International Journal of Research in Medical Sciences
Nair PS et al. Int J Res Med Sci. 2019 Nov;7(11):4084-4089
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20194971

Original Research Article

Analysis of cytology of germ cell tumors with histopathological and serum tumor marker correlation: a tertiary care centre experience

Sindhu Nair P.*, Jayasree Kattoor, Nileena Nayak, Preethi T. R.

Department of Pathology, Regional Cancer Centre, Trivandrum, Kerala, India

Received: 11 July 2019
Revised: 17 September 2019
Accepted: 26 September 2019

***Correspondence:**
Dr. Sindhu Nair P.,
E-mail: sindhunairp@yahoo.co.in

ABSTRACT

Background: Germ cell tumors are found primarily in children and young adults usually arising from gonads and rarely from extragonadal sites like mediastinum, retroperitoneum, pineal gland and sacrococcygeal region. Involvement of lymphnodes or bodycavities (pleural/peritoneal cavity) is usually associated with metastatic disease.
Methods: This is a retrospective analysis of 96 cases of germ cell tumor for which a primary diagnosis of germ cell tumor was given by cytology from primary and metastatic sites. The study period is from January 1993- December 2013. Pap stained and Romanowsky stained smears and cell block sections (10cases) were studied. Serum tumor markers (LDH, BetaHCG and AFP) were correlated in all cases along with histopathology in available cases.
Results: Among 96 cases 34 were diagnosed as seminoma/dysgerminoma,10 as embryonal carcinoma,9 as yolk sac tumor,6 as teratoma and 2 as mixed germ ell tumor. In 25 cases the cytology report was suggestive of germ cell tumor and in 10 cases malignant cells favouring germ cell tumor. Among the 10 cases the serum markers were high in six of the cases and the clinician after discussing with the pathologist treated them as germ cell tumors. 47 cases had histopathology and it correlated with cytology except in 14 cases which showed no residual neoplasm after chemotherapy. 15 cases expired immediately after the diagnosis or during the course of treatment 12cases were lost to follow up. Rest of the cases have completed the treatment. In our study the serum tumor markers showed a sensitivity of 92.75% and positive predictive value was 71.11%.
Conclusions: The study highlights the importance of picking up the diagnosis of germ cell tumors by fine needle aspiration cytology so that patient can get an early diagnosis, effective treatment and a multidisciplinary approach is essential in diagnosing a difficult case of germ cell tumor. Previous history, radiology, clinical features and serum tumor markers all aid in the cytological diagnosis of germ cell tumor.

Keywords: Fine needle aspiration cytology, Germ cell tumor, Serum tumor markers

INTRODUCTION

Germ Cell Tumors are found primarily in children and young adults. They commonly arise from gonads and extra gonadal sites like mediastinum, retroperitoneum, pineal gland and sacrococcygeal region and respond very well to chemotherapy. These tumors and their metastases may be found in various sites that are amenable to cytologic sampling like lymph nodes and body cavities. Incorrect interpretation of these neoplasms as poorly differentiated malignancies of other types may deprive the patient effective chemotherapy.[1]

Fine needle aspiration cytology is the first line diagnostic modality of superficial swellings. It is commonly employed for the diagnosis of primary gonadal and extra gonadal germ cell tumors and their metastases. It is a useful technique to differentiate germ cell tumor from

other malignancies in the proper clinical context although the utility of FNA in the diagnosis of testicular GCT has been questioned by controversy.[2] Gonadal germ cell tumors continue to be the cause of diverse, diagnostically challenging issues for the pathologist, and their correct resolution often has major important therapeutic and prognostic implications. They are academically interesting because of the biological diversity exhibited in the two gonads and variation in frequency of certain neoplasms.[3] However in extra gonadal location fine needle aspiration under radiological guidance offer a suitable alternative to biopsy especially in sites such as mediastinum and retro peritoneum. The role of clinical and radiological findings in the diagnosis of tumors located in the mediastinum is limited and fine needle aspiration cytology (FNAC) is adopted as the standard diagnostic procedure.[4] However the proximity to the heart and major vessels decrease the usefulness of FNAC and leads to failure to adequately describe cytomorphological characteristics of the tumor.[5] Effusion cytology also plays an important role in their diagnosis. An accurate diagnosis of GCT has important therapeutic and prognostic implication for the patient and this should be as rapid and reliable as possible. Correct preoperative cytological diagnosis may obviate the need of surgical excision of many of these lesions.[6] There are only limited case series or large studies in the literature about germ cell tumors and most of them share their experience in extra gonadal germ cell tumors.[7,8] So we have made an attempt to analyze the cases in which a primary diagnosis of germ cell tumor was given in cytology (both FNA and effusion cytology) and tried to correlate them with the serum tumor markers and histopathology wherever it was available.

The objectives of the study are to describe the characteristic cytologic features in different types of germ cell tumors and correlate with histopathology in available cases as this is considered as gold standard and to correlate with serum tumor markers in the right clinical context.

METHODS

This is a retrospective analysis of 96 cases for which initial diagnosis of germ cell tumor was given in cytology both from primary and metastatic sites in the time period January 1993-December 2013. The cases were retrieved from hospital medical records. Clinical details including age, sex, site of aspiration, serum tumor markers (βHCG, Alfa fetoprotein (AFP) and lactate dehydrogenase (LDH)) and follow up were recorded using a proforma. For the cytological study material was obtained by guided or direct aspiration from accessible sites like lymph nodes, chest wall masses etc. In a few of the cases the diagnosis was given from pleural and ascitic fluid cytology. Both ethanol fixed pap stained and air dried giemsa stained slides were studied. The slides were evaluated for cellularity, cell pattern, cytoplasmic, nuclear features and the background. In 10 cases we could study cell blocks which were prepared by Acid Alcohol formalin (AAF) technique. Immune histochemistry for PLAP (placental alkaline phosphatase), AFP (Alfa feto protein), C-KIT and OCT 3/4 were done wherever needed in cellblocks. Histopathology correlation was done in 47 cases.

RESULTS

The 96 patients included in the study had a wide age range from 1 year to 72 years.57.3% cases came under the age group of 20-40 years (Figure 1) with male predominance (2:1).

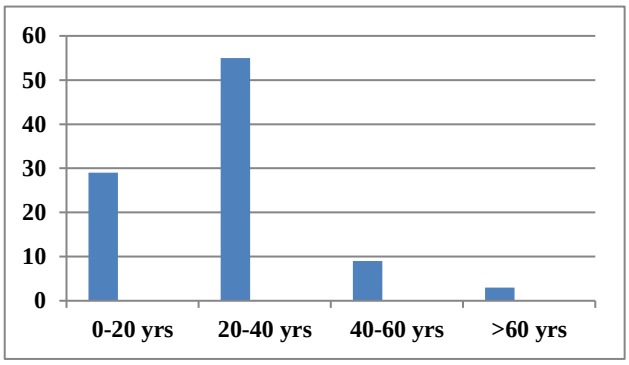

Figure 1: Age distribution.

A variety of aspiration sites were noted. Most common was from the gonads (n=38, testis-24 and ovary -14). The extra gonadal sites included mediastinum-18, lymphnodes-28, ascitic and pleural fluid-11 and sacrococcygeal region-1. (Table 1) In 61 cases only we could subtype the GCT and gave a specific diagnosis. Most common was seminoma/dysgerminoma followed by embryonal carcinoma, yolk sac tumor, teratoma and mixed germ cell tumor. In 25 cases the diagnosis was suggestive of germ cell tumor, further typing was not possible. In 10 cases we could diagnose them as malignant cells favour GCT after correlating with serum tumor markers, clinical and radiological features (Table 2).

Table 1: Sites of aspiration.

Gonads(n=38)	Testis(n=24) Ovary(n=14
Extra gonadal sites(n=58)	Lymphnodes (n=28) Mediastinum(n=18) Ascitic/pleural fluid (n=11) Sacrococcygeal region(n=1)

Sacrococcygeal teratoma was given in a 2-year-old child and in a case of adult teratoma in mediastinum when surgery was done after 6 courses of chemotherapy the specimen showed rhabdomyosarcoma along with residual teratoma. Among the 96 cases we could retrieve cytology slides only in 60 cases and histopathology slides in only 47 cases. Histopathology correlated with cytology except in 14 cases which showed no residual neoplasm after chemotherapy. 15 cases were lost follow up immediately

after the cytologic diagnosis and 12cases expired during the course of the disease without a histopathological diagnosis (Figure 2,3,4,5).

Table 2: Type and distribution of cases.

Seminoma/dysgerminoma	n=34
Yolk sac tumor	n=9
Embryonal carcinoma	n=10
Teratoma	n=6
Suggestive of germ cell tumor	n=25
Malignant cells favouring germ cell trumor	n=10
Mixed germ cell tumor	n=2
Total	n=96

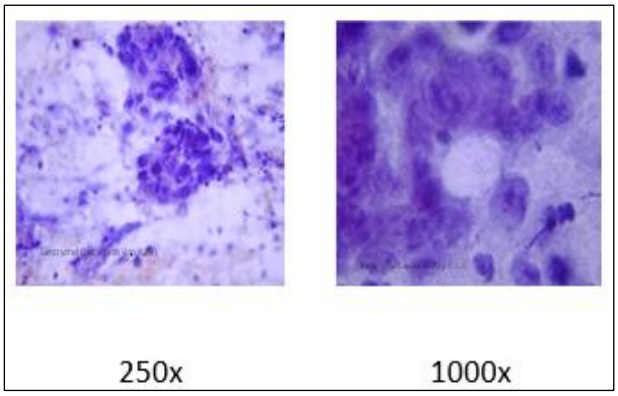

Figure 2: Embryonal carcinoma - Cells with prominent nucleoli and abundant necrotic material in the background.

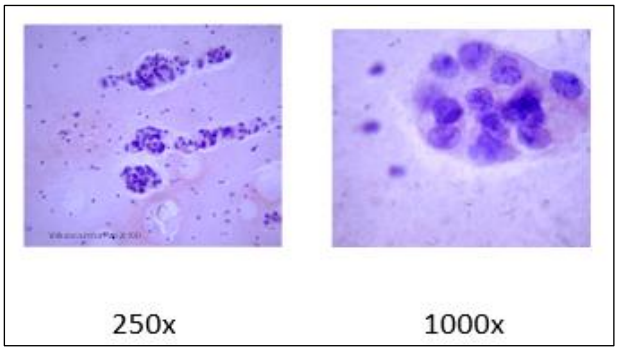

Figure 3: Yolk sac tumor - Cells in loose cluters and papillaroid pattern with a clear halo around it.

When authors analyzed serum tumor markers LDH was increased in seminoma/dysgerminoma and teratoma cases. In the unclassified group (n-25) and malignant cells favouring GCT (n-10), LDH didn't help much to give a diagnosis. But AFP was elevated in 78% of yolk sac tumors, 67% of embryonal carcinoma, 100% of mixed germ cell tumors, 77% of unclassified group (n=25) and 58% of malignant cells favoring GCT group (n=10). Beta HCG was increased in 65% seminoma/dysgerminoma cases, 50% of unclassified group and malignant cells favouring GCT group (Figure 6).

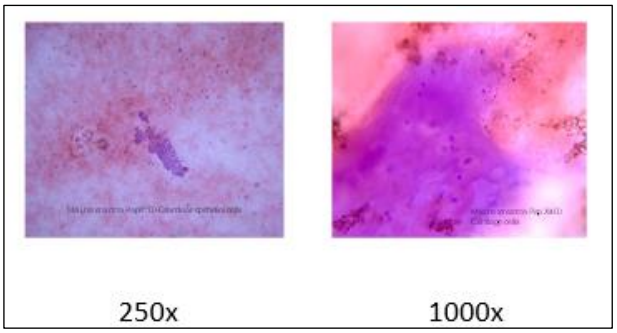

Figure 4: Teratoma - showing a sheet of mature glandular cells in first picture and mature cartilage in the second.

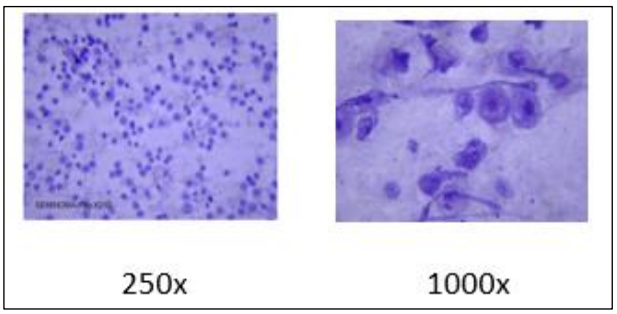

Figure 5: Seminoma - Large cells with indistinct fragile cytoplasm and large nuclei admixed with lymphocytes.

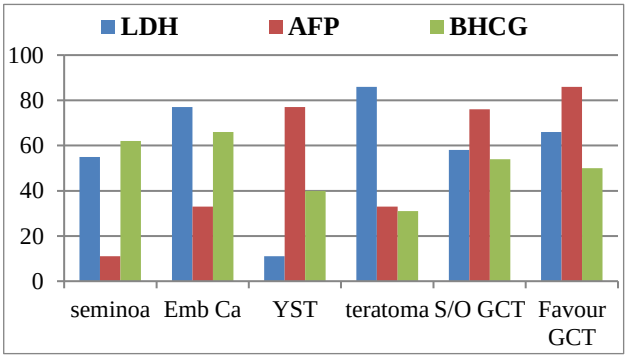

Figure 6: Correlation of cases with serum tumor markers.

So, beta HCG and AFP are the serum tumor markers that can be relied upon in giving a diagnosis of GCT. In our study the serum tumor markers showed a sensitivity of 92.75% and positive predictive value was 71.11%.

DISCUSSION

Germ cell tumors are neoplasms of young adults and children. Even though they primarily arise from gonads, they can also originate from extra gonadal sites like mediastinum, retroperitoneum, pineal gland and sacrococcygeal region. Extra gonadal primary germ cell tumors are more common in the mediastinum. The anterior mediastinum is the most common primary extra

gonadal site for germ cell neoplasms in adults and is second to the sacrococcygeal region for pathologically comparable tumors in children.[9] Among the theories explaining the pathogenesis of extra gonadal GCTs, the most widely accepted one assumes that the neoplasms arise from displaced primordial germ cells along the midline of the body during embryonic life.[10] FNA cytology compared with biopsy, is a rapid, reliable and less invasive technique with which to make a diagnosis of GCT. An earlier report by Garcia-Solano et al. on testicular GCTs also concluded that a reliable diagnosis of pure testicular germ cell tumor can be made by cytology.[11] Due to the high incidence of mixed histologic patterns, accurate typing of these neoplasms by FNAC may be very difficult. An accurate cyto diagnosis may be made by combining the cytological features, clinical features and lab findings. In this study we have made an attempt in this direction as FNAC is crucial in the early diagnosis of GCT which has a good response to chemotherapy.

In our study seminoma/dysgerminoma (34cases) was the most common germ cell tumor. Since most of these cases were from the primary gonadal sites (n=20, testis and ovary) the diagnosis was not a problem. The bimodal population of atypical large round cells with fragile cytoplasm and indistinct cell borders admixed with lymphocytes and plasma cells were identified in most of the cases. The characteristic tigroid background described in the textbooks we could see in majority of the cases (76%) from the primary gonadal sites. Not a single case from extragonadal sites (n=14) showed this background. This is in concordance with another Indian study done in 2008 by Gupta et al where they got tigroid background only in 36.8% of seminoma cases of mediastinum.[12] An earlier study by Caraway et al got the characteristic background only in less than 50% of their cases from the extra gonadal sites.[13] The absence of tigroid background is a problem in mediastinal germinoma where close differentials are thymoma and lymphoma especially in young adults.[14] Granulomas were noted in ten (29%) of our cases.

The embryonal carcinoma cases were highly cellular with abundant necrotic material in the background. The prominent nucleoli in embryonal carcinoma clinched the diagnosis in almost all cases and helped to differentiate it from yolk sac tumor wherever it came as a differential diagnosis. The presence of blood vessel fragments with adherent tumor cells also favoured it. The yolk sac tumor cases showed tumor cells predominantly in papillaroid pattern and in loose clusters.[15] A unique feature we observed in all our 9 yolk sac tumor cases was a clear halo around the cell clusters. We consider this as a characteristic feature for the diagnosis of yolk sac tumor even though an extensive literature search didn't describe it before.[16,17] The smears from teratoma were paucicellular consisting of mature squamous cells and columnar glandular cells. But to diagnose mature teratoma from cytology is quite difficult. Gupta et al also says that because of the variability of components, a definite diagnosis of mature teratoma may be difficult on aspiration cytology.[12] However, the presence of squamous cells, columnar cells, stromal fragments, and adipose tissue suggests mature teratoma. In all cases in which a diagnosis of teratoma is considered the smears have to be thoroughly screened for any immature component or another type of GCT. All our cases of teratoma had histopathology in which two showed another germ cell component, two had immature elements, one showed rhabdomyosarcoma along with teratoma and one had no residual neoplasm. The occurrence of rhabdomyosarcoma in treated cases of teratoma was reported earlier also.[18-20] For 2 cases we have given a diagnosis of mixed germ cell tumor .Both had features of teratoma and another germ cell component which was yolk sac tumor in one case and embryonal carcinoma in the second case. Histopathology confirmed our cytological diagnosis in both the cases. Literature says even though mixed tumors constitute 40-45% of all primary GCTs, they are difficult to diagnose by cytology alone.[21,22] Dagil et al and Chhieng et al says depending on the sampling, it may not be possible to see all components of the mixed germ cell tumors with the fine needle aspiration biopsy.[4,6] Among the 25 cases where we gave a diagnosis suggestive of germ cell tumor we were sure that it belonged to GCT group, but couldn't subtype. We could get histopathology in 17 cases in this group among which 6 showed no residual neoplasm, 5 cases were of mixed GCT and 6 cases were given a specific germ cell tumor type. When we analyzed cytology of these cases most of them had necrotic material and aspirate was bloody which might have prevented the subtyping. Another was the occurrence of mixed GCT. The presence of more than one cell type confused the cytopathologist and prevented further sub typing. All in this group were given chemotherapy for germ cell tumors and they responded well. Moving on to the group of 10 cases where a diagnosis of malignant cells favoring GCT was given only 3 cases were from gonads and rest of the 7 cases belonged to extra gonadal sites (4 were ascitic fluid cytology, 2 from mediastinum and 1 from lymph node). In all these cases the cytopathologist had no confusion about malignant cells but couldn't ascertain whether it belonged to GCT. All these patients were young, and 6 cases showed increased serum tumor markers. In three cases both AFP and Beta HCG were elevated, AFP was elevated markedly in one case and Beta HCG in another case. One case had elevated Beta HCG and LDH. In all these cases after serum marker correlation the diagnosis of GCT was favoured. In other 4 cases we really had problem. 2 were ascitic fluid, one was FNA from mediastinum and other was from lymph node. Aspirate from lymph node was cellular with plenty of malignant cells having fragile cytoplasm and eccentric nuclei. Serum LDH was high in this case. Since large cell lymphoma came as a differential diagnosis in this case, we advised biopsy. The histopathology report was metastatic seminoma. Meanwhile patient gave history of orchidectomy five

years back, Jyothi Raj et al also described cervical lymph node metastasis in a young man with testicular mixed germ cell tumor.[23] The 2 cases who presented with ascitis were young females below the age of 40years and both had ovarian masses. The smears were sparsely cellular and scattered atypical cells showed prominent nucleoli. In both cases cell blocks were prepared. The patient was sick in one case and started on chemotherapy for germ cell tumor based on the cytological diagnosis, But the patient expired and later on the cellblock showed PLAP positive cytokeratin and LCA negative tumor cells confirming our cytological diagnosis, In the other case cell block didn't show any material and was started on germ cell tumor chemotherapy based on cytology report. Ascitis subsided and patient went for oophorectomy later. The histopathological diagnosis came as malignant mixed germ cell tumor. In the fourth case the mediastinal aspirate was sparsely cellular showing a few scattered atypical cells with necrosis and calcified material. The presence of calcification and necrosis along with the young age of the patient and site of the tumor prompted us to give a diagnosis of malignant cells favouring germcell tumor. A trucut biopsy was attempted and it turned out to be inconclusive. The patient was started on chemotherapy against germ cell tumor and responded to treatment. According to Tanaka et al when the disease was difficult to diagnose by FNAC or trucut biopsy and if serum tumor markers were normal patients has to go for surgery before starting chemotherapy.[24] No cases of chorio carcinoma was included in our study may be because of the difficulty in diagnosing this entity cytologically as they present with abundant necrosis and haemorrhage which can obscure the smears.

Potential use of serum tumor markers for germ cell tumors may include screening, diagnosis, monitoring drug treatment and surveillance after therapy.[25] In our study serum AFP was the most specific marker which was elevated in 78% of yolk sac tumors. Beta HCG was elevated in seminoma/dysgerminoma and embryonal carcinoma. AFP and beta HCG were together elevated in 30% of our cases which also is a strong evidence for germ cell tumor.[26] The serum tumor markers can help to diagnose germ cell tumor whenever cytology smears are scanty as in our 6 cases from the group germ cell tumor favouring GCT where we relied upon serum tumor markers for the diagnosis. They also help when patient presents with metastatic disease either in lymph node or in body fluids.[27,28] In our study the serum tumor markers showed a sensitivity of 92.75% and positive predictive value was 71.11%.

CONCLUSION

To conclude a multidisciplinary approach is essential in diagnosing a difficult case of germ cell tumor. Previous history, radiology, clinical features and serum tumor markers all aid in the cytological diagnosis of germ cell tumor. The possibility of a malignant mixed germ cell tumor must be borne in mind whenever you are unable to subtype a case of germ cell tumor cytologically.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Stanley MW, Powers CN, Pitman MB, Korourian S, Bardales RH, Khurana K. Cytology of Germ cell tumors: Extragonadal, extracranial masses and intra operative problems.Cancer.1997;81(4):220-7.
2. Pandey A, Nandini N, Jha A, Manjunath G. Fine needle aspiration cytology and cell block in the diagnosis of seminoma testis. J Cytol. 2011;28(1):39-41.
3. Ulbright TM. Germ cell tumors of the gonads: a selective review emphasizing problems in differential diagnosis, newly appreciated, and controversial issues. Moder Patholo. 2005;18(2):S61-79.
4. Dagil AF, Pehllvan, Cihangiroglu G, Ozercan MR. Cytology of mixed germ cell tumor with mediastinal metastasis. J Cytol. 2009;26:120-2.
5. Shabb NS, Fahl M, Shabb B, Haswani P, Zaatari G. Fine-needle aspiration of the mediastinum: A clinical, radiologic cytologic, and histologic study of 42 cases. Diagn Cytopathol.1998;19(6):428-36.
6. Dey P. Diagnostic dilemma: Diagnostic algorithm in fine needle aspiration cytology of mediastinal tumors. Indian J Pathol Microbiol. 2010;53:395-402.
7. Chhieng DC, Lin O, Moran CA, Eltoum IA, Jhala NC, Jhala DN, et al. Fine-needle aspiration biopsy of nonteratomatous germ cell tumors of the mediastinum. Am J Clin Pathol. 2002;118(3):418-24.
8. Malagon HD, Montel DP. Mediastinal Germ Cell Tumors.Diagnostic Histopathol. 2010;16(5):228-36.
9. Dehner LP. Germ cell tumors of the mediastinum, Semin Diagn Pathol. 1990;7(4):266-84.
10. Willis R. Borderland of embryology and pathology. 2nd ed. Washington, DC: Butterworth and Company; 1992.
11. García-solano J, Sánchez-sánchez C, Montalbán-romero S, Sola-pérez J. Pérez-guillermo M. Fine needle aspiration (FNA) of testicular germ cell tumours; a 10-year experience in a community hospital. Cytopathol.1998;9(4):248-26.
12. Gupta R, Mathur SR, Arora KV, Sharma GS, Cytologic Features of Extra gonadal Germ Cell Tumors, A Study of 88 cases with aspiration Cytolog. Cancer. 2008;114(6):504-11.
13. Caraway NP, Fanning CV, Amato RJ, Sneige N, Fine-needle aspiration cytology of seminoma: a review of 16 cases, Diagnostic Cytopathol. 1995;12(4):327-33.

14. Yang GC, Hwang SJ, Yee HT. Fine needle aspiration cytology of unusual germ cell tumours of the mediastinum: Atypical seminoma and parietal yolk sac tumor. Diagn Cytopathol. 2002;27(2):69-74.
15. Bandyopadhyay A, Chakraborty J, Chowdhury AR, Bhattacharya A, Bhattachrya P, Chowdhury MJ. Fine needle aspiration cytology of ovarian tumors with histological correlation. Cytol. 2012;29(1):35-40.
16. Kataria SP, Singh G, Kumar S. Cytological findings of an extragonadal yolk sac tumor presenting at an unusual site J Cytol. 2015;32(1):62-4.
17. Akhtar K. Yolk Sac Tumor: A Rare Cytological Presentation. Int J clinical & case. 2017;1(6):131-3.
18. Ryu YJ, Yoo SH, Jung MJ, Jang S, CHO KJ. An Embryonal Rhabdomyosarcoma Arising from a Mediastinal Teratoma : An Unusual Case Report, J Korean Med Sci. 2013;28(3):476-9.
19. Kefeli M, Kandemir B, Akpolat I, Yildrim A, Kokcu A, Rhabdomyosarcoma Arising in a Mature Cystic Teratoma With Contralateral Serous Carcinoma: Case Report and Review of the Literature: International Journal of Gynecological Pathology : Official J Inter Soci of Gynecol Patholog. 2009;28(4):372-5.
20. N. Omezzine, C. Khouatra, S. Larivé, G. Freyer, S. Isaac-Pinet,L. Geriniere et al: Rhabdomyosarcoma arising in mediastinal teratoma in an adult man: a case report, Annals of Oncol. 2002;13(2):323-6.
21. Chao TY, Nieh S, Huang SH, Lee WH, Cytology of Fine Needle Aspirates of Primary Extragonadal Germ Cell Tumors: Acta Cytologica.1997;41:497-503.
22. Tickoo SK, Amin MB, Cramer HM, Hark LR, Ulbright TM. The testis, Paratesticular structures, and external genitalia. In Silverberg SG, De Lellis RA, Frable WJ, Li Volsi VA, Wick MR (Eds.): Silverberg's principles and practice of surgical pathology and cytopathology, 4 ed. China: Churchill Livingstone, Elsevier; 2006:731-789.
23. Raj JA, Sharmila PS, Mahanthachar V, Rajaram T. Metastatic Testicular Mixed Germ Cell Tumors. A Diagnostic Dilemma in Cytology.J Clin Biomed Sci.2013;3(2),90-5.
24. Tanaka Y, Okamura T, Ngai T, Kobayashi D, Kobayashi T, Akitha H, et al. A Study of Patients with Primary Mediastinal Germ Cell Tumors Treated Using Multimodal Therapy, Adv Urol.2017.
25. Milose JC, Filson CP, Weizer AZ, Hafez KS, Montgomery JS. Role of biochemical markers in testicular cancer: diagnosis, staging and surveillance. Open Access J Urol. 2011;30(4):1-8.
26. Mani R, Jamil K. Specificity of Serum Tumor Markers (CA125, CEA, AFP, Beta HCG) in Ovarian Malignancies. Trends in Medical research. 2007;2(3):128-34.
27. Gilligan TD, Seidenfeld J, Basch EM. American Society of Clinical Oncology clinical practice guideline on uses of serum tumor markers in adult males with germ cell tumors. J Clin Oncol. 2010;28(20):3388-404.
28. Barlow LJ, Badalato GM, McKierman JM. Serum tumor markers in the evaluation of male germ cell tumors, Nat Rev Urol. 2010;7(11):610-7.

Cite this article as: Nair SP, Kattoor J, Nayak N, Preethi TR. Analysis of cytology of germ cell tumors with histopathological and serum tumor marker correlation: a tertiary care centre experience. Int J Res Med Sci 2019;7:4084-9.