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LYMPHOMA ORBIT – A 4 YEAR STUDY AT A TERTIARY CARE CENTRE

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ABSTRACT

Over the last few decades, our understanding of orbital lymphoproliferative lesions has undergone profound change. The primary impetus has been the advent of increasingly sophisticated and specific immunodiagnostic and molecular techniques in tissue pathology. Accurate discrimination of orbital lymphoma from benign orbital lymphoproliferative disorders is crucial for treatment planning. A four year prospective study was carried out at tertiary care centre. All cases of clinically suspicious lymphomas, proven by CT and Histopathology were included. Incisional or excisional biopsy specimens were received for histopathological examination, and tissues were fixed in 10% formalin for processing. Sections were processed routinely with paraffin embedding and stained with haematoxylin and eosin. Immunohistochemistry was done wherever required. We analyzed in total of 86 cases with the clinical suspicion of orbital lymphoma from June 2016-May 2020. All these cases were included initially regardless of age and sex. Of these 49 cases proved to be cases of lymphoma orbit and the remaining 37 cases were excluded from the study as they were not lymphomas. The lymphomas were then subjected to immune histochemistry profiling which showed largely B cell neoplasms (87.77%)- with small lymphocytic lymphoma being the most common tumor - 21 cases (48.9%) followed by 11 cases (25.58%) of mixed small and large cell, 8 cases (18.6%) of large cell B cell lymphoma and 3 cases (6.9%) of Burkitts lymphoma. Primary lymphoid tumour of the orbit are not very uncommon. Histopathology remains the gold standard for diagnosis followed by proper immunohistochemistry profiling of the lesion for definitive treatment.

KEYWORDS: Orbital, Immunohistochemistry, Small lymphocytic lymphoma, Burkitts Lymphoma.

INTRODUCTION

Lymphomas of the orbit may arise spontaneously or may be associated with development of systemic lymphoma. Lymphoid tumours are the common neoplasm of ocular adnexa encountered by ophthamologist.[1] Orbital tumors form a very broad and a diverse spectrum, which is based on several demographic attributes.[2] Ocular adnexal non-Hodgkin's lymphoma (NHL), is seen to have a unique incidence pattern showing a steady and rapid increase in the past few decades with equal rates among both genders, and predominance among Asians/Pacific Islanders.[3] Hassan et al found that the incidence of orbital tumors in the United States according to the Surveillance, Epidemiology, and End Results (SEER) database was 1.59 per million person years. They also reported a significant increase in the overall incidence of orbital, conjunctival, and lacrimal gland tumors, with lymphoma having the highest incidence. Ocular adnexal lymphoid tumours may involve the eyelids, conjuctiva, orbital connective tissue and lacrimal gland. Overall orbital lymphomas account for more than 1% of all lymphomas.[4] Primary orbital NonHodgkin's Lymphoma is a rare presentation of extranodal Non-Hodgkin's lymphoma accounting for less than one percent of NHL. It affects primarily the lacrimal glands, conjunctiva, eyelids and orbits. Diffuse large B-cell lymphoma (DLCL) type of histology is much less commoner types of primary orbital NHL.[5] Primary orbital NHL affects all ages more commonly in 6th and 7th decade. The presenting clinical manifestations are a constellation of one or more of the following symptoms and signs; proptosis, visual field defect/loss of visual acuity, diplopia, strabism, pain (34%), lacrimation (23%), and conjunctival edema (22%).[6] Complete ophthalmologic and internist examination, ultrasound, computedtomography, cytologic and histologic examination with immunohistochemistry are used for diagnosis. They are highly radio sensitive and local recurrence is unsual. Radiotherapy seems to be effective in low grade lymphoma and chemotherapy is effective in intermediate and high grade lymphomas.[7]

MATERIALS AND METHODS

This is a prospective interventional study conducted during JUNE 2016 to MAY 2020 to study the incidence and spectrum of orbital lymphomas at Sarojini Devi Eye

Hospital/Osmania General Hospital /Osmania Medical College, Hyderabad.

All patients were subjected to a thorough history taking and clinical examination. Detailed ocular evaluation comprising of slit lamp examination of anterior segment, cover tests, ocular motility, Hertels exophthalmometry and fundus evaluation. Routine examination like CBP, ESR were done. Radiological investigations like CT scan brain and orbit was done. Clinically suspicious and radiologically confirmed cases were subjected to bone marrow aspiration with peripheral smear examination and ultrasound abdomen. Incisional / Excisional biopsy was done and sent for histopathological examination. Haemotoxylin and Eosin stained slides made from formalin fixed, paraffin embedded tissue sections were examined and reported by Pathologists. Immunohistochemistry was done wherever required. Informed consent was taken from the patients prior to surgery. Ethical clearance was taken for the study.

Procedure of bone marrow aspiration

- The patient is placed in the lateral decubitus position, with the top leg flexed and the lower leg straight.
- Palpate the iliac crest, and mark the preferred sampling site with a pen
- Aseptic technique is employed, including sterile gloves and gown
- The site is prepared with an antiseptic (eg, povidone-iodine or chlorhexidine gluconate), scrubbed, and draped, exposing only the site to be sampled.
- The skin and the underlying tissue to the periosteum are infiltrated with a local anaesthetic (eg approximately 10 mL of 1% Xylocaine [lidocaine]). A 10-mL syringe with a 25-gauge needle is used to inject an initial 0.5 mL directly under the skin, raising a wheal. A 22-gauge needle is used to penetrate deeper into the subcutaneous tissue and the underlying periosteum, an area roughly 1 cm in diameter.
- Adequacy of the anesthesia is tested by gently prodding the periosteum with the tip of the needle and questioning the patient for any painful sensation.
- A skin incision is made with a small surgical blade, through which the bone marrow aspiration needle, with a stylet locked in place, is inserted.
- Once the needle contacts the bone, it is advanced by slowly rotating clockwise and counterclockwise until the cortical bone is penetrated and the marrow cavity is entered. Contact with the marrow cavity is usually noted by a sudden reduction in pressure. The depth of the penetration should not extend beyond an initial 1 cm.
- Once within the marrow cavity, the stylet is removed. Using a 20 mL syringe, approximately 0.3 mL of bone marrow is aspirated. A volume greater than 0.3 mL may dilute the sample with peripheral blood and thus is not recommended. The material collected for bone marrow slides is generally not mixed with an anticoagulant, and it is processed immediately by a technologist; this avoids any cellular morphologic artifacts. If there is to be a delay in slide preparation, place the sample in EDTA (ethylenediaminetetraacetic acid) anticoagulant containing tube, preferably a pediatric-sized tube to avoid exposure to excess anticoagulant.
- The marrow needle is removed, and pressure is applied to the aspiration site with gauze until any bleeding has stopped, the specimen is processed by the hematopathology technician.

PROCEDURE FOR STAINING

Haematoxylin and Eosin

1. Bring the sections to water.
2. Stain for 4 minutes in alum hematoxylin (Harris).
3. Wash in tap water. Differentiate in 1% acid alcohol by dipping 3-4 times (3-4 secs).
4. Blueing done in running tap water for 10 minutes
5. Rinse in water.
6. Stain in acidified 1% eosin Y for 1 minute.
7. Wash in distilled water. Dehydrate in graded alcohols.
8. Clear in xylene. Mount in D.P.X.

Immunohistochemical staining protocol

- Fixation – formalin fixed, paraffin embedded sections
- Positive controls : cases of c- kit positive GIST
- Negative controls : case of schwannoma
- Solutions and reagents

Primary antibody

Rabbit anti-CD 117, c-kit (dako cytomation, cat # a 4502) optimal dilution 1:200

Secondary antibody.
Goat anti- rabbit IgG (H+L), biotinylated (vector laboratories, cat # BA – 1000)
Optimal dilution 1:500

- **Procedure**

Paraffin section to distilled water,

Epitope retrival: Use tris – ethylene diamino tetraacidic acid buffer epitope retrival method. briefly, pre – heat steamer or water bath with staining dish containing tris – EDTA buffer (pH 9.0) until temperature reaches 95-100 degree centigrade.

Immerse slides in the staining dish and place the lid loosely on the staining dish. Incubate for 20 minutes and turn off the steamer or water bath. Place the staining dish at room temperature and allow the slides to cool for 20 minutes.

Rinse sections in 2 changes of washing buffer, 2 minutes each. Serum blocking: incubate sections with normal

goat serum blocking solutions for 30 minutes to block non- specific binding of immunoglobulin.

Primary antibody: Incubate sections with rabbit anti – CD 117, c- kit (Dako Cytomation, Cat # A 4502) diluted in 1:200 in primary antibody dilution buffer for 1 hour at room temperature. Rinse in washing for 2x2 min.

Peroxidase blocking: Incubate sections in peroxidase blocking solution for 10 minutes to block endogenous peroxidase activity. Rinse in washing buffer for 3x2 minutes.

Secondary antibody: incubate sections with biotinylated Goat Anti- rabbit IgG diluted in secondary antibody dilution buffer for 30 minutes at room temperature. Rinse in washing buffer for 3x2 min.

Chromogen/substrate: Incubate sections in DAB peroxiase substrate solution fir 5-10 minutes. Rinse in distilled water briefly. Counter stain in Gill's haematoxylin solution or Mayer's haematoxylin solution if desired. Rinse in running tap water for 5 minutes. Dehydrate through 95% ethanol for 2 minutes, 100% ethanol for 2x3 min. Clear in xylene for 2x3 min. Cover slip with permanent mounting medium.

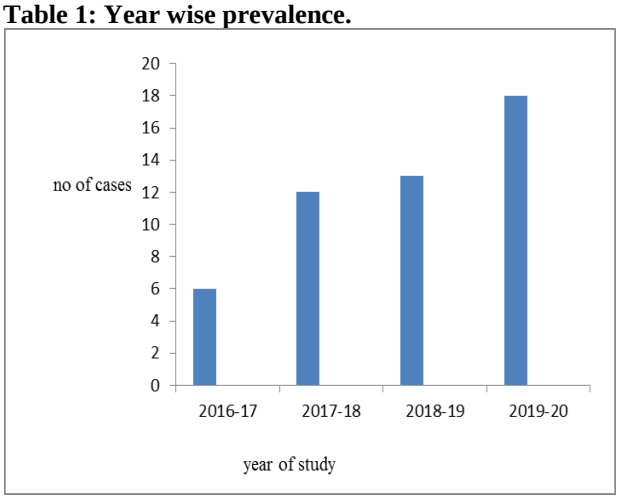
Results: staining pattern – cytoplasmic
A standard IHC was performed using the polyclonal anti CD 20 antibody in 1: 200 dilution. A standard technique was performed using polymer HRP detection system.

RESULTS AND DISCUSSION

Upon analyzing the data and knowing the results appropriate conclusions are drawn. The data was analyzed for the following facts regarding orbital lymphoma
a. Incidence
b. Age groups
c. Sex distribution
d. Mode of presentation
e. Histological types

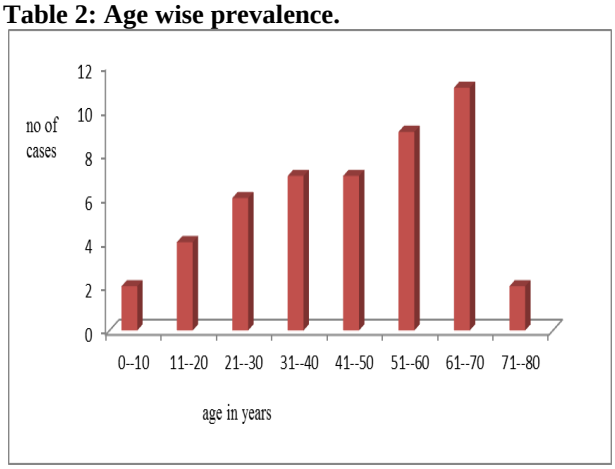
We analyzed in total 86 cases with the clinical suspicion of orbital lymphoma from June 2016- May 2020.All these cases were included initially regardless of age and sex. Of these 49 cases proved to be cases of lymphoma orbit and the remaining 37 cases were excluded from the study
Total no of cases studied - - - - - - - 86
Proven cases of lymphoma - - - - - - - -49
Cases excluded from study - - - - - - - 37
Of the excluded
No follow ups - - - - - - - - - - -05
Idiopathic orbital inflammations - - - - 09
Meibomian gland carcinoma - - - - - - -08
Squamous cell carcinoma - - - - - - - - --13
Inconclusive - - - - - - - - - -- - - - -- - - - --02

Table 1: Year wise prevalence.



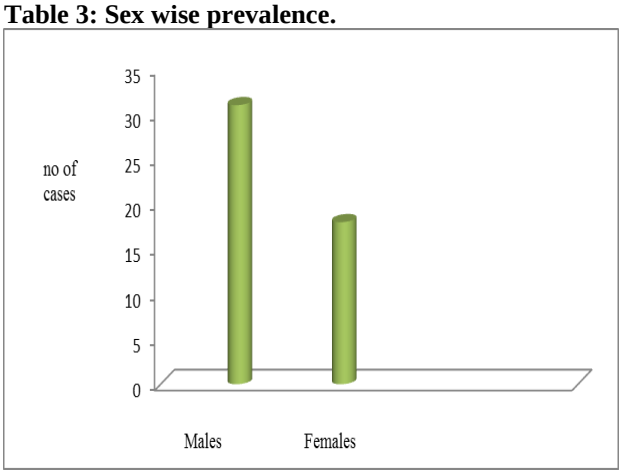
Increasing incidence of orbital lymphoma is observed per year, with maximum number of cases in 2020.

Table 2: Age wise prevalence.



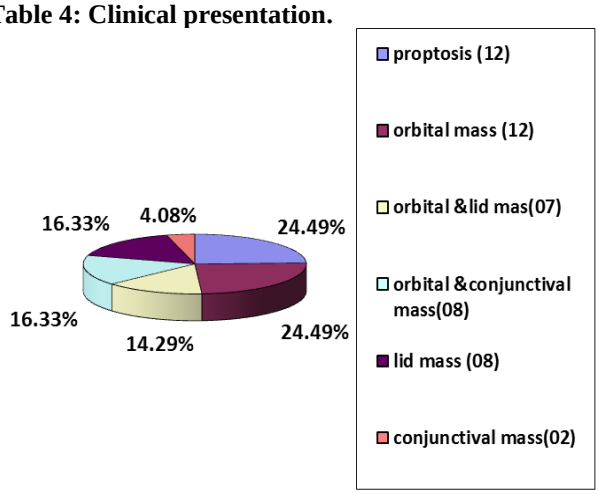
Lymphoma orbit is more common in fourth to seventh decade, with seventh decade being the most common age group in our study.

Table 3: Sex wise prevalence.



In our study male predominance is observed, Males were 31 (69%) and females were 18 (31%) of cases.

Table 4: Clinical presentation.



The most common presentation observed in our study is orbital mass and proptosis.

Orbital mass in combination with conjunctival and lid mass is also observed.

All the forty nine cases studied were Non Hodgkins Lymphoma type and not a single case of Hodgkins lymphoma occurred.

The histological sub types according to Working Formulation Classification observed were as Follows.

Histological types	No of cases
Low grade lymphomas Small lymphocytic lymphoma	24 (49 %)
Intermediate grade lymphoma Diffuse mixed small & large cell	11 (22.4%)
High grade lymphoma Diffuse large cell	09(18.3%)
Burkitt's lymphoma	05(10.2%)

The majority of the lymphomas were low grade small lymphocytic type, followed by intermediate grade. There were nine cases of diffuse large cell and five cases of Burkitt's lymphoma.

Of the fortynine cases of Non Hodgkin's Lymphoma 43(87.77%) were B – cell type and 6(12.24%) were T cell lymphomas.

All cases of lymphoma were diagnosed histopathologically. Immuno histochemistry was done where ever necessary.

B cell marker used was CD 20, T cell marker used was CD 3.

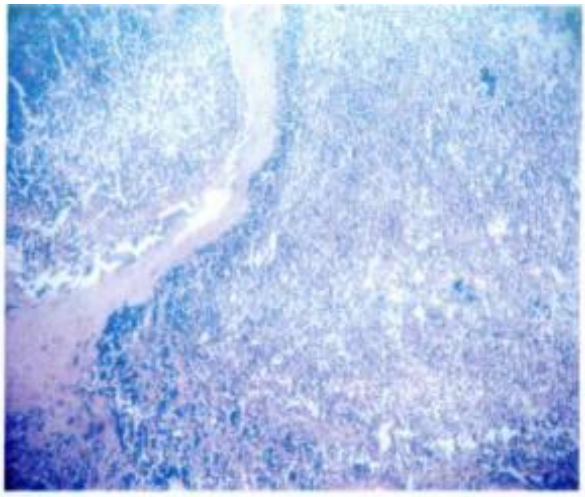


Figure 1: Small lymphocytic lymphoma.

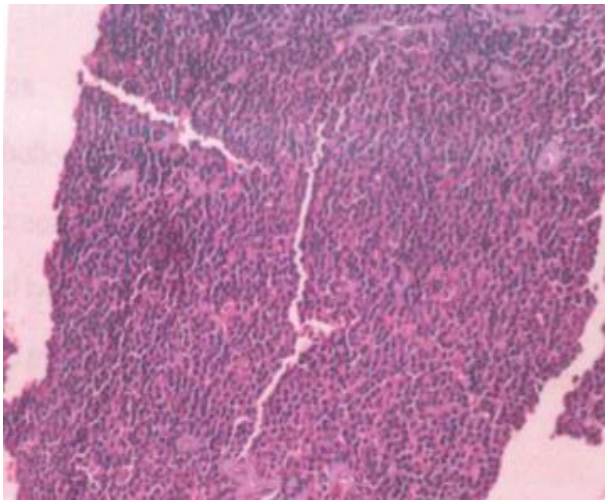


Figure 2: Diffuse mixed lymphoma.

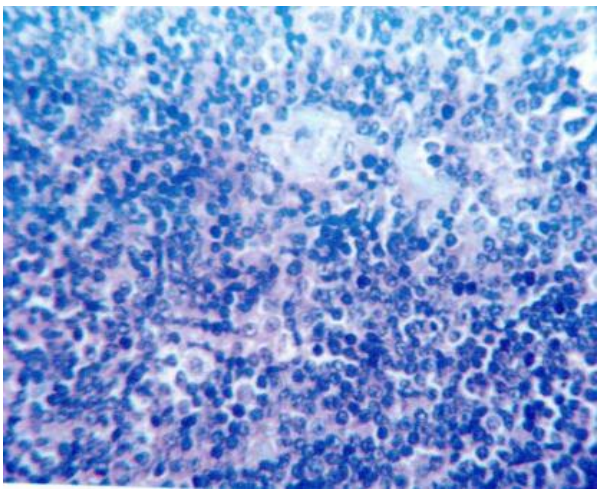


Figure 3: Diffuse large B cell.

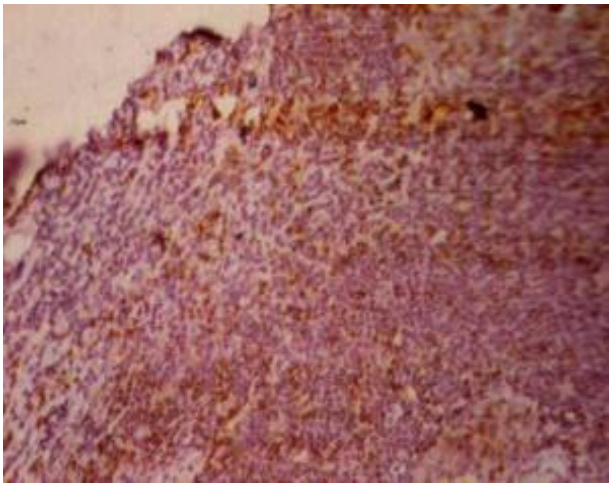


Figure 4: IHC CD 20 –Positive.

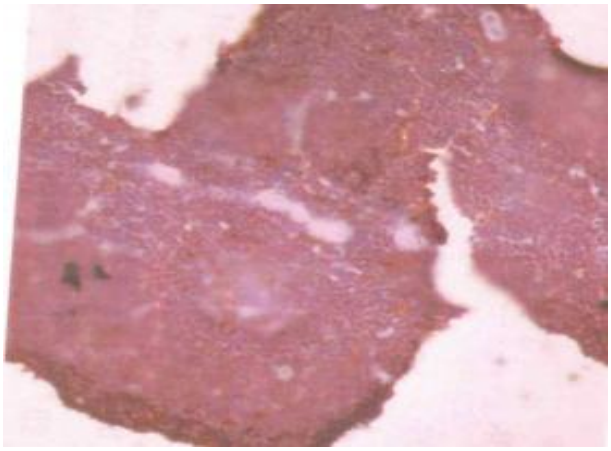


Figure 5: IHC CD 3.

All the cases in our study had no systemic involvement of lymphoma, the peripheral smear and bone marrow studies done were normal with no lymphoproliferative evidence.

Most of the cases were surgically treated by excision followed by radiotherapy. Few of them were treated by chemotherapy following excisional biopsy. No case of recurrence was observed in our study.

DISCUSSION

Orbital lymphoma refers to a lymphoma occurring in the conjunctiva, lacrimal gland, eyelid and ocular musculature. Primary non-Hodgkin's lymphoma (NHL) of the orbit is a rare presentation, representing 8-10% of extranodal NHL and only 1% of all NHL. Generally, it has an indolent course.[7] Conclusions reached by any orbital survey vary according to the source of material and the age group studied. Percentage of biopsy proven entities, geographical area encompassed, the specialty and type of practice of the researchers, and the scope of diagnostic modalities used to evaluate the patient enrolled in the series. Two diagnostic modalities have revolutionized the scope and accuracy of orbital evaluation—USG and CT scan. Although the unequivocal diagnosis of orbital disease can be made only by histopathological examination.[8]

Studies have emphasized that benign and malignant lymphoproliferative disorders cannot be distinguished by clinical examination alone and others have found it impossible to make a definitive diagnosis even with histopathological examination and believe that it can be concluded only after prolonged follow up.

The question of whether the ocular tissues can be primary site of malignant lymphoma or mererly an atypical presentation of systemic lymphoma continues to be debated. Orbital tumors in the senior adult population are malignant in 63% of cases. Malignant lymphoma is the most common tumor in this age group, accounting for 24% of cases.[9]

We analyzed 86 cases between June 2016 – May 2020 of these 49 cases of orbital lymphomas were analyzed and compared with other current data available from around the world. One of the studies that was conducted at Sri Sankaradeva Netralaya, Guwahati Assam by Dipankar Das had similar findings as proptosis as the most common symptom and B cell type of Non hodgkins lymphoma was found to be in 89% of cases[1] like wise our study showing 87.7% of B cell type of Nonhodgkins lymphoma. Most of the observations of our study correlated with theirs, with no recurrence of the disease. The histopathological subtype and the clinical stage of the disease are the best indicators of prognosis and patient outcome. Low-grade lymphomas such as extranodal marginal zone B-cell lymphoma and FL have a good prognosis, whereas high-grade lymphomas (diffuse large B-cell lymphoma and mantle cell lymphoma) are associated with a poor prognosis.[10]

Orbital and adnexal lymphomas are associated with systemic lymphoma in 30%–35% of cases. Hence, all patients with ocular lymphoma should have a complete workup to rule out systemic lymphoma.[11] Almost 80%of orbital and adnexal lymphomas are of low-grade variety, with B-cell lymphomas and extranodal marginal zone lymphoma of the mucosa-associated lymphoid tissue type (MALT lymphoma) being the most common histological diagnosis.[11]

In our cases recorded, all had orbital involvement without any systemic manifestation.

Our study correlated in terms of sex incidence and histology with Manuel F Rosado et al.[12] The treatment options for orbital lymphoma are chemotherapy, immunotherapy, targeted therapy, radiation therapy, stem cell transplant, and in rare cases, surgery. Prognosis depends on the histological type, grade and stage and treatment modality employed, but the overall 5-year survival rate is approximately 60%.[13]

In general terms the findings of our study are very similar to those dscribed in literature. It is important for the ophthalmologist to br familiar with this type or orbitary pathology because in many occasions they are the first specialists to examine the patients who suffer it.

CONCLUSION

There has been a progressive increase in the incidence of lymphomas over the years with frequent reports of varying and atypical presentations and a bewildering number of confusing classification which still do not fit all cases. In our study certain interesting points are noted with wide age group 10 - 80 years. Males are more common than females. Age at time of presentation is one decade earlier for males when compared to female counterparts. But the majority are between 4^{th} – 7^{th} decade. The most common presenting symptoms was orbital mass followed by proptosis seen in most of the cases followed by upper lidmass along with orbital mass. In conclusion we would say that early detection is very important because it is a potentially curable disease and it is important to identify this entity to avoid it going unnoticed. Primary lymphoid tumour of the orbit are not very uncommon, although proptosis is the most common presentation, patient may also present with lid swelling. Histopathology remains the gold standard for diagnosis. Timely diagnosis and prompt management can result in excellent prognosis, radiotherapy is the treatment of choice for primary orbital lymphomas recurrences are unusual.

REFERENCES

1. Dipankar Das, MS et al Ocular adnexal lymphoma in the Northeast Indian population, Indian J Ophthalmol, 2008 Mar-Apr; 56(2): 153–155.
2. Honavar, Santosh G. Manjandavida, Fairooz P. MD Recent Advances in Orbital Tumors—A Review of Publications from 2014-2016 Asia-Pacific Journal of Ophthalmology, March, 2017; 6(2): 153-158.
3. Roxana Moslehi, Maria J Schymura, Seema Nayak, and F Bruce Coles - Ocular adnexal non-Hodgkin's lymphoma: a review of epidemiology and risk factors Expert Rev Ophthalmol, Apr, 2011; 6(2): 181–193.
4. John T. LucasJr., Kathryn McConnell Greven - Orbital, Ocular, and Optic Nerve Tumors, Orbital lymphoma Clinical Radiation Oncology (Third Edition), 2012; 529-542.
5. Parikshit et al Case report: Primary Non-Hodgkin's Orbital lymphoma of diffuse large B-cell type: a rare presentation Bangladesh Journal of Medical Science, 2016; 15(04).
6. Mohammed Jaber Al-Mamoori Orbital tumors: A prospective study of 95 cases Medical journal of Babylon, 2018; 15(4): 334-340.
7. Yadav, B S; Sharma, S C Orbital lymphoma Role of radiation Indian Journal of Ophthalmology, Mar–Apr, 2009; 57(2): 91-97.
8. Siddharth Karan, Manisha Nathani, M. Tanveer Ali Khan, Aftab Abdul Khader, Suchetha Ireni, Abdul Basith Syed A Non-Randomised Prospective Study Of Thyroid Orbitopathy In A Tertiary Care Centre JEBMH, May, 2016; 3(38): 1910-1914.
9. HakanDemirciMD Carol LShieldsMD - Orbital tumors in the older adult population Ophthalmology, February 2002; 109: 243-248.
10. Olsen TG, Heegaard S Orbital lymphoma. Survey of Ophthalmology, 23 Aug, 2018; 64(1): 45-66.
11. Baskararajan Gopalakrishnan, B Bhuvanasundari - Orbital lymphoma case series - TNOA, Journal of ophthalmic research, 2017; 55(2): 140-144.
12. Manual F Rosado et al Cohort study on ocular adnexal lymphoma, University of Miami, Florida. Blood, 15, 2006; 107(2): 467-72.
13. Sharma T, Manjunath Kamath M. Eyenet magazine. In: Fekrat S, Scott IU, editors. Diagnosis and Management of Orbital Lymphoma. AAO.org: American Academy of Ophthalmology, 2015.