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Original Research Article

Significance of Serum Markers - AFP, β-hCG, LDH in Reporting of Testicular Tumors, According to CAP Guidelines

Rashi Gupta[1], Ashok Panchonia[2], Pankaj Shinde[3], Gurvani Rehill[4], Vandana Pahadiya[5]

[1]Senior Resident, Department of Pathology MGMMC Indore.
[2] Professor, Department of Pathology MGMMC Indore.
[3]Associate Professor, Department of Pathology MGMMC Indore.
[4]Junior Resident, Department of Pathology MGMMC Indore.
[5]Senior Resident, Department of Pathology MGMMC Indore.

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Corresponding Author: Dr. Vandana Pahadiya
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Abstract:

Introduction: Testicular cancer is the most common malignancy in men aged 15 to 45 years, with a variation in incidence worldwide from below 1 to 12 per 100000 males. It represents 1% of male tumors and 5% of urological malignancies. Majority of testicular tumors are germ cell tumors (GCTs).

Aims and Objectives: To study the role of serum markers (AFP, β-hCG, LDH) in testicular tumors and reporting them according to recent CAP guidelines (2017). To assess the level of serum tumor markers preoperatively and postoperatively for diagnosis and staging of testicular tumors. To correlate serum tumor marker level with histopathology of testicular tumor.

Materials and Method: Prospective and observational cross-sectional study, conducted in the department of Pathology, M.Y. Hospital, during the study period of one year, included 26 cases of suspected testicular cancer. (Reduced Sample size due to covid) which were grossed according to standard guidelines and reported as per CAP protocol. Pre and post operative tumor marker levels- AFP, β-hCG and LDH were obtained for each of these cases and compared.

Results: Maximum cases in our study were mixed germ cell tumor followed by seminoma. In cases of seminomatous germ cell tumor AFP was not elevated whereas β-hCG and LDH were raised, post operatively there was reduction in these two markers in few cases, in others it remained elevated. In non seminomatous tumors, all the three markers were raised in almost 50% cases, the declination in marker level was significant for β-hCG and LDH, whereas it was insignificant for AFP.

Introduction

Testicular cancer is the most common malignancy in men aged 15 to 45 years, with a variation in incidence worldwide from below 1 to 12 per 100000 males. [1,2] It represents 1% of male tumors and 5% of urological malignancies. [4] Testicular cancer incidence has doubled over the past 40 years. [5]

Testicular cancers are defined based on their cell type. 2016 updated WHO histopathological classification characterizes testicular cancers with the following classifications:[6]

- Germ Cell Tumors
- Derived from Germ Cell Neoplasia in Situ (GCNIS)
- Germ Cell Tumors Unrelated to GCNIS
- Sex Cord/Stromal Cell Tumors
- Miscellaneous Non-specific Stromal Cell Tumors

Majority of testicular tumors are germ cell tumors (GCTs). [7] Both environmental and genetic factors have been studied in the development of testicular cancers. [3]

Ultrasonography helps further narrow down the diagnosis and radical inguinal orchiectomy is the definitive modality for diagnosis. [3]

Serum tumor markers AFP, β-HCG, and LDH represent valuable tools for the diagnosis and clinical management of testicular GCTs. [8] According to current guidelines, serum tumor markers are used to assist timely diagnosis of GCT, to accurately stage the disease, to assess the prognostic category of metastasized GCTs, to monitor treatment success, and finally to detect relapse during follow-up. [9,10]

Only few original data are available relating to associations of marker positivity before and after treatment, thus it is incompletely understood.

Our study attempts to understand the frequency of tumour marker elevations in primary GCT patients and to analyse the associations of marker positivity before and after orchiectomy and correlate it with histological type.

Table 1: Tumor Marker Characterstics

Tumor marker	Size (daltons)	Half-life	Normal range	Tumor type
AFP	70,000	5–7 days	40 ug/I	Embryonal, teratoma, yolk sac
Hcg	38,000	24–36 hours	5 IU/I	Seminoma, embryonal choriocarcinoma,
LDH	134,000	Varies	1.5–3.2 ukat/I	Any

Material and Methods

The study was conducted during the study period of one year and included 26 cases (Reduced Sample size due to covid) of suspected testicular tumor specimen submitted to the Department of Pathology, M.G.M Medical College and M.Y. Hospital, Indore for Histopathological study. The most common specimen received was radical high inguinal orchiectomy specimen wherein the testis is removed with the tunica, epididymis, and a length of spermatic cord via an inguinal approach. [11]

The patients' information was taken from their pathology and hospital records. Pre-operative and post-operative (after5-7days)level of tumour markers-AFP, LDH, β-hCG was taken. After receiving orchiectomy specimen and taking relevant clinical details, grossing of the specimen was done according to standard guidelines and were reported as per CAP protocol

Inclusion Criteria: Suspected cases of testicular tumors.

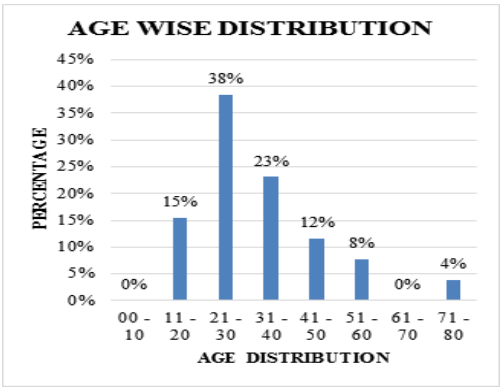
Exclusion Criteria: Para testicular malignancies (consider Soft Tissue protocol)

- Non-testis germ cell tumors (consider Ex-tragonadal Germ Cell protocol)
- Lymphoma and sarcoma.

Observation & Results

Table 2: Age Wise Distribution of Cases

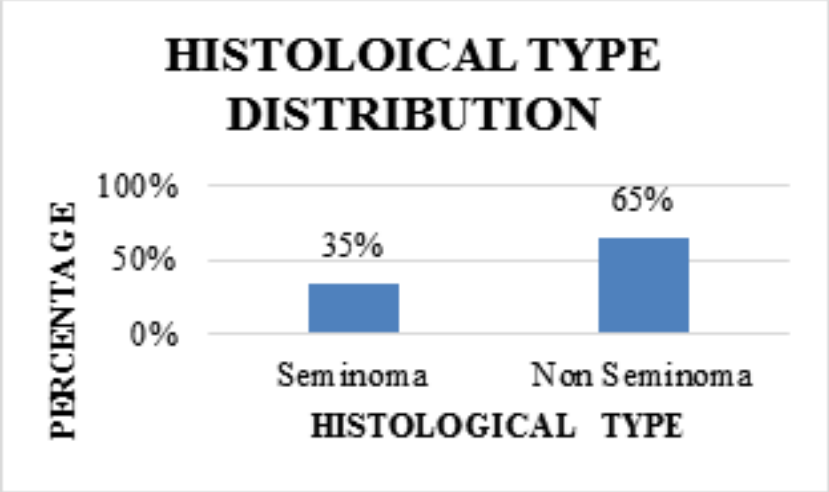
Sr. No.	Age Distribution	Case Distribution	Percentage
1	00 -10	0	0%
2	11 - 20	4	15%
3	21 - 30	10	38%
4	31 - 40	6	23%
5	41 - 50	3	12%
6	51 - 60	2	8%
7	61 - 70	0	0%
8	71 - 80	1	4%
	TOTAL	**26**	**100%**



Maximum number of cases i.e. 10 (38%) were found in the age group of 21 to 30 and second highest number of cases i.e. 6 (23%) were found in the age group of 31 to 40. Thus making 21-40 years as most common age group for testicular cancer.

Table 3: Histological type distribution of cases

Sr. No.	Histological Type	Case Distribution	Percentage
1	Seminoma	9	35%
2	Non Seminoma	17	65%
	TOTAL	**26**	**100%**



Out of total 26 cases, 9 cases (35%) had seminoma, whereas non seminomatous tumor was seen in 17 (65%) cases.

Table 4: Histological subtypes wise distribution of cases

Sr. No.	Histological Types	Case Distribution	Percentage
1	Embroynal Carcinoma	3	12%
2	Yolk Sac Tumor	1	4%
3	Teratoma	1	4%
4	Mixed Germ Cell Tumor (Embryoral, Yolk Sac, Teratoma)	3	12%
5	Mixed Germ Cell Tumor (Embryonal, Teratoma)	4	15%
6	Mixed Germ Cell Tumor (Yolk Sac, Teratoma)	1	4%
7	Mixed Germ Cell Tumor (Seminoma, teratoma)	1	4%
8	Mixed Germ Cell Tumor (Seminoma, Yolk sac)	2	8%
9	Poorly differentiated Mixed GCT	1	4%
10	Seminoma	9	35%
	TOTAL	**26**	**100%**

Out of 26 cases, 09 cases were of seminoma and 17 cases were of non seminomatous germ cell tumor. Among non seminomatous, embryonal carcinoma is most common histological type, in pure form as well as with mixed germ cell tumor followed by teratoma.

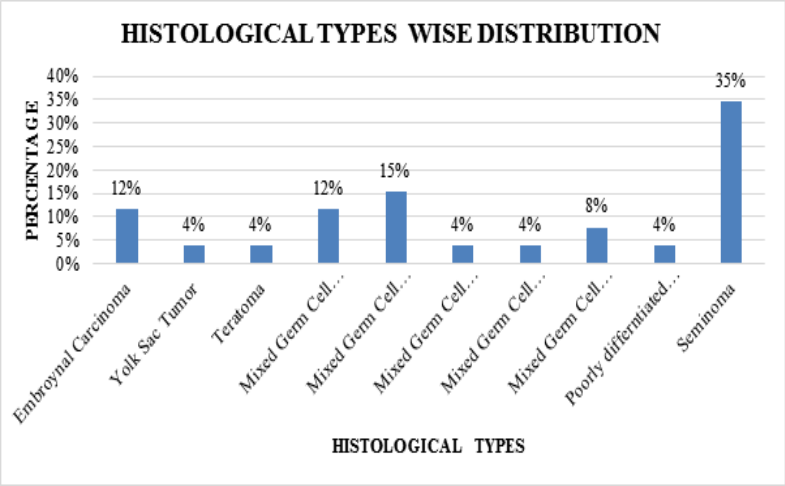


Table 5: Tumor extension wise distribution of cases

Tumor is limited to testis in 65% of cases, whereas it invades to rete testis, epididymis, tunica albugenia and vaginalis and spermatic cord in less than 05% of cases.

Sr. No.	Tumor Extension	No. of Cases	Percentage
1	Invades Epididynis	2	8%
2	Invades Spermatic Cord	1	4%
3	Invades Tunica Albugenia	1	4%
4	Invades Tunica Vaginalis	1	4%
5	Involve Rete Testis Hilar Soft Tissue Epididymis	2	8%
6	Limited To Testis	17	65%
7	Involve Perihilar Soft Tissue Epididymis	1	4%
8	Retro Peritoreum	1	4%
	TOTAL	**26**	**100%**

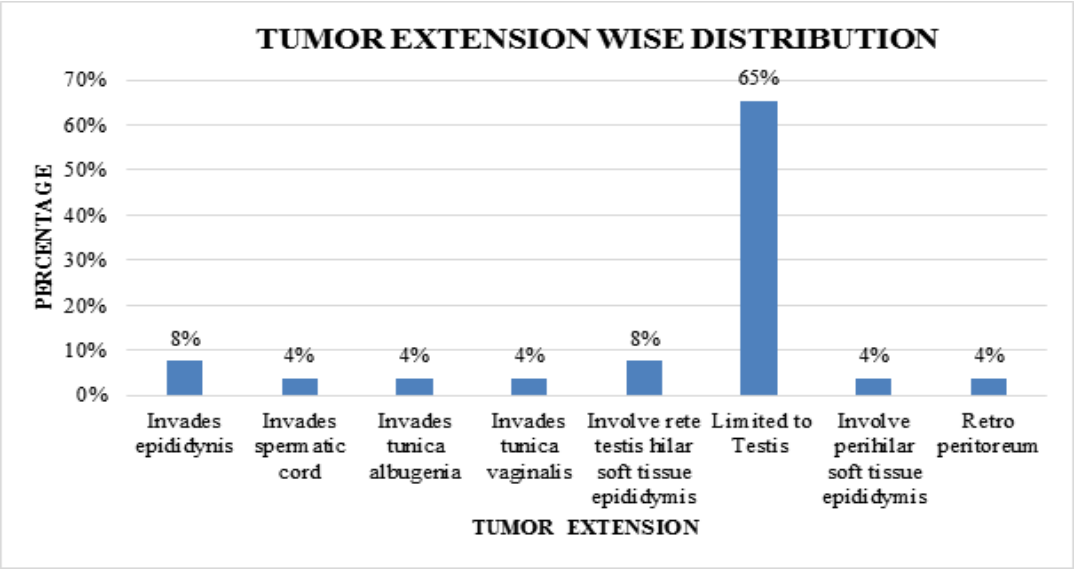
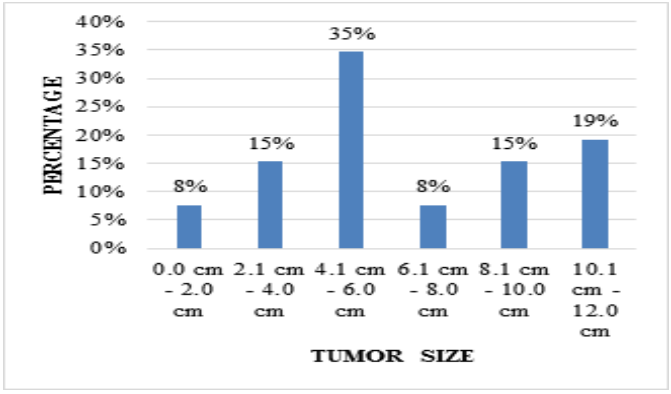


Table 6: Tumor size wise distribution of cases

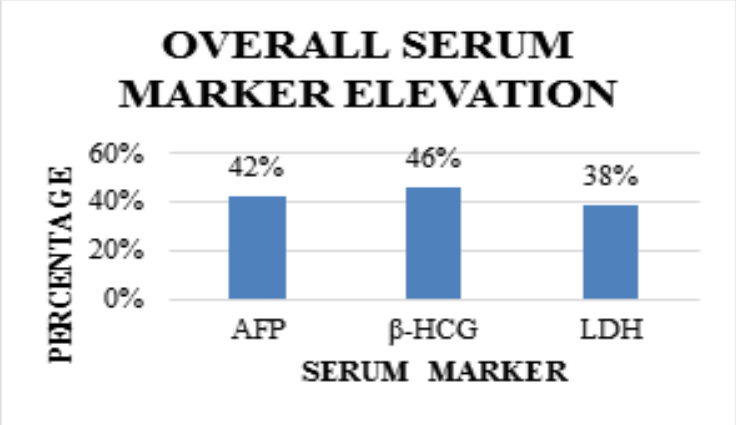
Sr. No.	Tumor Size (in cm)	Case Distribution	Percentage
1	0.0 - 2.0	2	8%
2	2.1 - 4.0	4	15%
3	4.1 - 6.0	9	35%
4	6.1 - 8.0	2	8%
5	8.1 - 10.0	4	15%
6	10.1 - 12.0	5	19%
	TOTAL	**26**	**100%**



Maximum number of cases (09) have median size range of 4.0-6.0 cm, followed by size range of 10.0-12.0cm.

Table 7: Overall serum marker elevation in entire case group

Sr. No.	Serum Marker	No. of elevated cases	Percentage
1	AFP	11	42%
2	β-hCG	12	46%
3	LDH	10	38%



In our study, in the entire group of patients, it was observed that elevations of β-hCG, LDH, and AFP in 46%, 38%, and 42%, respectively.

Table 8: Pre-orchiectomy serum marker elevations wise distribution of cases

Sr. No	Histological types	Total Cases	Pre	AFP	β-hCG	LDH
1	Seminoma	9	**No. of Cases**	0	2	3
			Percentage	0%	22%	33%
2	Non Seminoma	17	**No. of Cases**	11	10	7
			Percentage	65%	59%	41%

Patients affected with seminoma, AFP was not elevated in any case, β-hCG was elevated in 22% cases and LDH was elevated in 33% cases, whereas in patients affected with non seminomatous tumors, AFP was the most frequently elevated marker (65%), followed by β-hCG in(59%) and LDH in 41% cases.

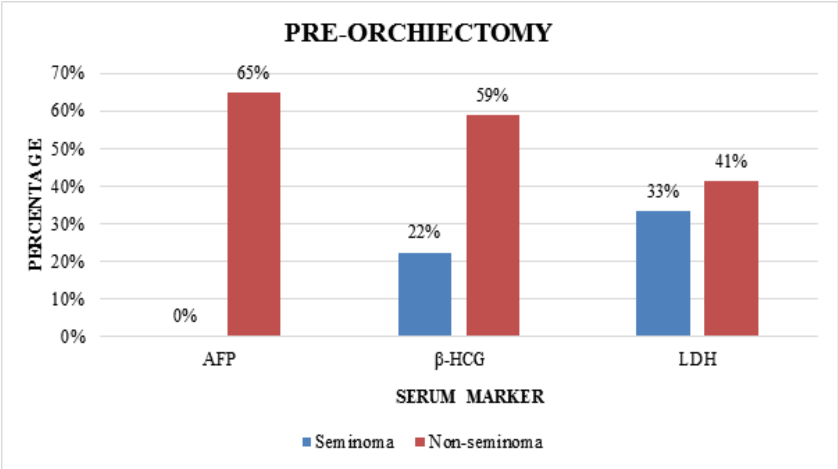


Table 9: Post-orchiectomy serum marker elevations wise distribution of cases

Sr. No	Histological types	Total Cases	Post	AFP	β-hCG	LDH
1	Seminoma	9	**No. of Cases**	0	1	2
			Percentage	0%	11%	22%
2	Non Seminoma	17	**No. of Cases**	8	3	1
			Percentage	47%	18%	6%

Post Orchiectomy in patients affected seminoma, AFP was not elevated in any case, β-hCG was elevated in 11% cases and LDH was elevated in 22% cases, whereas in patients affected with non seminomatous tumors, AFP was the most frequently elevated marker 47%, followed by β-hCG in 18% and LDH in 6% cases.

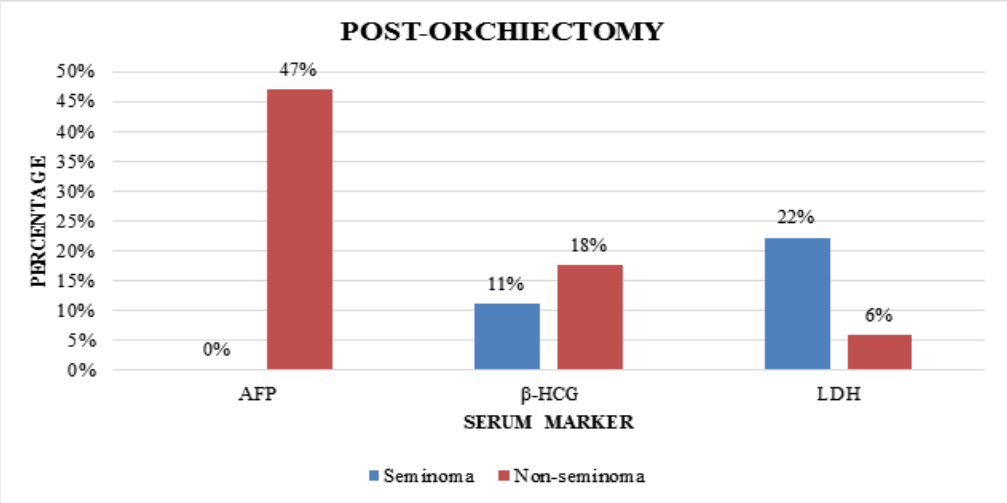


Table 10: Significance of orchiectomy on elevated marker levels in testicular tumor

Sr. No	Pre & Post	Seminoma		Non-Seminoma		
		β-hCG	LDH	AFP	β-hCG	LDH
1	Markers elevated Pre or-chiectomy	2	3	11	10	7
2	No. of Cases with reduced marker levels post or-chiectomy	1	1	3	7	6
3	p-Valve	1.000	1.000	0.250	0.016	0.031
4	Significance	Non Significant	Non Significant	Non Significant	Significant	Significant

It was observed that post orchiectomy the reduction in the marker elevation was insignificant for β-hCG and LDH in Seminoma cases and was insignificant for AFP in Non Seminoma cases. However, significant reduction of level was seen for β-hCG and LDH in Non seminoma cases.

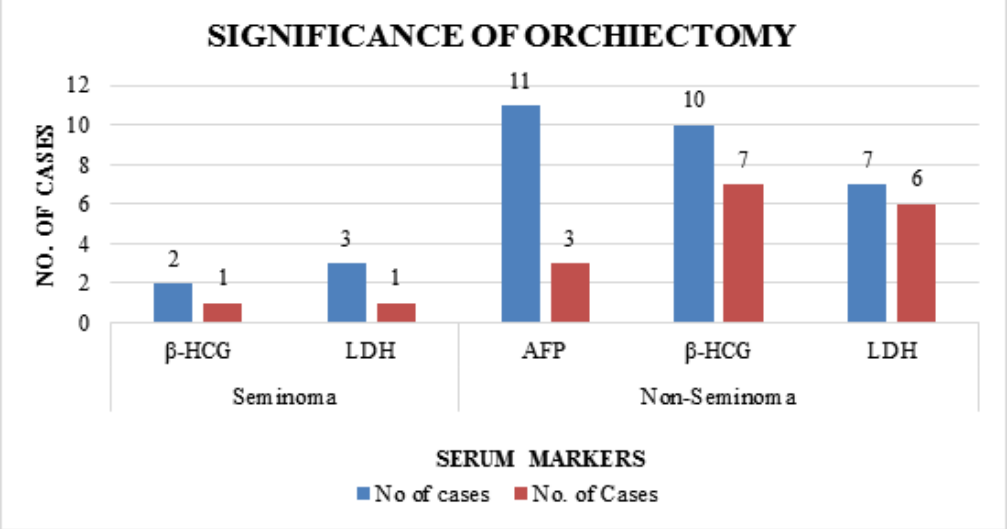


Table 11: Frequencies of elevated marker levels in relation to treatment

Sr. No	Pre & Post	Seminoma			Non Seminoma			
		Total Cases	β-hCG	LDH	Total Cases	AFP	β-hCG	LDH
1	Pre (No. of Cases)	9	2	3	17	11	10	7
	Pre (Percentage)		22%	33%		65%	59%	41%
2	Post (No. of Cases	9	1	1	17	8	3	1
	Post (Percentage)		11%	11%		47%	18%	6%

It was observed that post orchiectomy the level in the marker elevation was still found to be 11% each for β-hCG and LDH in Seminoma cases and in Non seminoma cases post orchiectomy the level in the marker elevation was still found to be 47% for AFP, 18% for β-hCG and 6% for LDH.

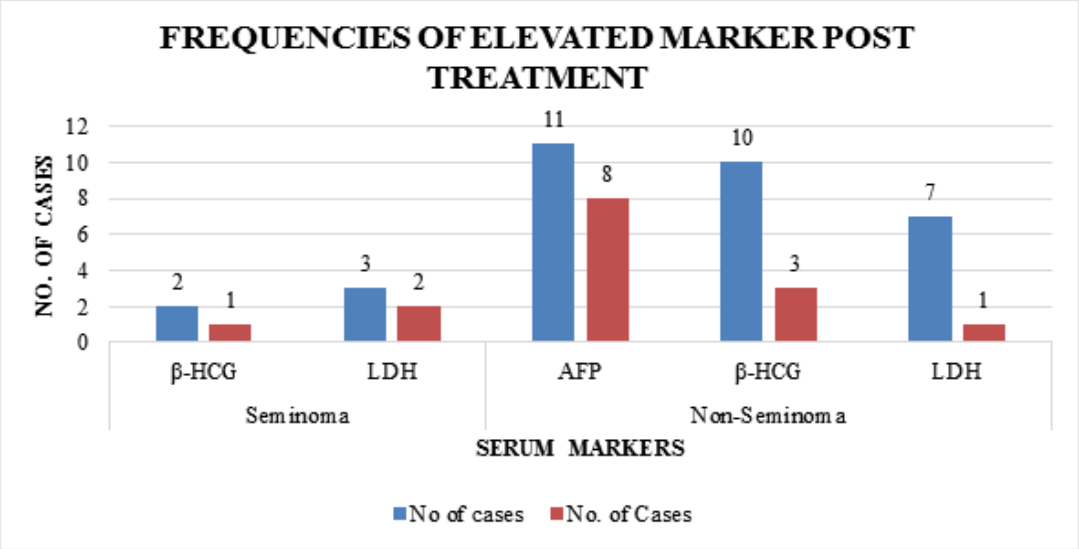
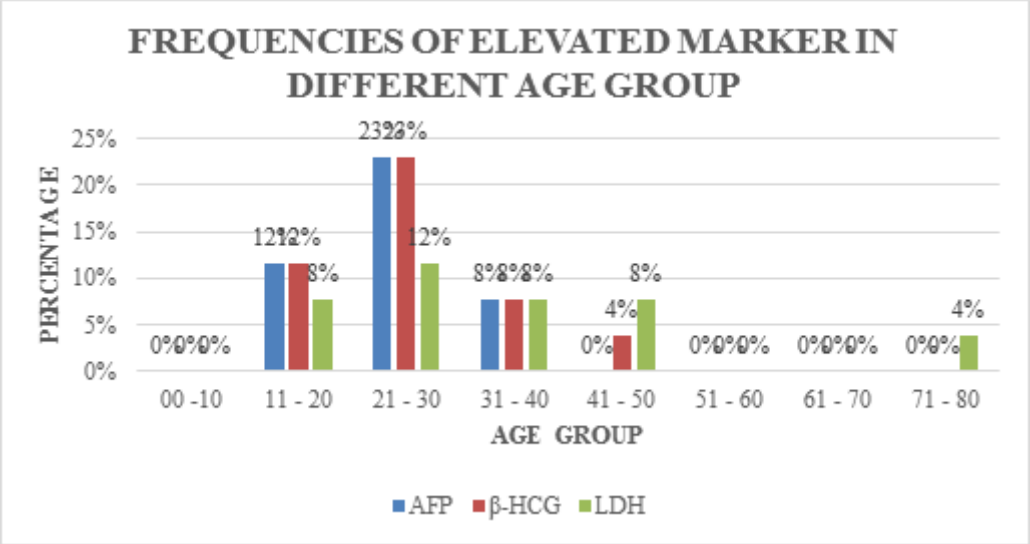


Table 12: Frequencies of elevated marker in different age group

		AFP		β-hCG		LDH	
Sr. No	Age	Case Distribution	Percentage	Case Distribution	Percentage	Case Distribution	Percentage
1	00 -10	0	0%	0	0%	0	0%
2	11 - 20	3	12%	3	12%	2	8%
3	21 - 30	6	23%	6	23%	3	12%
4	31 - 40	2	8%	2	8%	2	8%
5	41 - 50	0	0%	1	4%	2	8%
6	51 - 60	0	0%	0	0%	0	0%
7	61 - 70	0	0%	0	0%	0	0%
8	71 - 80	0	0%	0	0%	1	4%

Marker elevation was higher in younger age group, the elevation rates were lower in age group above 50 years.



Results

1. In the age group of 21 to 30 maximum number of cases i.e. 10 (38%) were found and in the age group of 31 to 40 second highest number of cases i.e. 6 (23%) were found. Thus, making 21-40 years as most common age group for testicular cancer. Jee Soo Park et. al. in their studies on recent global trend on testicular cancer [18] also found that testicular cancer is more common in adolescence young adult group i.e. 15 – 40 years of age.
2. Study analysis shows that right testis is slightly more affected than the left testis. This study finding is similar to finding seen in the publication done by Leslie et. al. [19]
3. Most of the cases in our study were in the pT_1 (62%) stage, suggesting that testicular tumors are diagnosed in early stages. However, there could be a bias due to less no of cases in ou study (due to covid).
4. All 26 cases in our study were Germ Cell Tumor. Venkata S. et. al. in their study also quoted the same. [20] Histologically maximum no of cases were of Mixed Germ Cell Tumor followed by Seminomas.
5. In our study tumor were limited to testis in 65% of the cases and extension to each of epididymis, spematic cord, rete testis, peri hilar soft tissue, retroperitoneum was seen in less than 10% of cases.
6. Most common size range of the tumor was between 4.0cm to 6.0cm.
7. Lymphovascular invasion was seen in only one case.
8. In Patients affected with seminoma, AFP was not elevated in any case, β-hCG was elevated in 22% cases and LDH was elevated in 33% cases, whereas in patients affected with non seminomatous tumors, AFP was the most frequently elevated marker (65%), followed by β-hCG in(59%) and LDH in 41% cases

Gross and Microscopic Images

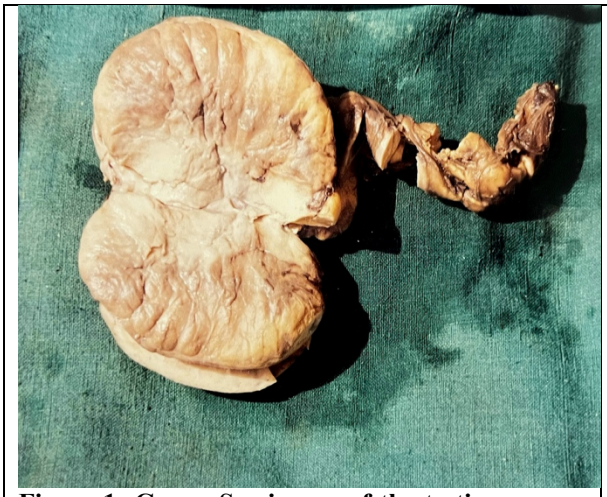


Figure 1: Gross- Seminoma of the testis, appearing as well circumscribed, pale, fleshy, homogenous mass on surface

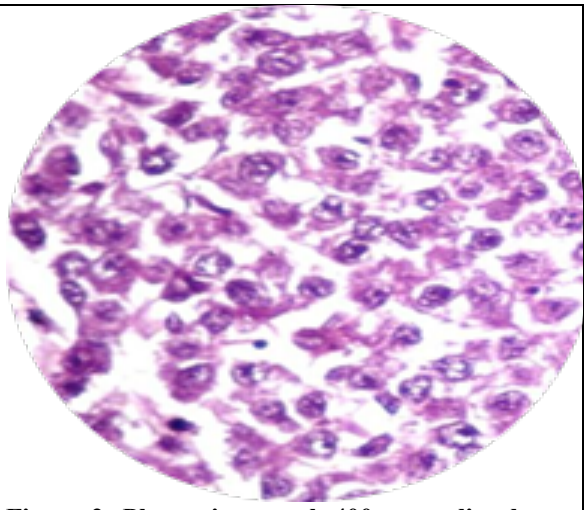


Figure 2: Photomicrograph 400x revealing large cells with distinct cell borders, pale nuclei, prominent nucleoli and abundant cytoplasm

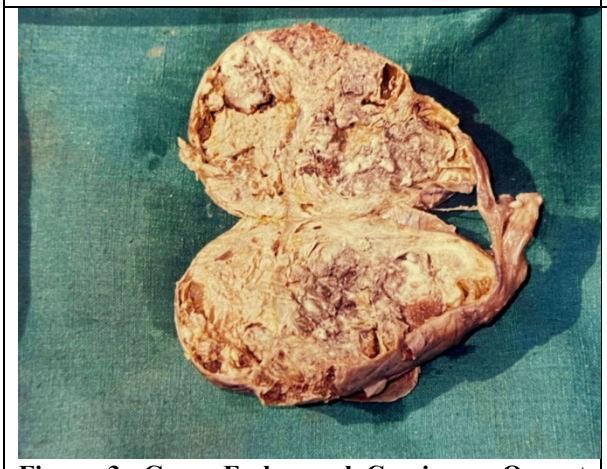


Figure 3: Gross Embryonal Carcinoma-On cut has variegated appearance due to presence of foci necrosi and haemorrhage

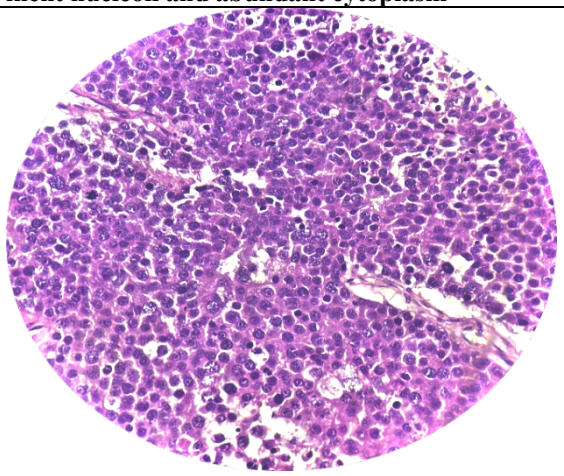


Figure 4: Photomicrograph (400x) Embryonal carcinoma composed of cells with large, hyperchromatic nuclei arranged in sheets and poorly formed glands.

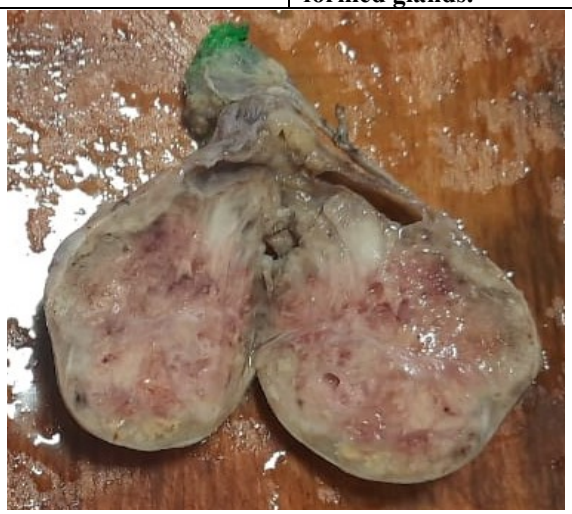


Figure 5: Gross yolk sac tumor. On cut, tumor is solid cystic, grayish white heterogenous in texture with few mucinous areas

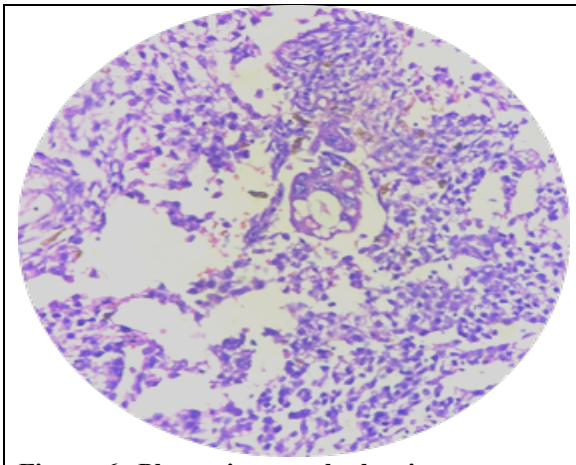


Figure 6: Photomicrograph showing presence of schiller duval body in yolk sac tumor. There is presence of core with a central capillary and visceral and parietal layers of cell containing primitive glomeruli.

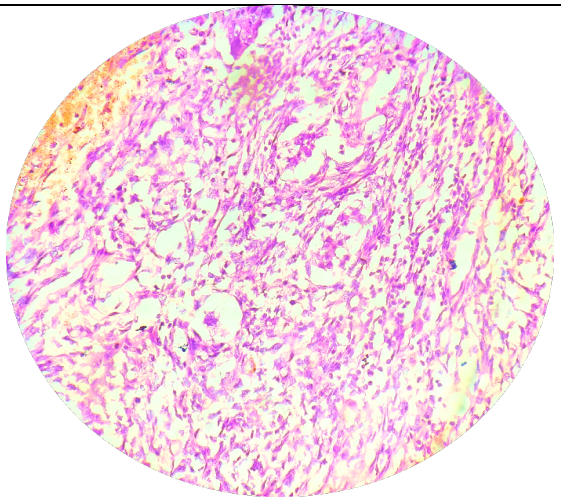


Figure 7: Photomicrograph (400x) showing microcystic pattern (spider web like architecture).

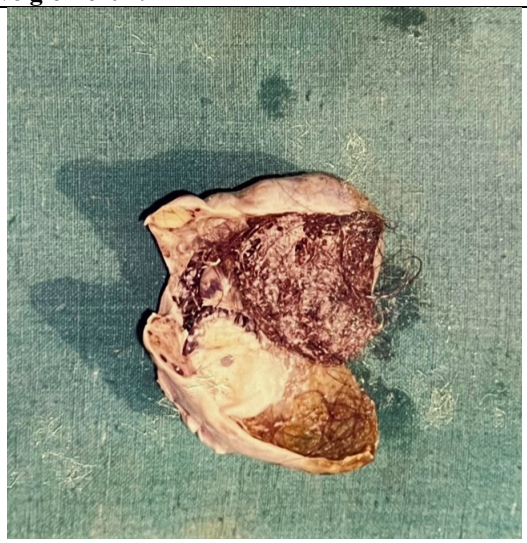


Figure 8: Gross Teratoma of testis. The variegated cut surface with cysts and hairs can be appreciated.

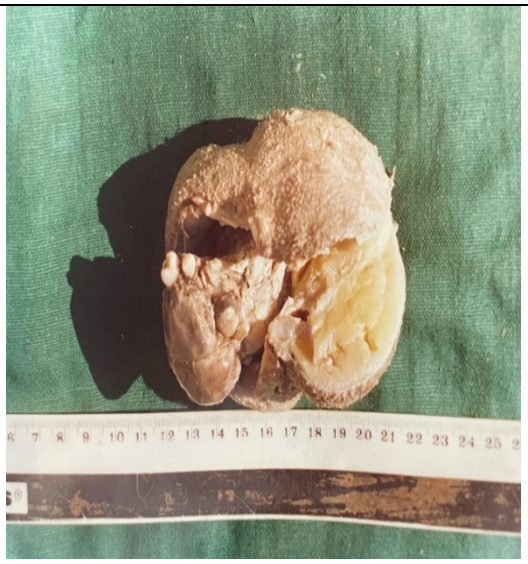


Figure 9: Gross Mixed Germ Cell tumor showing presence of mature tooth and fat along with homogenous bulging mass.

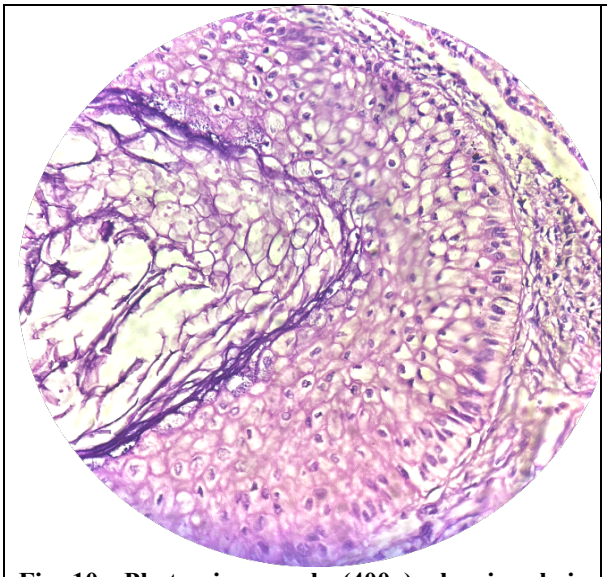


Fig 10: Photomicrograph (400x) showing hair follicle differentiation.

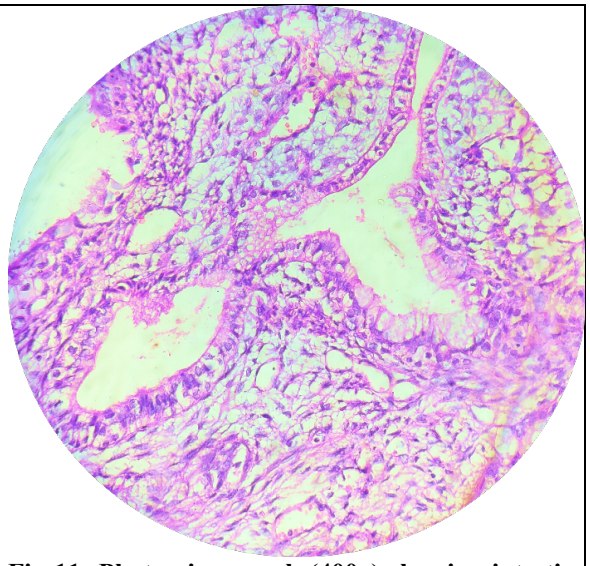


Fig 11: Photomicrograph (400x) showing intestinal gland (containing mucin) differentiation.

Discussion

This study analyses serum tumor marker elevation rates before and after orchiectomy and discusses their role in reporting of testicular tumor according to CAP guidelines.

Rate of Marker Elevation in Entire Groups: In our study , in the entire group of patients, it was observed that elevations of β-hCG, LDH, and AFP in 46%, 38%, and 42%, respectively. The results in our study are slightly higher to German study by Klaus – Peter Dieckman et al, where elevations of β-hCG, LDH, and AFP in 37.9%, 32.9%, and 25.6%, respectively. This difference most probably relates to the higher proportion of seminomas in that series.

Rate of Marker Elevation Different Histologic Groups: In seminoma, we found AFP was not elevated in any case, β-hCG elevations was in 22% of cases. [21-25].

Table 21: Tumor marker elevation rates in seminoma

First Author	Year	n	B-hCG (%)	LDH (%)
Germa-Lluch [22]	2002	852	21	
Neumann [23]	2011	73	18.8	
Sanchis Bonet [25]	2011	72	29.3	
Rothermundt [26]	2018	107	18.8	20.3
Dieckmann [24]	2019	187	31	29.9

The LDH elevation rate of 33% in our patients accords with the rate of 34% reported by Weissbach [13] but a Norwegian study reported a higher rate of 46% [12]. Overall, patients with seminoma have significantly lower elevation rates of all tumour markers than patients with nonseminoma.

Accordingly, the median serum level of β-hCG was significantly lower in seminoma than in nonseminoma because many of the seminoma patients have only slightly elevated serum levels of this marker. The data accumulated here suggest that about 40% of seminoma patients have elevated levels of β-hCG or LDH. Thus, these markers are helpful only for a minority of patients and from a clinical point of view a more sensitive marker would be desirable.

Regarding tumour marker elevation rates in nonseminomas, AFP had the highest rate with 65% of our cases. β-hCG and LDH were elevated in 59% and 41%, respectively. Our data are also in line with the results of a large Spanish study where AFP was found to be the most prevalent marker in nonseminoma with 70% while β-hCG was elevated in 53% [22].

There is a marked paucity of systematic investigations of tumour markers in GCT reported in this century [14]. Table 25 summarizes 5 studies of reasonable size that provide data on marker measurements in nonseminoma patients [22-24, 26].

Table 22: Tumour marker elevation rates in nonseminoma patients

First Author	Year	n	AFP (%)	β-hCG (%)
Germa-Lluch [22]	2002	852	70	52.9
Neumann [123]	2011	73	66.7	
Rothermundt [26]	2018	107	55.2	55.1
Dieckmann [24]	2019	187	59.7	63.6

In our study we found that higher rates of marker elevation was seen in younger age group for AFP and β-hCG. Similar finding of inverse association of elevation rates of AFP and β-hCG with age in GCT patients have higher rates of elevated tumour markers than the older ones in Klaus-Peter Dieckmann et. al [27] studies. This result contrasts with the reported higher rates of β-hCG elevations in the older age groups of the healthy male population [28]. LDH is not associated with age.

Marker Elevation Rates in Response to Treatment: A premier role of serum tumor markers is to monitor the course of clinical management and to early herald success or treatment failure [29-32]. Accordingly, marker decline indicating response to therapy has been documented in the very early reports after the upcoming of the three markers [33-36]. In accordance with these reports, we observed significant decreases of elevation rates of markers after orchiectomy in non seminomatous patients.

In Seminomas the rate of declination of both β-hCG and LDH was not significant in our study. Thus, suggesting that post orchiectomy marker level is not much needed. However marker level may fall after cycles of chemotherapy.

In Non Seminomatous tumor the rate of declination of AFP was not significant in our study, whereas the rate of declination of both β-hCG and LDH was significant (p valve < 0.05). Thus, suggesting that post orchiectomy marker level is not much needed. However marker level may fall after cycles of chemotherapy.

In accordance with these reports, the result of studies conducted by Klaus-Peter Dieckmann et. al also showed significant decreases of elevation rates of all three markers after orchiectomy in less than 50% patients regarding β-hCG and LDH. Notably, the AFP elevation rate did not drop to that extent. [27].

Limitation of Present Study: Some of our findings of our study relate to a possible bias because of low sample size (due to covid).

Advantage of Present Study: Our study helped us in understanding the correct method of handling and grossing orchiectomy specimen. It also helped us understanding the role of serum marker pre operative and post operative testicular tumor.

Conclusion

Being well versed with recent grossing guidelines is very crucial for pathologists, so as to make correct diagnosis for proper treatment. AFP, LDH and β-hCG are valuable tools for the diagnosis, clinical management and follow up of GCTs.

A major shortcoming of these markers is the low frequency of elevated serum levels in less than 50% in the entire group of patients.

The frequencies of elevated marker levels rates are associated with histology and other clinical factors such as younger age. Serum levels of AFP, LDH and β-hCG

decrease in response to therapy but also continues to be expressed in around some patients after orchiectomy.

Current guidelines recommend the measurement of the three serum markers discussed herein as one cornerstone of the clinical management of testicular cancer [15,16]. However, new epigenetic markers particularly serum levels of microRNA-371a-3p outperform the classical markers by far with a sensitivity of 90.1% and a specificity of 94.0% [24, 17]. Clearly, the traditional markers of GCT are currently indispensable for the clinical management of GCT despite their limitations.

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