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Mediastinal Tumors: A Clinicopathological Study with Special Reference to Immunohistochemistry

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Abstract:
Introduction: *Mediastinum is located in the central part of the chest cavity splitting into the superior, anterior, middle and posterior compartments. They represent a wide diversity of disease states. So, the location and composition of a mass is significant in confining the differential diagnosis.*
Aims: *To study the frequency, location and distribution of various pathologically diagnosed masses and correlate their clinical, histological and immunohistochemical (IHC) findings.*

Settings and Design: *This was a cross-sectional observational study conducted for two years at a tertiary care hospital with a sample size of 40.*
Materials and methods*: Detailed history taking, clinical examination and investigations were carried out. The tumor masses were excised and were sent for histopathological reporting. Immunohistochemistry was also performed.*

Statistical analysis used: *Microsoft excel 2016 and SPSS 18 (SPSS, Inc., Chicago, IL, USA).*
Results: *The present study includes total 40 cases with male: female ratio 1.22:1. Most common age group of mediastinal disease was 31-50 years (Mean: 32.2). Anterior mediastinum was the most common site (35.00%). The most common nature of the disease was benign 28 cases (70 %) followed by malignant 12 cases (30%).*
Conclusions: *Mediastinum is a small narrow limited area in central region of thoracic cavity but it houses a broad array of lesions. Acquaintance regarding the lesions and the topographical distribution helps in the diagnosis of the case. Clinical and imaging features both are very much essential along with histopathology and immunohistochemistry to confirm the diagnosis.*
Keywords:*Mediastinum, benign, malignant, histopathology, immunohistochemistry*

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I. Introduction

Mediastinum is located in the central part of the chest cavity splitting into the superior, anterior, middle and posterior compartments. Every compartment has its own structures; so, the masses originating from these structures are different and each cyst or tumor has a tendency for a specific compartment. Mediastinal primary masses as well as tumors and metastatic tumors arise in all four parts of the mediastinum and include benign to malignant lesions. They represent a wide diversity of disease states.[1]So, the location and composition of a mass is significant in confining the differential diagnosis.

The aim was to studythe frequency, location and distribution of various pathologically diagnosedmasses and correlate their clinical, histological and immunohistochemical (IHC) findings.

II. Materials and methods

This was a cross-sectional observational study conducted for two years from January 2018 to January 2020ata tertiary care hospital with a sample size of 40. All biopsy based diagnosed cases of mediastinal mass in

pre-treatment phase at the Cardio-Thoracic-Vascular Surgery (CTVS) clinic were included. Patients solely diagnosed on the basis of radiology or cytology, with serious illness and unwillingness for the biopsy or surgery were excluded. Written informed consent was obtained from all patients. Approval of the study was taken from the Institutional Ethics Committee.

Detailed history taking and clinical examination were carried out with a predesigned proforma. X-Ray chest and CT scan reports or MRI were studied in all cases followed by excision of the tumor masses and the further treatment plan. The excised specimen was sent for gross examination and the histopathological reporting. For each case paraffin blocks were available to perform immunohistochemistry. S100 (Monoclonal Mouse antibody, clone: 15E2E2, positive control: Melanoma, cytoplasmic staining), Oct-3/4 (Monoclonal Mouse Anti-Human antibody, clone: SEMGC, positive control: Known case of seminoma, nuclear staining), LCA (Monoclonal Mouse Anti-Human antibody, clone: PD 7/26 &2B11, positive control: tonsil, membrane staining), CD99 [Monoclonal Mouse Antibody; clone:12E7, positive control: esophagus, membranous staining] , vimentin [Monoclonal Mouse Antibody; clone: V9, positive control: tonsil, cytoplasmic staining] were used for the confirmation of the diagnosis.

III. Statistical analysis

Statistical software was used to analyze data. Demographic, clinical,laboratory data for each patient was recorded in statistical forms. Microsoft excel 2016 and SPSS 18(SPSS, Inc., Chicago, IL, USA), was used. Quantitative datawas presented as mean ± standard deviation (SD) for normally distributed data, including the standard error of the mean (SEM) or as median and inter-quartile range for non-normally distributed data.Comparisonsbetween two groups were performed using an independent samplet-test. For categorical/nonparametric variables, Chi square test and Kruskal–Wallis test was performed. A P-value <0.05 was considered indicative ofstatistically significant differences. Correlation was calculated using appropriatetest of association and regression analysis.

IV. Results

The present study includes total 40 cases. There were 22 (55%) male and 18(45%) female patients. Thus male: female ratio was 1.22:1. Mean age of presentation was 32.2 years. Most common age group of mediastinal disease was 31-50 years (Table 1).Amongmale population mean age was 25.85 years with SEM 3.97 and SD 14.86.Mean age of female population was 33.72 years with SEM 3.58 and SD 15.18.

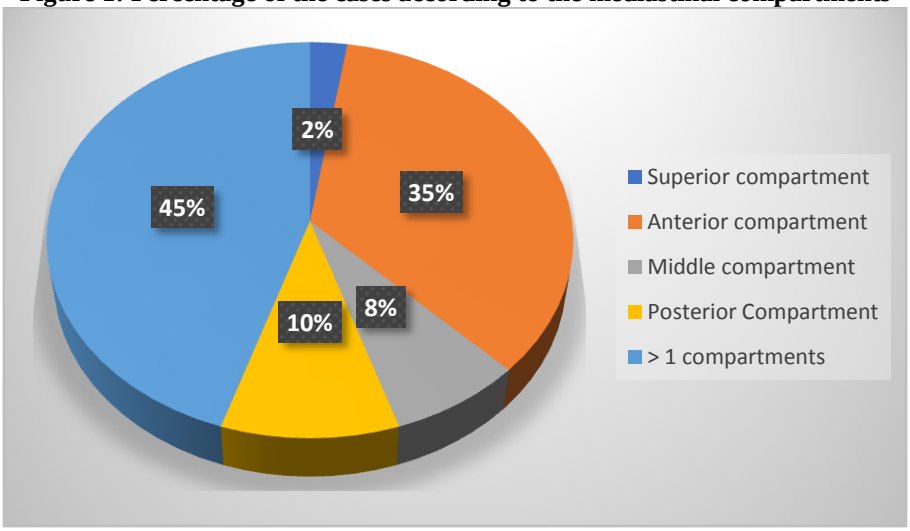
Table 1: Age and sex distribution of mediastinal lesions (N=40)

Age group	**Male**	**Female**	**Total**	**Percentage (%)**
1- 10yrs	4	3	7	17.5
11yrs – 20yrs	2	0	2	5.0
21yrs – 30yrs	4	4	8	20.0
31yrs – 40yrs	4	7	11	27.5
41yrs – 50yrs	6	2	8	20.0
51yrs – 60yrs	1	1	2	5.0
61yrs and above	1	1	2	5.0
Total	22	18	40	100
Percentage (%)	55	45		

Shortness of breath (80% of total patients) was the most common symptom followed by chest pain (60% of total patients). Cough and dysphagia were also very common among the patients (47.5% of total patients each). In majority of cases presence of multiple symptoms were seen. All the symptoms,in the study population were mostly seen in cases where multiple compartment of the mediastinum was involved. Anterior mediastinal lesions showed most of the symptoms independently.

More than one mediastinum was most commonly (18 cases out of total 40 cases) involved comprising 45% of total cases. As single site involvement, anterior mediastinum was the most common site (35%) (Figure 1).Onlysuperior compartment involvement is less common, but it is more commonlyinvolved along with anterior compartment.

Figure 1: Percentage of the cases according to the mediastinal compartments



Among 28 patients (70%) was benign which was the most common nature of the disease in this study.12 patients (30%) were of malignant nature. In 21-50 years, age group 27 cases were involved, among them 23 cases were benign (85.2%) and 4 cases were malignant (14.8%). On the other hand, malignant lesion was seen mostly in extreme age groups. 4 cases (out of 7 cases) are malignant in 1-10 years age group (57.1%). And 3 cases (out of 4 cases) were seen malignant above 50 years (75%).

Here, 20-50 years of age group were more affected from mediastinal lesion.11 patients (out of 40 cases) are in 30-40 yrs of age group whichwere most commonly affected from thymic tumors. Soft tissue tumors were alsoaffected in most common prevalent age group of mediastinal diseases.Lymphoproliferativedisease was equally distributed in all age group except 1-10 years of age group. Germ cell tumors affected the younger age group mostly.3cases (out of 8 cases) were present in pediatric age group(1-10 years).

Soft tissue tumor was the most common etiologycomprising 22.5% of total cases (Table 2). Among them almost all cases(8 cases, out of 9cases) were benign in nature except one case of synovial sarcoma. Germ cell tumor and neurogenic tumor [schwannoma (Figure 2A, 2B), neurofibroma (Figure 2D), ganglioneuroma (Figure 2F)]jointly took the second most common position(20%) in the etiological diagnosis of the study. Out of eight germ cell tumors,5cases (62.5%) were benign mature cystic teratoma of thymus (Figure3A, 3B), 2 cases(37.50%)ofmixed malignant germ cell tumor (Figure 3C, 3D) and one yolk sac tumor (Figure 3E) were there.

Table 2: Etiological Diagnosis of the patients in the study (N=40)

Diseases	**Number of cases**	**Percentage (%)**
Thymic tumors	06	15.0
Thymic neuroendocrine tumors	03	7.5
Germ cell tumors	08	20.0
Neurogenic tumors	08	20.0
Lymphoproliferative diseases	05	12.5
Soft tissue tumors	09	22.5
Others	01	2.5

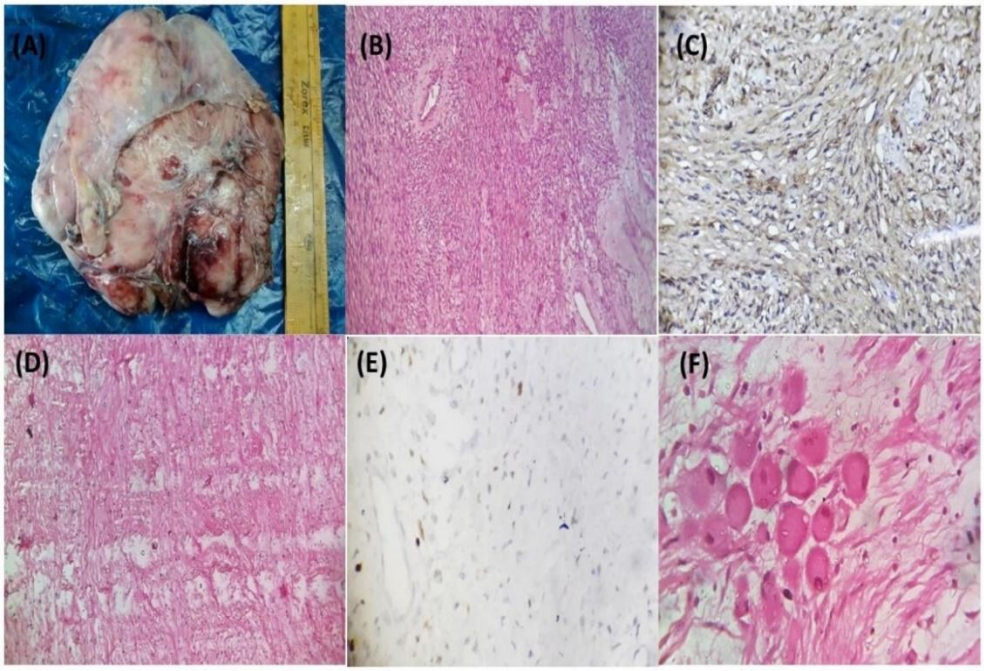


Figure 2: Schwannoma (A) Gross specimen (B) Section shows more cellular areas (Antoni A) and less cellular edematous stroma (Antoni B) (x100, H&E) (C) IHC staining for S100 showing positivity (x400). Neurofibroma (D) Section shows collagen fibrils with wavy nuclei and serpentine ends (x100, H&E) (E) IHC staining for S100 showing positivity (x400). Ganglioneuroma (F) Section shows ganglion cells with eosinophilic cytoplasm and eccentric nucleus along with schwann cells (x400, H&E)

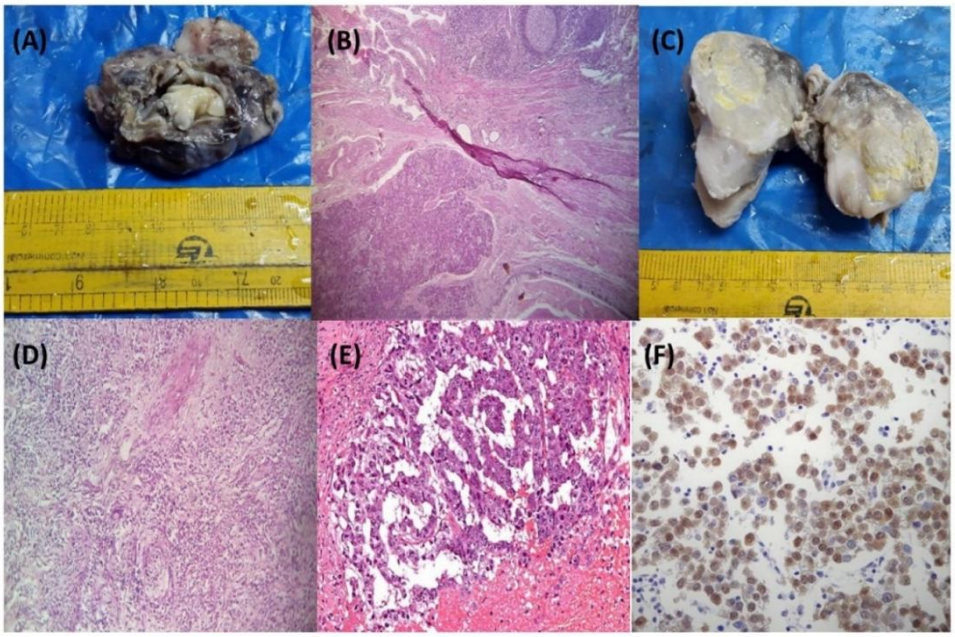


Figure 3: Mature Cystic Teratoma (A) Gross specimen (B) Section shows pancreatic tissue (x100, H&E). Malignant Mixed Germ Cell Tumour (C) Gross specimen (D) Section shows the histology of dysgerminoma and yolk sac tumour (x100, H&E) (E) Section shows the histology of yolk sac tumour (x100, H&E) (F) IHC staining for Oct-3/4 showing positivity (x400)

In neurogenic tumor, all were benign in nature except 3 cases which were malignant primitive neuroectodermal tumor (PNET) (Figure 4A, 4B) in pediatric age group (10-13yrs). Thymic tumor was seen in 15% of total cases. Among them most tumors were benign thymoma (Figure 4C, 4D) of different WHO variant and only one case (out of six cases) was thymic carcinoma (Figure 4E). Six cases of thymic neuroendocrine tumors (Figure 4F). Five cases (12.5%) were lymphoproliferative disease. 2 cases were Hodgkin's tumor

(Nodular sclerosis type)(Figure 5A) and each one of, diffuse large B celllymphoma (Figure 5C), Castleman disease (Figure 6A) and small lymphocytic lymphoma. Others included one case of parathyroid adenoma (Figure 6B) and one case solitary fibrous tumor (Figure 6C, 6D).

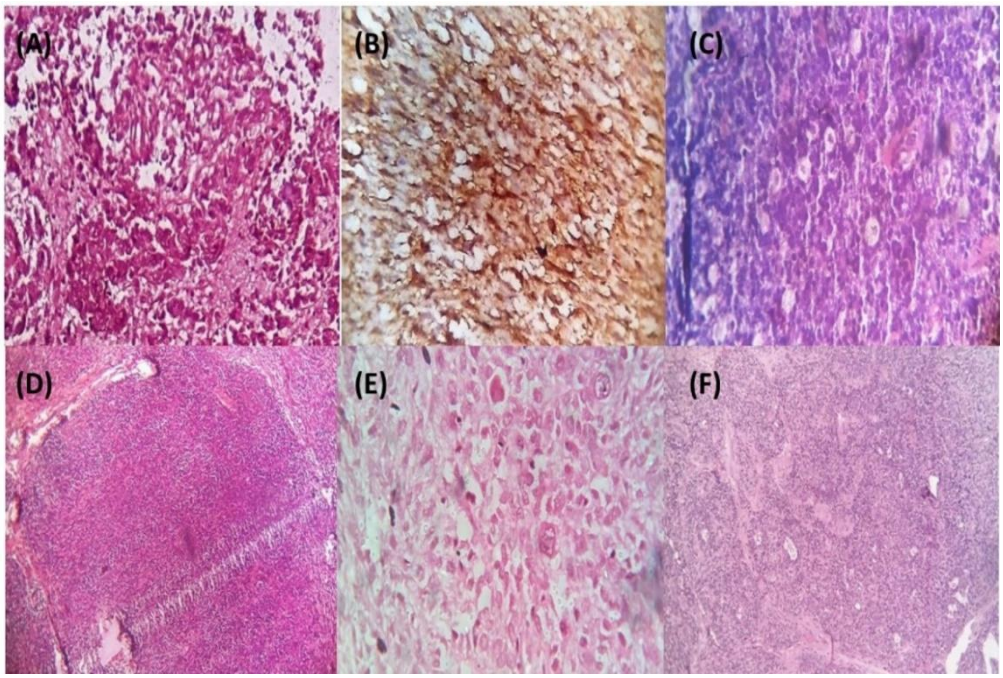


Figure 4: PNET (A) Section shows sheets of small round cells (x400, H&E) (B) IHC staining for CD99 showing positivity (x400). Thymoma A (C) Section shows bland spindle cells (x400, H&E). Thymoma B1 (D) Section shows medullary islands (x100, H&E). Thymic Carcinoma (E) Section shows cohesive growth with oval nuclear outlines and eosinophilic nucleoli (x400, H&E). Neuroendocrine tumour (F) Section shows trabecular pattern with small to medium cells and finely granular cytoplasm (x400, H&E).

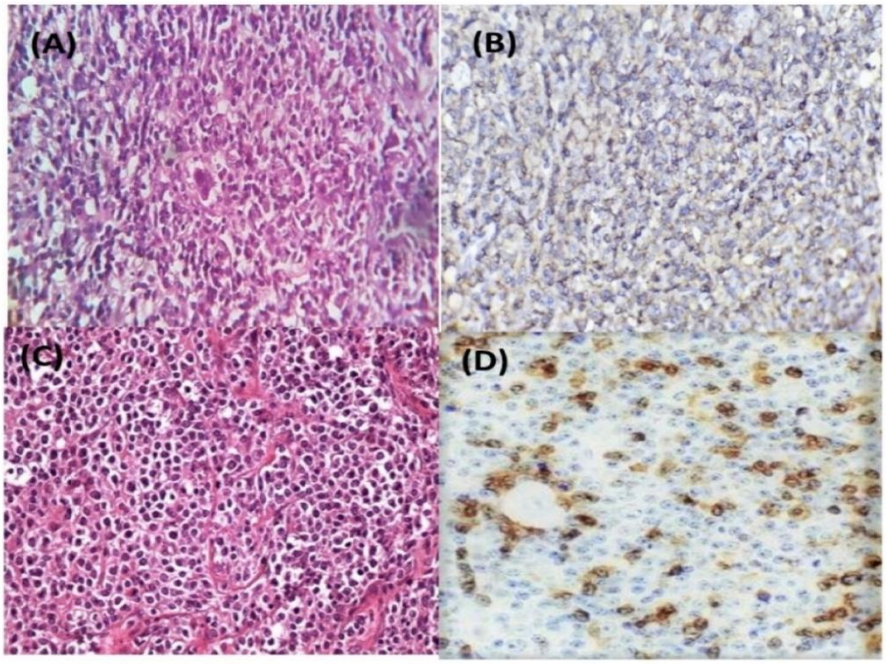


Figure 5: Hodgkin Lymphoma (A) Section shows Reed Sternberg (RS) cell (x400, H&E) (B) IHC staining for LCA (CD45) showing positivity (x400) Diffuse large B-cell Lymphoma (C) Section shows diffuse growth pattern with large cells (5x normal lymphocytes) (x400, H&E). Small Lymphocytic Lymphoma (D) IHC staining for LCA (CD45) showing positivity (x400)

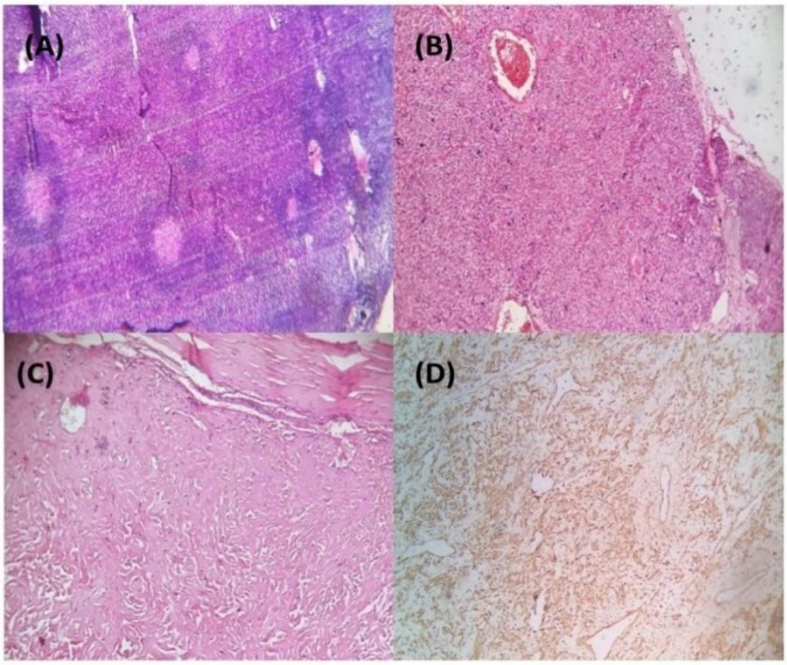


Figure 6: Castleman disease (A) Section shows multiple germinal centre (x100, H&E) Parathyroid adenoma (B) Section shows chief cells with some oxyphil cells in delicate capillary network (x100, H&E). Solitary fibrous tumor (C) Section shows hypocellular and hypercellular areas separated by hyalinized collagen with cracking artifact and staghorn vessels (x100, H&E) (D) IHC staining for vimentin showing positivity (x400).

The immunohistochemical findings are listed in the table (Table 3).

Table 3: **Immunohistochemical study of the selected cases**

Tumour	**Oct-3/4**	**LCA**	**S100**
Neurofibroma	NA*	NA	Positive
Malignant mixed germ cell tumour	Positive	NA	NA
Schwannoma	NA	NA	Positive
Schwannoma	NA	NA	Positive
Neurofibroma	NA	NA	Positive
Small lymphocytic lymphoma	NA	Positive	NA
Hodgkin lymphoma	NA	Positive	NA
Malignant mixed germ cell tumour	Positive	NA	NA
Diffuse large B cell lymphoma	NA	Positive	NA
Ganglioneuroma	NA	NA	Negative
Hodgkin lymphoma	NA	Positive	NA
Yolk sac tumour	Negative	NA	NA

*NA- Not Applicable

V. Discussion

The mediastinum is the portion of the thoracic cavity located between the pleural cavities, extending antero-posteriorly from the sternum to the spine and sagittally from the thoracic inlet to the diaphragm. Etiology and clinical spectrum of mediastinal diseases are very wide. Clinical presentation may vary depending upon size and location of mediastinal disease. The most common causes of a superior mediastinal mass include thymoma and thymic cyst, malignant lymphoma, thyroid lesions, parathyroid adenoma; anterior mediastinal mass includes thymoma and thymic cyst, germ cell tumour, thyroid and parathyroid lesion, lymphoma, paraganglioma, hemangioma, lipoma. Masses of the middle mediastinum are typically congenital cysts, including foregut andpericardial cysts, while those in the posterior mediastinum areneurogenic tumours and gastroenteric cyst.[2]
In the present study among 40 patients, 22 (55%) male and 18(45%) female patients. Male: female ratio was 1.22:1. Most common age group of mediastinal disease was 31-50 years.Thiswas compared to other studies (Table 4).

Table 4: **Age and sex incidences from different study as compared to present study**

Name of the study	**Mean age (years)**	**Male: Female ratio**
Karki S, *et al.* [3]	35.5	1: 1.1

Bagheri R, *et al*. [4]	35.4	1: 1.1
Dasgupta S, *et al*. [5]	18.5	2:1
Bekele A, *et al*. [6]	35.9	2:1
Aroor AR, *et al*. [7]	45.4	2.2:1
Al-Khalifa M, *et al*. [8]	48.1	1.3:1
Wani AM, *et al*. [9]	35.4	1.3:1
Present study	32.2	1.2:1

Present study revealed different clinical symptoms of mediastinal diseases. In majority multiple symptoms were present in each case. Among them shortness of breath (80%),chestpain(60%),cough(47.5%)anddysphagia(47.5%) were the leading symptoms. Certain clinical features were prevalent in certain topographical position. After studying topographical distribution of symptoms, it was revealed that multiple compartment involvement causes majority of symptoms. Anterior mediastinallesions showed most of the symptoms independently.Percentageof various clinical symptoms in different studies including present study are compared (Table 5).

Table 5:Presenting features of mediastinal lesions in various studies

Symptoms	Dasgupta S, *et al*. [5]	Aroor AR, *et al*. [7]	Wani AM, *et al*. [9]	Baram A, *et al*. [10]	Vaziri M, *et al*. [11]	Present study
Dyspnea (%)	100	45.71	-	53.8	41	80
Chest pain (%)	100	-	31.63	42.8	30	60
Cough (%)	22.7	57.14	55.1	34	40	47.5

By means of imaging methods topographical distribution of mediastinal diseases were assessed. In this study, mediastinum was separated into 4 compartments-superior, anterior, middle and posterior. More than one mediastinum was most commonly (18 cases out of 40 cases) involved, comprising 45% of total cases. As a single site involvement, anterior mediastinum was the most common site (35%).This was coherent with other studies.[5,7-12]

Present study revealed that,all lymphomas were seen in relation to anteriormediastinum either alone in anterior compartment or in combination withsuperior and middle mediastinum. All mature cystic teratoma was seen in anterior compartment.Allthymic tumors were presented in anterosuperiorcompartment.Most of neurogenic tumors were seen in posterior compartment.In this study, thymic tumors were the most common lesioninanterosuperior mediastinum and this was similar to other studies.[5,9,13-15]Majority of germ celltumors were seen in anterior or anterosuperior compartment and so as in other studies.[7,16,17] Lymphomas was seen in 12.5% which was comparable to Wani AM, *et al*. [9] and Shrivastava PC, *et al*. [13] but Aroor AR, *et al*.[7], Vaziri M, *et al*. [11]and Adegboye VO, *et al*. [17] have found a higherincidence of lymphomas than thymomas.

Thymomas are the most common neoplasm of the anterior mediastinum with an incidence of 0.15 cases per 100,000. Although rare in children, thymomasrepresent 20% of anterior mediastinal neoplasms in adults.[18]Thymomas as a group have a wide spectrum of histologic diversity and are classified based on cell type predominance as lymphocytic, epithelial, or spindle cell variants. There is a strong association between histologic subtype and invasiveness as well as prognosis. As a result, the World Health Organization[19] devised a new classification system consisting of type A, AB, B1, B2, B3, C. Myasthenia gravis is most frequent in women and is associated with thymoma. Symptoms include diplopia, ptosis, dysphagia, weakness, and fatigue. 30% to 50% of patients with thymomas have myasthenia gravis, compared to 10% to 15% of patients with myasthenia gravis who have a thymoma.[20]

Thymic carcinomas are a heterogeneous group of aggressive, invasive epithelial malignancies.[21]Their incidence is rare, occurring predominantly in middle-aged men. Histologically, thymic carcinomas are large, firm, infiltrating masses with areas of cystic change and necrosis. They are classified as low grade or high grade, with squamous cell-like and lymphoepithelioma-like variants being the most common cell types.Thymic carcinoid is a malignant tumor, which is histologically similar tocarcinoid tumors found at other sites. Its highest incidence is in the fourth andfifth decades of life. Thymolipoma is a rare, benign, slowly growing tumor of the thymus gland that occurs in young adults of both sexes.[22]CT scans and MRI studies show a characteristic fat density.

Germ cell tumors are derived from primitive germ cells that fail to migrate completely during early embryonic development. They are found in young adults and represent 15% of anterior mediastinal masses found in adults.[23]Malignant Germ cell tumors are more common (> 90%) in men. Germ cell tumors are classified into the following three groups based on cell type: benign teratomas; seminomas; and embryonal tumours.

The mediastinum is the most common location at which an ectopic parathyroid tumor may develop. Overall, 20% of parathyroid adenomas develop in the mediastinum, with 80% occurring in the anterior

mediastinum.[13]Primary mediastinal lymphoma is a rare entity comprising only 10% of lymphomas in the mediastinum. Hodgkin lymphoma representsapproximately 50% to 70% of mediastinal lymphomas, while non-Hodgkinlymphoma comprises 15 to 25%. The three most common types of mediastinallymphoma include nodular sclerosing Hodgkin lymphoma, large B-cell lymphoma, andlymphoblastic lymphoma.[13]Microscopically the presence of Reed-Sternberg cell is pathognomonic of Hodgkin lymphoma. These cells contain bilobed nuclei containing prominent eosinophilic nuclei.

Neurogenic tumours are derived from tissue of the neural crest, including cells of the peripheral, autonomic, and paraganglionic nervous systems. They are classified on the basis of cell type and comprise approximately 12 to 21% of all mediastinal masses, although 95% occur in the posterior compartment.[24]Ganglioneuromasare benign tumors composed of oneor more mature ganglionic cells. Arising from the nerve ganglion cells they are the most benign and differentiated of the autonomic ganglionictumors. Most patients are asymptomatic and receive diagnoses in thesecond or third decade of life.[25]The most common nature of the disease wasbenign (70 %) followed by malignant (30%).Thiswas compared to other studies (Table 6).

Table 6: Table showing the comparison of benign and malignant tumors with previous studies

Name of the study	Benign tumors (%)	Malignant tumors (%)
Karki S, *et al.* [3]	66.6	33.4
Bagheri R, *et al.* [4]	36.8	63.2
Dasgupta S, *et al.* [5]	63.6	36.4
Aroor AR, *et al*. [7]	31.4	68.6
Al-Khalifa M, *et al*.[8]	52.7	47.3
Wani AM, *et al*. [9]	68.3	31.6
Baram A, *et al.*[10]	43.5	56.5
Vaziri M, *et al.* [11]	40	60
Shrivastava PC, *et al*. [13]	41.5	58.5
Present study	70	30

In the present study we used immunostains selectively, toconfirm histopathological diagnosis. Oct-3/4, leukocyte common antigen (LCA) (CD45) and S100 was used for germcell tumor, lymphoma and neurogenic tumors respectively.

In this study, S100 immunostain positivity was seen in neurogenic tumors.Schwannomacases showed strong positivity (Figure 2C) but neurofibroma was seen to stainweak to intermediate positive (Figure 2E). Ganglioneuroma also showed positivity for S100immunostain.Neural crest derivedtissue: Schwann cells, melanocytes, glial cells stain positive for S100 protein.GogoiG, *et al*. [26]revealed S100 positivity in a similar studyas seen in this present study.

Oct-3/4 has been reported as anexcellent nuclear marker of classical seminoma and embryonal carcinoma. Oct-3/4antibody has excellent sensitivity and specificity for these two tumours and canbe effectively used as an aid to screen for these neoplasms. In our study, weexamined 3 cases for Oct-3/4 immunostain, of which, 2 cases of mixed malignantgerm cell tumour were reported positive containing seminomatous component (Figure 3F).Onecase of yolk sac tumour was reported negative. These finding corroboratedwith the study done by de Jong J, *et al*.[27]Looijenga LH, *et al*.[28] stated that, POU5F1 (Oct-3/4) identifies cells with pluripotent potential in human germ cell tumors.

Here, one case of Diffuse large B cell lymphoma, two cases of Hodgkin's lymphoma and one case of small lymphocytic lymphoma were taken. All the cases stained positive for LCA (Figure 5B, 5D). Peter VE, *et al.* [29] showed in their study over nine cases of lymphoblastic lymphoma and eight cases of small, noncleaved undifferentiated non-Burkitt's lymphoma where all cases stained positive with monoclonal antibodies, directed to the LCA.

Data regarding epidemiology and clinical profile and evaluation modalities are limited in eastern India, so it is difficult in understanding the changing pattern of mediastinal tumors. Low study population for a shorter time period is another limitation of this study. Multi-institutional methodologies may improve our experience.

VI. Conclusion

Mediastinum is a small narrow limited area in central region of thoracic cavity but it houses a broad array of lesions.Acquaintanceregarding the lesions and the topographical distribution helps in the diagnosis of the case. Clinical and imaging features both are very much essential along with histopathology and immunohistochemistry to confirm the diagnosis.

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