper diagnosis of these neoplasms is essential for appropriate treatment and maintenance of fertility wherever possible.

Most ovarian tumors are readily characterized by morphological examination but at times the histologic findings may be confounding and significant problems may occur due to neoplasms of similar or even diverse histogenetic origins.[4,5] Hence, immunohistochemical confirmation becomes necessary to support or confirm a morphological diagnosis. Although, much has been published about GCTs of the ovary, there are few retrospective cross sectional studies from India to understand the epidemiology of this disease. Therefore we undertook a retrospective study of all GCTs of the ovary operated at single tertiary care centre over the period of 25 years to estimate the same in current scenario.

Previously, several markers have been used to facilitate the diagnosis of GCTs like PLAP, CD30, C-KIT and alpha fetoprotein (AFP). But all these markers lack sensitivity and have restrictive uses.[6,7,8,9] Thus there is need for more reliable markers with better predictive value so that an unequivocal diagnosis can be reached in most cases. OCT3/4 and SALL4 have recently surfaced as new biomarkers for GCTs.

OCT3/4 is a transcription factor that is critically involved in the self-renewal of undifferentiated embryonic stem cells.[10] It has been tested for both gonadal and extragonadal GCTs. Its positivity has been detected mainly in the undifferentiated GCTs such as seminomas and embryonal carcinomas.

Spalt-like transcription factor 4 (SALL4) is a transcription factor that not only regulates development and maintains embryonic stem cell pluripotency but it is also involved in some tumor progression.[11] SALL4 is said to maintain embryonal stem cell pluripotency by forming a regulatory network with OCT3/4, NANOG, and SOX2.[12] SALL4 and OCT3/4 work antagonistically to balance the expressions of other SALL gene family members.[13] The utility of SALL 4 has been investigated in all GCTs. However, the utility of SALL 4 over OCT3/4 or vice versa had not been studied much. It is essential to have a marker which is more specific and sensitive for confirmation of diagnosis esp on small biopies in low resource settings. In this study we also studied the role of OCT3/4 and SALL4 as a diagnostic immunohistochemical marker in GCTs.

MATERIALS AND METHODS

CLINICOPATHOLOGICAL PROFILE

This is a retrospective study of all the GCT of the ovary received in the department of pathology at a tertiary care hospital, India. It included all the consecutive cases of ovarian GCT received in the department over a period of 25 years from 1993 to 2018. The data regarding age, size, laterality, gross, morphological features, complications and surgery performed was retrieved from the pathological archives and Descriptive statistics were used for analysis using SPSS version 22 software.

OCT3/4 AND SALL4 IMMUNOSTAINING

Sample selection: For the purpose of immunostaining 32 cases of malignant ovarian

GCTs received in past five years were included in the study. Ten cases each of malignant surface epithelial tumors and sex cord stromal tumors were included to determine the specificity of antibodies for GCTs.

Immunohistochemical Staining and Evalution

The clinical data of the cases was recorded and Hematoxylin and Eosin (H&E) Stained slide and block were retrieved from the records. All the slides available were reviewed to study the histological features of tumors. 3-4 micrometer thick sections were taken from representative block of each case to check for expression of OCT3/4 and SALL4 on poly-L-lysine coated slide.

Immunostaining for OCT3/4 (spring Bioscience; 7.0 ml pre-diluted immunogen affinity purified rabbit polyclonal antibody in TBS/1% BSA buffer pH 7.6 with less than 0.1% sodium azide.)and SALL4 (biocare medical, Concentrated and Prediluted Mouse Monoclonal Antibody) was done using Avidin-Biotin technique. Well-established cases of seminoma wereused as positive controls. For negative controls, primary antibody was replaced by TRIS buffer. The slides were examined at 400x magnifications.

Since OCT3/4 and SALL4 are nuclear transcription factors involved in gene regulation so only nuclear staining was considered as positive. The staining intensity was recorded as weak, moderate or strong. For yolk sac tumors, AFP immunostaining was done and was weighed against OCT3/4 and SALL4. To find out the sensitivity and specificity of immunostaining for OCT3/4 and SALL4 taking histopathology as gold standard chi square test and Fischer's exact test were used and a p value of <0.05 was considered as significant.

RESULTS CLINICOPATHOLOGICAL PROFILE

A total of 1102 ovarian neoplasms were received in our department during a period of 25 years from 1990 to 2014. 25.7% (284/1102) of these were GCTs. Mature cystic teratoma (MCT) was the most common tumor accounting for 20.2% of the total ovarian neoplasms and 78.5% of all the GCTs. The mean age of cases was 29.4 ± 14.5 years (mean ± standard deviation) ranging from 4 to 70 years of age. While 53.5% of all cases were seen in age group <30 years rest occurred in older age groups. In > 40 years of age dysgerminoma and yolk sac tumors were more frequent. It was worth noting that malignant transformation of MCT was seen in patients > 30 years of age. Immature teratoma was however seen in patients <30 years of age. The incidence of malignant transformation of mature teratoma in our study was 3.5%. Squamous cell carcinoma was the common form of malignancy arising in a teratomain our study. Lump abdomen followed by pain abdomen was the most common presenting complaint in our study. ***TABLE 1 summarizes the number of cases and their laterality, size of the tumors found in this study.***

Table 1: Showing the laterality and size of ovarian germ cell tumors found in our study.

Laterality	**Right**	**Left**	**Bilateral**
	61.6%	32.6%	5.8%
Tumor size	**Minimum**	**Maximum**	**Mean size**
	2.5 cm	32 cm	10.4cm ± 4.5 cm
Tumor Type and numberof cases	MCT	n= 231	
	Dysgerminoma	n= 15	
	Immature teratoma	n= 11	
	Yolk sac tumor	n= 9	
	Mixed germ	n= 9	
	cell Tumor		
	Embryonal carcinoma	n= 1	
	Malignant Transformationof MCT	n= 8	

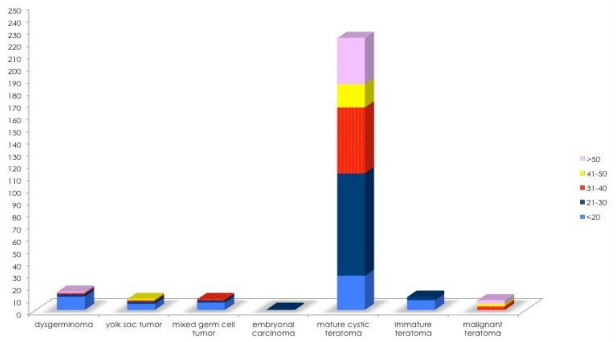

Figure 1: Represents the age wise distribution of various germ cell neoplasmsfound in our study.

Immunohistochemical evaluation

A total of 32 cases of malignant GCTs received in the last 5 years were included in the study. They comprised of 13 dysgerminomas, 5 yolk sac tumors, 7 immature teratomas, 4 mixed tumors and 3 teratomas with malignant transformation. All the yolk sac tumors included in the study displayed reticular pattern with microcystic areas. 3 cases showed presence of well-formed Schiller-duval bodies and hyaline globules. All four cases of mixed GCTs were composed of dysgerminomatous and yolk sac components. Of the seven immature teratomas included in the study, two were grade 3, one grade 2 and four grade 1. Table two summarizes the immunopositivity for OCT ¾ and SALL4 in all cases included in this study. The sensitivity of OCT3/4 for GCTs was 72.41% and specificity was 100% with a positive predictive value of 1 and negative predictive value of 0.71. (Figure 2) SALL4 was positive in all the cases of Yolk sac tumors with moderate to strong staining intensity (3+ to 4+). AFP immunostain was also done in all the five cases. Although all five cases were positive, the positivity was focal and only of weak to moderate intensity. Sensitivity of SALL4 for GCTs was 82.75% and specificity was 100%. The positive predictive value was 1 and negative predictive value was 0.8. Fischer's exact test and chi square test were used for analysis and a significant p value of 0.005 was obtained. ***Figure 3*** *compares the immunopositivity of malignant GCTs for OCT3/4 and SALL4.* Though OCT3 / 4 and SALL4 both are equally specific for malignant GCTs, SALL 4 is more sensitive than OCT 3 / 4 for the same.

Table 2: OCT and SALL4 Immunostain analysis in malignant GCTs.

Tumor	IHC STAIN	NEG#	+ WEAK	++ (Mild)	+++ (MOD*)	++++ (Strong)	TOTAL +VE $
Dysgerminoma (n=13)	OCT 3/4	0	1	1	4	7	13
	SALL 4	1	0	0	4	8	12
Yolk Sac Tumor (n=5)	OCT 3/4	4	1	0	0	0	1
	SALL 4	0	0	0	3	2	5
Immature Teratoma (n=7)	OCT 3/4	4	3	0	0	0	3
	SALL 4	4	3	0	0	0	3
Mixed Germ Cell Tumor (n=4)	OCT 3/4	0	0	1	1	2	4
	SALL 4	0	0	0	2	2	4

(#: Negative, * Moderate, $ Positive)

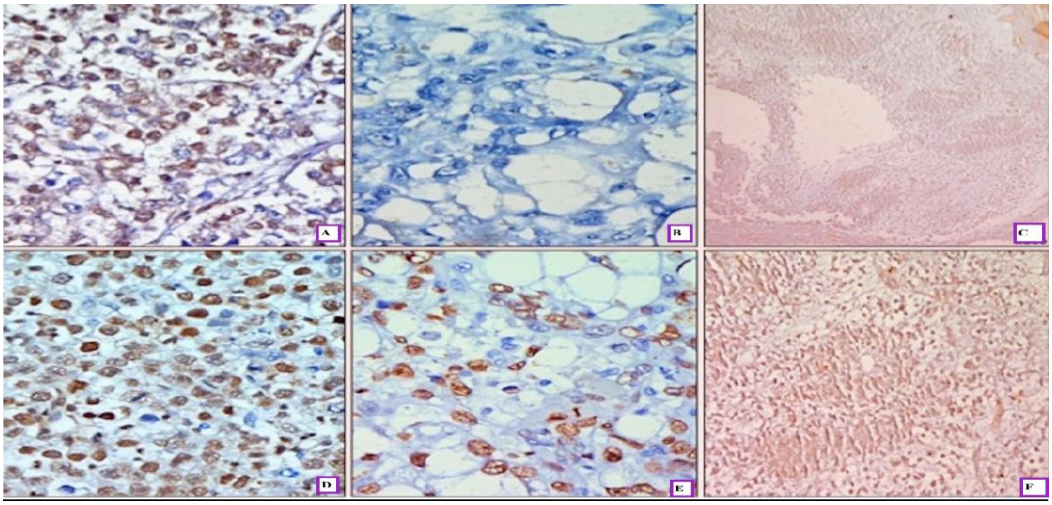

Figure 2: OCT3/4 and SALL 4 Immunostain: [A] Dysgerminoma showing strong nuclear positivity (4+) of OCT3/4 {IHC: 400X} [B] Yolk Sac Tumor showing negative staining for OCT3/4 {IHC: 400X}.[C] Immature teratoma showing focal nuclear positivity for OCT3/4 {IHC: 100X}. [D] Dysgerminoma showing strong nuclear positive (4+) staining for SALL4. {IHC: 400X}E: Yolk Sac Tumor showing strong nuclear positivity for SALL4 (4+) {IHC: 400X}F: Immature neuroepithelium displaying focal positivity for SALL4 (1+) {IHC :200X}.

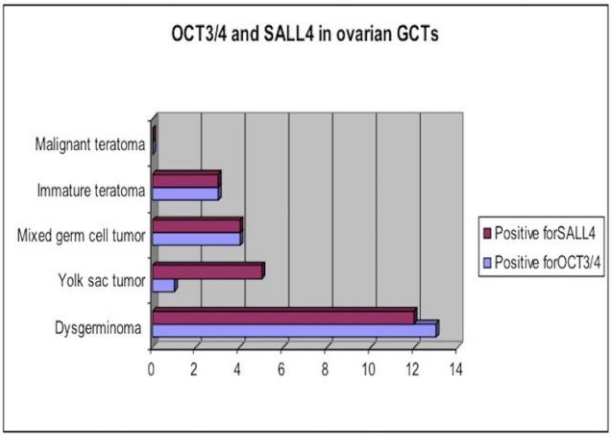

Figure 3: Comparison of OCT3/4 and SALL 4 staining in Malignant GCTs.

DISCUSSION

Ovarian GCTs principally affects young women. They are derived from primitive germ cells of the embryonic gonad, and may undergo germinomatous or embryonic differentiation.[14]

In our study also, incidence of GCT (25.7%) was in concordance with studies conducted by Gupta et al and Ranu sarkar with incidence of 31.18%and 27.9% respectively in their studies.[15,16]

GCT was also the most common malignant ovarian neoplasm accounting upto 71.1% of all the malignant tumors of the ovary occurring in paediatric and adolescent girls upto 20 years of age. While immature teratoma was the most common malignant GCT in this age group in our study as has been reported earlier by Rathore et al,[18] dysgerminoma was most common malignant GCT in the study conducted by Mukhopadhyay et al[19] from India.

These tumors are frequently unilateral and if found and treated earlier, they are generally curable. Only 5.8% of the tumors included in our study were bilateral. It is the use of combination chemotherapy after initial surgery which has drastically improved the prognosis for many women with these tumors.[20-22] since myriad varieties of GCT differ in their clinical presentation and histology correct diagnosis is of utmost importance for their early treatment. As gross and microscopy is still the mainstay of diagnosis in these cases, use of immunohistochemistry becomes essential for confirmation of these tumors. The role of various immunohistochemical markers like CD117, CD133, SALL4, OCT4, TCL1 and glypican-3 has been studied in various studies.[23]

OCT3/4 is a transcription factor expressed in embryonic stem cells and germ cells. Various studies have established its role in maintaining the pluripotency in undifferentiated tumors cells in testicular tumors.[10,24,25] It has been found to be consistently expressed in testicular seminomas and embryonal carcinomas.

Cheng et al[26] found OCT3/4 to be consistently expressed in 100% of dysgerminomas whereas they reported the cases of non-dysgerminomatous GCTs to be negative for OCT3/4. The negative cases included yolk sac tumors, teratomas, sex cord stromal tumors and epithelial malignancies except for some focal positivity seen in clear cell adenocarcinomas. In our study also, 80% of yolk sac tumors, sex cord stromal tumors and surface epithelial tumorswere negative for OCT3/4.

We also observed positivity in 3 cases of immature teratomas. Positivity was seen primarily in neural tissues (both mature and immature). Immature neuroepithelium showed a stronger positivity as compared to the mature glial tissue. Cases displaying OCT3/4 positivity were grade 3 and grade 2 teratomas while all grade 1 teratomas being negative. Similar findings were noted by Abiko et al[27] who found OCT3/4 expression in high grade cases of immatureteratomas.

Thus the expression of OCT3/4 is directly related to the degree of differentiation of these tumors. Hence dysgerminomas and embryonal carcinomas show highest positivity followed by immature elements in teratomas.

SALL4 is a zinc finger transcription factor and homologous to the Drosophila spalt (sal) gene.[28] SALL4 is located on chromosome 20q13.13-13.2.5 and is essential to human development. Mutations in SALL4 lead to acro–renal-ocular and Okihiro syndromes.[29,30] In human embryonic stem cells, SALL4 is essential to

maintain embryonal stem cell pluripotency and self-renewal by forming a regulatory network with OCT3/4, NANOG, and SOX2.[31] In embryonic stem cells SALL4 acts upstream of OCT3/4. It is not known if similar relationship exists in ovarian GCTs.

In our study SALL4 was strongly expressed in 92.3% of dysgerminomas, 100% cases of mixed GCTs, 100% of yolk sac tumors and 42% immature teratomas. These results are concordant with those obtained by Cao and Guo(2009)[32] who found SALL4 to be 100% positive in yolk sac tumors, dysgerminomas, embryonal carcinoma, & mixed GCTs with a yolk sac component. They also found 73% positivity in immature teratomas with primitive neuroepithelial tissue, blastema like stroma and teratomatous glands showing SALL4 expression.

Cao et al(2009)[32] investigated SALL4 expression in testicular tumors and reported that 100% of seminomas and embryonal carcinomas expressed SALL4. Definite SALL4 positivity was also seen in all of spermatocytic seminomas and choriocarcinomas (mononucleate trophoblastic cells). Seminoma cases were used as positive controls in our study. Similar expression of OCT3/4 and SALL4 was noted at extragonadal sites by Mei et al(2009),[33] Wang et al (2009)[34] & Liu et al (2010).[35] SALL4 was found to be particularly useful, covering a wider spectrum of tumors and was especially useful in Yolk sac tumor.

Most commonly used markers for GCTs include PLAP and AFP (yolk sac tumors). PLAP is neither highly sensitive nor specific for GCTs. Many of the non-GCTs have been found to be positive for PLAP.[6] AFP is considered a useful marker for yolk sac tumor but its utility is limited by its low sensitivity. Moreover the staining is focal and patchy and may even be lost in metastatic tumors.[9] In our study also, although all the cases of yolk sac tumor were positive with AFP, the staining was weak and low in intensity. In contrast staining with SALL4 was moderate to strong in intensity with >60% cells showing positivity in most cases. These observations suggest that SALL4 immunostain evaluation may prove to be a significant tool for small biopsy and specially in cases of metastatic tumors. Particular utility of SALL4 has been established by Cao and Guo in differentiating Yolk sac tumor from Clear cell carcinoma, which may mimic each other histologically but differs from widely in treatment and prognosis.

With the advent of cisplatin-based chemotherapy, cure rates of 85-90% have been obtained even in advanced cases of ovarian GCTs.[36-40] In yolk sac tumors, after the introduction of VAC regime, 2-year survival rate has improved from 25% to 60-70%.[41,42] Thus, for preservation of fertility wherever indicated and for better prognosis of the patient a correct diagnosis is of utmost importance.

CONCLUSION

GCT Is the second largest group of ovarian tumors after surface epithelial tumors of the ovary. While MCT is the most common type of GCT in all age groups, Dysgerminoma is the most common malignant GCT. However in the girls upto 20 years of age the incidence of Immature teratoma is higher then dysgerminoma amongst the malignant ovarian GCT.

The present study and review of existing literature also points out that both OCT3/4 and SALL4 supersede all the available markers for ovarian GCTs. They are particularly valuable in histologically challenging cases and in small biopsies from metastatic lesions. SALL4 is superior to OCT3/4 as, though both are equally specific for malignant GCTs, SALL 4 is more sensitive (82.75%) then OCT3/4 (72.41%). Since SALL 4 also covers a wider spectrum of tumors, it does fulfill the need for a pan germ cell marker for ovarian GCTs.

REFERENCES

1. Boyle P, Maisonneuve P, Autier P. Update on cancer control in women. Int J Gynaecol Obstet, 2000; 70: 263-303.
2. Shaaban AM, Rezvani M, Elsayes KM, Baskin Jr H, Mourad A, Foster BR, Jarboe EA, Menias CO. Ovarian malignant germ cell tumors: cellular classification and clinical and imaging features. Radiographics, 2014; 34(3): 777-801.
3. Chen LM, Berek JS. Ovarian and fallopian tubes, Cancer treatment, 5th ed. Philadelphia, PA: WB Saunders, 2001: 55: 900–932.
4. Ulbright TM. Germ cell tumors of the gonads: a selective review emphasizing problems in differential diagnosis, newly appreciated, and controversial issues. Mod Pathol, 2005; 18: 61-79.
5. Scully RE, Young RH, Clement PB. Tumors of the ovary, maldeveloped gonads, fallopian tube, and broad ligament. In: Atlas of tumor pathology. Washington, DC: Armed Forces Institute of Pathology, 1998; 23: 3.
6. Wick MR, Swanson PE, Manivel JC. Placental-like alkaline phosphatase reactivity in human tumors: an immunohistochemical study of 520 cases. Hum Pathol, 1987; 18: 946-54.
7. Berny DM, Shamash J, Pieroni K, Loss of CD30 expression in metatstatic embryonal carcinoma the effects of chemotherapy. Histopathology, 2001; 39: 382-385.
8. Polski JM, Janney CG. Ber-H2 (CD30) immunohistochemical staining in malignant melanoma. Mod Pathol, 1999; 12: 903-6.
9. Niehans GA, Manivel JC, Copland GT et al. Immunohistochemistry of germ cell and trophoblastic neoplasms.Cancer, 1988; 62: 1113-23.
10. Niwa H, Miyazaki J, Smith AG. Quantitative expression of OCT3/4-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. Nat Genet, 2000; 24(4): 372-6.
11. Yang M, Xie X, Ding Y. SALL4 is a marker of poor

prognosis in serous ovarian carcinoma promoting invasion and metastasis. Oncology reports, 2016; 1, 35(3): 1796-806.
12. Zhou Q, Chipperfield H, Melton DA et al. A gene regulatory network in mouse embryonic stem cells. Proc Natl Acad Sci U S A, 2007; 104: 16438-16443.
13. Yang J, Gao C, Chai L et al. A novel SALL4/OCT3/4 transcriptional feedback network for pluripotency of embryonic stem cells. PLoS One, 2010; 5(5).
14. Gershenson DM. Update on malignant ovarian germ cell tumors. Cancer, 1993; 71: 1581–1590.
15. Gupta S.C., Singh P.A., Mehrotra T.N., et al. — A Clinicopathologic study of ovarian tumors. Ind J Pathol Microbiol, 1986; 29: 354 - 362.
16. Sarkar R. — Ovarian neoplasms – A 14 years study. J Obstet Gynecol India, 1996; 46: 156 –160..
17. Ruchi Rathore, Sonal Sharma, Deepshikha Arora. Clinicopathological Evaluation of 223 Cases of Mature Cystic Teratoma, Ovary: 25-Year Experience in a Single TertiaryCare Centre in India. Journal of Clinical and Diagnostic Research, 2017; 11(4): EC11-EC14.
18. Rathore R, Sharma S, Arora D. Spectrum of Childhood and Adolescent Ovarian Tumors in India: 25 Years Experience at a Single Institution. Open Access Macedonian Journal of Medical Sciences, 2016; 4(4): 551-555.
19. Mukhopadhyay M, Shukla RM, Mukhopadhyay B, et al. Ovarian cysts and tumors in infancy and childhood. Journal of Indian Association of Pediatric Surgeons, 2013; 18(1): 16-19.
20. Gershenson DM: Update on malignant ovarian germ cell tumors. Cancer, 1993; 71 (4): 1581-90.
21. Segelov E, Campbell J, Ng M, et al.: Cisplatin-based chemotherapy for ovarian germ cell malignancies: the Australian experience. J Clin Oncol, 1994; 12 (2): 378-84.
22. Williams S1, Blessing JA, Liao SY, Ball H, Hanjani P: Adjuvant therapy of ovarian germ cell tumors with cisplatin, etoposide, and bleomycin: a trial of the Gynecologic Oncology Group. J Clin Oncol, 1994; 12 (4): 701-6..
23. Trinh DT[1], Shibata K, Hirosawa T, Umezu T, Mizuno M, Kajiyama H, Kikkawa F. Diagnostic utility of CD117, CD133, SALL4, OCT4, TCL1 and glypican-3 in malignant germ cell tumors of the ovary. J Obstet Gynaecol Res, 2012; 38(5): 841-8. doi: 10.1111/j.1447-0756.2011.01798.x. Epub 2012 Mar 26.
24. Looijenga LH, Stoop H, de Leeuw HP et al. POU5F1 (OCT3/4) identifies cells with pluripotent potential in human germ cell tumors. Cancer Res, 2003; 63(9): 2244-50.
25. Cheng L, Sung MT, Cossu-Rocca P et al. OCT3/4: biological functions and clinical applications as a marker of germ cell neoplasia. J Pathol, 2007; 211(1): 1-9.
26. Cheng L, Thomas A, Roth LM et al. OCT3/4: a novel biomarker for dysgerminoma ofthe ovary. Am J Surg Pathol, 2004; 28(10): 1341-6.
27. Abiko K, Mandai M, Hamanishi J et aI. OCT3/4 expression in immature teratoma of the ovary: relevance to histologic grade and degree of differentiation. Am J Surg Pathol, 2010; 34(12): 1842-8.
28. Reuter D, Schuh R, Jackle H. The homeotic gene spalt (sal) evolved during Drosophila speciation. Proc Natl Acad Sci U S A, 1989; 86: 5483-5486.
29. Jurgens G. Head and tail development of the Drosophila embryo involves spalt, a novel homeotic gene. EMBO J, 1988; 7: 189-196.
30. Warren M, Wang W, Spiden S, et al. A SALL4 mutant mouse model useful for studying the role of SALL4 in early embryonic development and organogenesis.Genesis, 2007; 45: 51-58.
31. Zhou Q, Chipperfield H, Melton DA et al. A gene regulatory network in mouse embryonic stem cells. Proc Natl Acad Sci U S A, 2007; 104: 16438-16443.
32. Cao D, Li J, Guo CC et al. SALL4 is a novel diagnostic marker for testicular germ cell tumors. Am J Surg Pathol, 2009; 33(7): 1065-77.
33. Mei K, Liu A, Allan RW et al. Diagnostic utility of SALL4 in primary germ cell tumors of the central nervous system: a study of 77 cases. Mod Pathol, 2009; 22(12): 1628-36.
34. Wang F, Liu A, Peng Y et al. Diagnostic utility of SALL4 in extragonadal yolk sac tumors: an immunohistochemical study of 59 cases with comparison to placental-like alkaline phosphatase, alpha-fetoprotein, and glypican-3. Am J Surg Pathol, 2009; 33(10): 1529-39.
35. Liu A, Cheng L, Du J et al. Diagnostic utility of novel stem cell markers SALL4, OCT3/4, NANOG, SOX2, UTF1, and TCL1 in primary mediastinal germ cell tumors. Am J Surg Pathol, 2010; 34(5): 697-706.
36. Williams S, Blessing JA, Liao S, et al. Adjuvant therapy of ovarian germ cell tumors with cisplatin, etoposide, and bleomycin: a trial of the Gynecologic Oncology Group. J Clin Oncol, 1994; 12: 701–706.
37. Gershenson DM, Morris M, Cangir A, et al. Treatment of malignant germ cell tumors of the ovary with bleomycin, etoposide, and cisplatin. J Clin Oncol, 1990; 8: 715–720.
38. Bekaii-Saab T, Einhorn LH, Williams SD. Late relapse of ovarian dysgerminoma: case report and literature review. Gynecol Oncol, 1999; 72: 111–112.
39. Kurtz JE, Jaeck D, Maloisel F, et al. Combined modality treatment for malignant transformation of a benign ovarian teratoma. Gynecol Oncol, 1999; 73: 319–321.
40. Williams SD, Kauderer J, Burnett A, et al. Adjuvant therapy of completely resected dysgerminoma with carboplatin and etoposide: a trial of the Gynecologic Oncology Group. Gynecol Oncol, 2004; 95: 496–499.

41. Wong LC, Ngan HYS, Ma HK. Primary treatment with vincristine, dactinomycin, and cyclophosphamide in non-dysgerminomatous germ cell tumour of the ovary. Gynecol Oncol, 1989; 34: 155–158.
42. Slayton RE, Park RC, Silverberg SC, et al. Vincristine, dactinomycin, and cyclophosphamide in the treatment of malignant germ cell tumors of the ovary: a Gynecologic Oncology Group study (a final report). Cancer, 1985; 56: 243–248