IMMUNOHISTOCHEMICAL ANALYSIS
OF ORBITAL LYMPHOMA IN A
TERTIARY EYE CARE CENTRE

Dissertation Submitted for
MS Degree (Branch III) Ophthalmology
April 2012

The Tamilnadu Dr.M.G.R.Medical University
Chennai – 600 032.
CERTIFICATE

Certified that this dissertation entitled “IMMUNOHISTOCHEMICAL ANALYSIS OF ORBITAL LYMPHOMA IN A TERTIARY EYE CARE CENTRE ” submitted to the Tamilnadu Dr M.G.R Medical university, Chennai December 2011 is the Bonafide work done by DR.R.SANKARANANTHAN under our supervision and guidance in the orbit and occuloplasty Department of Aravind Eye Hospital and post graduate institute of ophthalmology, Madurai during his residency programme from May 2011 to April 2012.

Dr. USHA KIM
CHIEF ORBIT AND OCCULOPLASTY.
ARAVIND EYE HOSPITAL
MADURAI

DR.M.SRINIVASAN
DIRECTOR
ARAVIND EYE HOSPITAL
MADURAI
ACKNOWLEDGEMENT

At the outset, I would like to take this opportunity to pay my respects to Dr. G. Venkataswamy, the founder of the Aravind Eye Care System and the source of inspiration that continues to drive the work performed here.

I would like to thank Dr. Usha Kim, Head of the Department, orbit & Occuloplasty, Aravind Eye Hospital, Madurai for her enthusiasm, guidance and constant support for this project.

I am deeply indebted to Dr. T. Venkatesh Prajna, Chief, Department of Medical education for all his help, support and above all, kindness that he has extended me throughout my residency programme at Aravind.

I sincerely thank Dr. Namperumalsamy, Chairman Emeritus, Dr. G. Natchiar, Director-Human Resources, Dr. M. Srinivasan, Director Emeritus and Dr. R. D. Ravindran, Chairman whose untiring dedication to the prevention of needless blindness in this country has, and will continue to inspire innumerable young ophthalmologists like me.
I am grateful to Dr. Shilpa Taneja, orbit & Occuloplasty, Aravind Eye Hospital, for her help in this study. I would also like to thank Mr. Vijayakumar, Biostatician for his assistance in the statistical analysis of data. I am grateful to the staff of the Aravind Medical Records Department without whose help, it would have not been possible to complete this study.

I would also like to thank the librarians Mrs. Kumaragurupari and for their valuable support during this project.

Finally, I would like to thank my family for their selfless love and sacrifices without which I would not be what I am today.

DR.R.SANKARANANTHAN
## CONTENTS

<table>
<thead>
<tr>
<th>S.No</th>
<th>Content</th>
<th>Page No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2.</td>
<td>INTRODUCTION TO LYMPHOMA</td>
<td>3</td>
</tr>
<tr>
<td>3.</td>
<td>HODGKIN’S LYMPHOMA</td>
<td>4</td>
</tr>
<tr>
<td>4.</td>
<td>NON- HODGKIN’S LYMPHOMA</td>
<td>9</td>
</tr>
<tr>
<td>5.</td>
<td>INTRODUCTION TO ORBITAL LYMPHOMA</td>
<td>15</td>
</tr>
<tr>
<td>6.</td>
<td>INTRODUCTION TO IMMUNOHISTOCHEMISTRY</td>
<td>28</td>
</tr>
<tr>
<td>7.</td>
<td>AIM AND OBJECTIVES</td>
<td>46</td>
</tr>
<tr>
<td>8.</td>
<td>METHODOLOGY</td>
<td>47</td>
</tr>
<tr>
<td>9.</td>
<td>RESULTS</td>
<td>49</td>
</tr>
<tr>
<td>10.</td>
<td>DISCUSSION</td>
<td>57</td>
</tr>
<tr>
<td>11.</td>
<td>CONCLUSION</td>
<td>63</td>
</tr>
</tbody>
</table>

ANNEXURE

BIBLIOGAPHY
INTRODUCTION

Orbit is a socket situated in the skull which contains the eyeball, protective pad of fat, various blood vessels, extra ocular muscles and nerves surrounded by bone, nasal sinuses and intracranial structures. Hence wide spectrum of diseases may manifest in orbit of which Orbital tumours, and in turn, orbital lymphomas constitute a significant proportion, the Diagnosis of which is a challenge to the clinicians.

The lymphoid system comprises of lymph nodes, spleen, mucosa associated Lymphoid tissue –MALT, pharyngeal lymphoid tissue and thymus are closely interlinked With the function of the bone marrow $^1$.

Lymphoid tumours constitute the largest sub group among orbital tumours. Lymphoid tumours can occur in the orbit with or without associated systemic diseases.

The anterior location of the eye ball limits direct observation and palpation of orbital lesions. Even ,the knowledge of size, location and consistency of orbital lesion does not settle the issue of localisation of the lesion.
The various clinical and non clinical methodologies detect the presence of the tumour, the size and extent of it. But, the exact histological picture can be predicted only if the usage of various histopathological studies in particular Immunohistochemistry comes into use.

Immunohistochemistry techniques can be applied to various orbital lesions to aid in differential diagnosis.
LYMPHOID SYSTEM

The lymphoid system comprises of lymph nodes, spleen, mucosa associated lymphoid tissue –MALT, pharyngeal lymphoid tissue and thymus are closely interlinked with the function of the bone marrow ¹.

Functionally, the lymphocytes are divided into T and B lymphocytes depending upon whether they are immunologically active in cell-mediated immunity or in humoral antibody respectively. B cells proliferate and mature into plasma cells.¹⁴

LYMPHOMAS

Lymphomas are malignant tumours of the lymph reticular origin i.e. from lymphocytes and histiocytes and their precursor cells. ²

Two clinically distinct clinicopathological groups can be seen

1. HODGKINS LYMPHOMA OR HODGKIN’S DISEASE.

2. NONHODGKIN’S LYMPHOMAS.
HODGKIN’S DISEASE

Arises primarily within the lymph nodes and involves the extra nodal sites secondarily. ⁴

INCIDENCE

Shows bimodal peak ⁵

Young adult between the age of 15 and 35 years of age and the other peak after ⁵ᵗʰ decade of life. More prevalent in young males than females ⁴, ⁵

The characteristic finding of Hodgkin’s disease is the presence of REED STERNBERG CELLS ⁶, ⁷

MODIFIED REES CLASSIFICATION ⁴

<table>
<thead>
<tr>
<th>HISTOLOGIC SUBTYPE</th>
<th>INCIDENCE</th>
<th>MORPHOLOGY</th>
<th>REEDSTERNBERG CELLS</th>
<th>PROGNOSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CLASSICAL HD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i) Lymphocyte predominance</td>
<td>5%</td>
<td>Proliferating lymphocytes, few histiocytes</td>
<td>Few classic and polyploidy type. CD15</td>
<td>Excellent</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>--------</td>
<td>--------------------------------</td>
<td>--------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>ii) Nodular sclerosis</td>
<td>70%</td>
<td>Lymphoid nodules and collagen bands</td>
<td>Frequent lacunar type. CD15, CD30</td>
<td>Very good</td>
</tr>
<tr>
<td>iii) Mixed cellularity</td>
<td>22%</td>
<td>Mixed infiltrate</td>
<td>Numerous, classic type, CD15+, CD30+</td>
<td>Good</td>
</tr>
<tr>
<td>iv) Lymphocyte depletion</td>
<td>1%</td>
<td>Scanty lymphocytes, atypical histiocytes</td>
<td>Numerous pleomorphic type, CD15+, CD30+</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>NODULAR LYMPHOCYTE PREDOMINANCE TYPE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2%</td>
<td>Proliferation of small lymphocytes, nodular pattern of growth</td>
<td>Sparse number of RS cells CD45+, EMA, CD15+, CD30+</td>
<td>Chronic relapsing may transform into large B cell NH</td>
</tr>
</tbody>
</table>

**REED STERNBERG CELLS (RS)**\(^6\), \(^7\)

The diagnosis of RS cells in Hodgkin’s lymphoma is an important pathological finding; it can at times be confusing with the cells found in infectious mononucleosis.
In this case the RS cells are classified into 4 variants which are \(^5\,^6\):

1. **Classical RS cell** – Blobbed nucleus. each nucleus has a prominent owl like eye appearing nucleolus

2. **Lacunar type RS cell** – characteristic of nodular sclerosis HD

3. **Polyploidy type RS cell** – lymphocyte predominance type of HD. large nucleus in the shape of popcorn.

4. **Pleomorphic RS cells** – are features of lymphocyte depletion type.

   It’s important to know that the number of RS cells is inversely proportional to the number of lymphocytes in a particular number of HD.

**CLINICAL FEATURES:**

Common among young and middle aged adults. \(^7\,^8\),

Common among males except the nodular sclerosis type.

The disease usually begins with superficial lymph node enlargement and subsequently spreads to other lymphoid and non lymphoid structures \(^7\,^8\).

1. Painless movable and firm lymphadenopathy. the cervical and mediastinal lymph nodes are most frequently involved.

2. Approximately half the patients develop spleenomegaly during the course of the disease. liver enlargement too may occur. \(^8\)
3. Most common are fever, night sweats, weight loss, fatigue, malaise weakness and pruritus.

LABORATORY DIAGNOSIS:

Hematologic abnormalities:
1. A moderate, normocytic and normochromic anaemia is often present.

2. Serum iron and TIBC are low but marrow iron stores are normal or increased.

3. Marrow infiltration by the disease may produce marrow failure with leucoerythroblastic reaction.

4. Routine blood counts reveal moderate leukamoid reaction

5. Platelet count is normal or increased

6. ESR is invariably elevated.
ANN ARBOR STAGING OF HODGKINS LYMPHOMA
<table>
<thead>
<tr>
<th>STAGE 1</th>
<th>1 involvement of a single lymph node region (^9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A or B)</td>
<td>1E involvement of a single extra lymphatic organ or site</td>
</tr>
<tr>
<td>STAGE 2</td>
<td>2 involvement of two or more lymph node regions on</td>
</tr>
<tr>
<td>(AorB)</td>
<td>the same side of the diaphragm</td>
</tr>
<tr>
<td></td>
<td>2e (or) with localised contiguous involvement of an Extra nodal</td>
</tr>
<tr>
<td></td>
<td>Organ or site</td>
</tr>
<tr>
<td>STAGE 3</td>
<td>3 involvement of lymph node regions on both sides of the</td>
</tr>
<tr>
<td>(A or B)</td>
<td>diaphragm (^{10})</td>
</tr>
<tr>
<td></td>
<td>3e (or) localised contiguous involvement of an extra Nodal site</td>
</tr>
<tr>
<td></td>
<td>or organ</td>
</tr>
<tr>
<td></td>
<td>3s (or) with involvement of spleen.</td>
</tr>
<tr>
<td></td>
<td>3es (or) with involvement of both 3e and 3s</td>
</tr>
<tr>
<td>STAGE 4</td>
<td>4 Multiple or disseminated involvement of one or more</td>
</tr>
<tr>
<td>(A or B)</td>
<td>Lymphatic organs or tissues with or without lymphatic</td>
</tr>
<tr>
<td></td>
<td>involvement</td>
</tr>
</tbody>
</table>

**NON HODGKIN’S LYMPHOMA**
Are the malignant neoplasms of the immune system of the body and
are more common than the Hodgkin’s lymphoma.  

Non Hodgkin’s lymphoma is more common among the young adults
(20-40) years. Majority of the NHL arise in lymph nodes (65%) while the
remaining (35%) take origin in the extra nodal lymphoid tissues.

All forms of NHL have potential to spread to other lymph nodes,
liver, spleen and bone marrow.

**ETIOLOGY**

1. **Infections** :

   Epstein barr virus – Burkett’s lymphoma

   Human-T-cell virus type 1 in adult T cell lymphoma – leukaemia

   HIV in diffuse large B cell lymphoma and Burkett’s lymphoma.

2. **Immunodeficiency disease**

3. **Autoimmune deficiency disease**

   - Sjogren’s syndrome

   - Non tropical sprue
- Rheumatoid arthritis
- SLE
- Chemical and drug exposure Phenytoin
- Radiation
- Prior chemotherapy
- Agricultural chemicals

CLASSIFICATION OF NON HODGKIN’S LYMPHOMAS

RAPPORT’S CLASSIFICATION

NODULAR NHL:

1. lymphocytic poorly differentiated
2. mixed, lymphocytic and histolytic
3. histolytic

DIFFUSE NHL

1. lymphocytic well differentiated
2. lymphocytic poorly differentiated
3. mixed lymphocytic and histiocytic
4. histiocytic

5. Diffuse Undifferentiated: Burkitt’s and Non Burkitt’s

**LUKES-COLLINS CLASSIFICATION** ¹², ¹³

<table>
<thead>
<tr>
<th>1. UNDEFINED CELL</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. T – CELL . B-CELL</td>
</tr>
<tr>
<td>- small lymphocyte - small lymphocyte</td>
</tr>
<tr>
<td>- convoluted lymphocyte - plasmocytoid lymphocyte</td>
</tr>
<tr>
<td>- cerebriform cell of Sezary’s - follicular center cell (FCC)</td>
</tr>
<tr>
<td>and mycosis fungoides small cleaved</td>
</tr>
<tr>
<td>- Lymphoepitheloid cell Large cleaved</td>
</tr>
<tr>
<td>- immunoblastic sarcoma - immunoblastic sarcoma</td>
</tr>
<tr>
<td>- histolytic Small non cleaved</td>
</tr>
<tr>
<td>- cell of uncertain classification Large non cleaved</td>
</tr>
</tbody>
</table>
NEW WORKING FORMULATION FOR CLINICAL USE

LOW GRADE:

A. small lymphocytic

B. follicular predominantly cleaved cell

C. follicular, mixed, small cleaved and large cleaved cell

INTERMEDIATE GRADE:

D. follicular predominantly large cell - cleaved cell or non – cleaved cell.

E. diffuse, small cleaved cell.

F. diffuse, mixed, large and small cell.

G. diffuse, large cell, cleaved or non-cleaved

HIGH GRADE

H. large cell immunoblastic

I. lymphoblastic
J. Small non cleaved cell (Burkitt’s or Non Burkitt’s )

REAL CLASSIFICATION 12, 13

I. Leukemias and lymphoma of B cell origin

(Pan B CD19 , CD 20 positive )

A. Indolent B – cell malignancies

- Chronic lymphocytic leukemia / small lymphocytic lymphoma .
- Hairy cell leukemia .
- Follicular lymphoma (grade 1 small cleaved . grade 2 mixed small and large ).
- Lymphoplasmacytoid lymphoma / waldenstorm’s macroglobulinaemia .
- Marginal zone lymphoma (lymphoma of mucosa associated lymphoid
tissue (MALT), spleenic lymphoma)

B. Aggressive B cell malignancies

- Diffuse large cell lymphoma
- Follicular large cell lymphoma (grade 3)
- Mantle cell lymphoma
- Burkitt’s lymphoma
- Plasmacytoma / myeloma

II. Leukaemias and lymphomas of T cell origin

(CD 2,7 Positive)

A. Indolent, T cell malignancies

- T – CLL, T prolymphocytic leukemia
- Cutaneous T cell lymphoma

B. Aggressive T-cell malignancies

- Peripheral T cell NHL
- Angio immunoblastic T cell lymphoma
- Intestinal T cell lymphoma
- Adult T-ALL
ORBITAL LYMPHOMA

Introduction

Orbital lymphoma is a common type of non-Hodgkin lymphoma that occurs near or on the eye.

Common symptoms include decreased vision and uveitis. Orbital lymphoma can be diagnosed via a biopsy of the eye and is usually treated with Radiotherapy or with combination with chemotherapy.

Symptoms
Primary visible symptoms of ocular lymphoma include proptosis and a visible mass in the eye. Other symptoms are due to mass effect.

**Pathophysiology**

Recent studies have detected the presence of viral DNA in ocular lymphoma cells.

This implies that pathogens play a role in ocular lymphoma. Other studies have found that the ageing population, the increasing number of immunosuppressive drugs, and the AIDS epidemic have also contributed to the increased incidence of Non-Hodgkin lymphomas. Ocular MALT lymphomas may also be associated with Chlamydia psittaci\textsuperscript{14,15} although whether or not this is the case is still debated.

Follicular lymphoma, diffuse large B cell lymphoma, mantle cell lymphoma, B-cell chronic lymphocytic leukaemia, peripheral T-cell lymphoma, and natural killer cell lymphoma have also been reported to affect the orbit.\textsuperscript{16}

**Epidemiology**

55% of all lymphoma cases in adults and 10% in older patients is a form of ocular lymphoma.
In 2008, a prediction by the National Cancer Institute Surveillance, estimated that in 1,340 men and 1,050 women would be diagnosed with eye cancer and 240 people would die of the disease that year. Orbital lymphoma is more prevalent in Asia and Europe than in the United States. 17

Although intraocular lymphoma is rare, the number of cases per year is rising, affecting mainly people in their seventies and immunocompromised patients. A recent study has shown that ocular lymphoma is more prevalent in women than men. 17

The survival rate is approximately 60% after 5 years.

Types

There are two types of ocular lymphomas: intraocular lymphomas and adnexal lymphomas.

An intraocular lymphoma occurs within the eye, while an adnexal lymphoma occurs outside, but adjoined to the eye.

OCULAR ADNEXAL LYMPHOMA

Lymphoproliferative tumours of the ocular adnexa encompass a wide spectrum of lesions that range from reactive benign hyperplasia to malignant lymphoma. Ocular adnexal lymphoma (OAL) is a localized form
of systemic lymphoma affecting the orbit, the lacrimal gland, the lids and/or the conjunctiva. It comprises 6-8% of orbital tumours, and 10-15% of adnexal lesions. OAL affect both genders, with a slight female predilection. Ocular adnexal lymphoma (OAL) is considered primary if it involves the ocular adnexa alone and secondary if it is accompanied by a lymphoma of identical type at another site. OAL is defined as solitary if it involves one or both orbits only, extension if it involves contiguous sites such as the sinuses, and systemic if remote sites are involved. The majority of lesions in this area are non-Hodgkin lymphoma (NHL), 80% of which arises from B-lymphocytes, 14% from T cells and only 6% from natural killer cells. The most common primary OAL is the low-grade malignant extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (ENZL or MALT), but other types can occur: follicular lymphoma (FL), diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL) and lymphoplasmatic lymphoma (LPL). Secondary OALs arise from systemic disease and are represented by intermediate or high-grade follicular lymphomas. The reported frequency of involvement of periocular sites are: conjunctiva, 20% to 33%; orbit, 46% to 74%; and eyelid, 5% to 20%. ²⁰
Tumours arise from germinal center cells (follicular lymphoma), mantle cells (mantle cell lymphoma) or memory B cells (extranodal marginal zone lymphoma) all of which have undergone antigen exposure. The infection/inflammation/mutation model (IMM) of lymphopathogenesis explains why the ocular adnexa is so often affected by lymphoma, occurring as a result of mistakes during normal lymphocyte response to infection or inflammation.18

**Signs**

In the conjunctiva: the typical lesion is salmon or flesh-pink color. In the orbit, eyelid or lacrimal gland: when palpable, the masses are firm. 18

**Symptoms**

Many lesions are asymptomatic but patients can complaint of exophthalmos, pain or diplopia, as well of conjunctival, eyelid, orbital or lacrimal gland mass.

**Management**

**Radiation:** It has been the most frequently used modality for treating OAL, because many patients present with localized disease. Electron or photon irradiation can be used depending on the site, extent of disease and the tumor grade or type. A wide variation of doses has been recommended, ranging
from 15–20 Gy up to 40 Gy. Typical doses are 28 to 36 Gy for low-grade OAL and 30 to 40 Gy for high-grade disease. Recurrences of low-grade types are often treatable with local modalities. \textsuperscript{21}

**Chemotherapy:** With solitary OAL, systemic chemotherapy is not indicated, except for diffuse large B-cell lymphoma (DLBCL). Chemotherapy for OAL when it is part of stage II or greater disease has included the use of standard regimens for systemic lymphoma. \textsuperscript{22}

**Immunotherapy** - The local use of IFN-a for OAL is not yet established. - Antilymphocyte antibodies represent the newest form of lymphoma treatment. Using antibodies to CD20 (Rituximab), destruction of B cells can take place based on the induction of apoptosis, complement-mediated cytolysis, and antibody-dependent cyto-toxicity. \textsuperscript{22}

In microbial-associated MALT lymphomas, antimicrobial treatment can lead to remission. Further studies are needed to verify this infectious association and those with other possible inciting pathogens. \textsuperscript{23}

**PRIMARY INTRA OCULAR LYMPHOMA**

Ophthalmic masquerade syndromes are a group of disorders that classically present as ocular inflammation, but in fact are often infectious or
malignant processes. The first case described in the ophthalmic literature in which the term masquerade syndrome was applied was one in which a conjunctiva carcinoma presented as chronic conjunctivitis. Since then, a wide variety of both malignant and non-malignant diseases have been grouped under this heading. Any disorder that presents with either inflammatory cells in the eye or inflammation of the adnexa, but is not a primary immune-mediated disease itself may thus be called masquerade. The list of intraocular masquerades alone is quite long and includes such diverse diseases as lymphoid malignancy, retinoblastoma, ocular melanoma, ocular metastatic disease, juvenile xanthogranuloma, chronic retinal detachment, retinal degenerations, trauma, ocular ischemic syndrome, and infection. Of these, intraocular lymphoma is the most common, but can also pose the most difficult diagnostic challenge. Intraocular cells are often predominantly lymphocytes and the clinical differentiation between classic uveitic processes and malignancy can be impossible.

**Nomenclature**

Primary intraocular lymphoma is the preferred term for the condition that has been variously referred to as primary central nervous system lymphoma (PCNSL) involving the eye, histolytic lymphoma, intraocular
large cell lymphoma, microglioma, periepithelial sarcoma, and Reticulum cell sarcoma.\textsuperscript{23,25}

PIOL is considered a subcategory of PCNSL.\textsuperscript{25} The disease can occur without concurrent involvement of the central nervous system (CNS). Although the cytological features of PCNSL and PIOL are identical,\textsuperscript{25} we will focus on the disease that presents clinically with ophthalmic findings, most commonly posterior vitritis...

The median age of onset of PCNSL/PIOL in immunocompetent patients is the late 50s and 60s, with a reported range of 15 to 85 years of age for PIOL. The male-female ratio is 1.2-1.7:1.\textsuperscript{25} Half of the patient population with PCNSL has multifocal disease at the time of initial presentation, with ocular involvement found in 15% to 25%. On the other hand, 60% to 80% of patients in whom PIOL is initially diagnosed develop CNS disease within a mean of 29 months. Ocular disease is bilateral in 80% of cases.\textsuperscript{26}

**Clinical Features**

Intraocular lymphoma most commonly presents as chronic posterior uveitis with vitritis.\textsuperscript{27} Eye pain, blurred vision, floaters, or even a foreign body sensation may be the presenting symptom. Some patients are
asymptomatic and are found to have signs of intraocular lymphoma on routine slit-lamp examination.\textsuperscript{28} Patients with intraocular lymphoma may manifest a myriad of ophthalmic signs.\textsuperscript{29,30} These include anterior chamber cell with or without keratin precipitates, hyphaema, hypopyon, iris neovascularisation with or without resultant glaucoma, a mass of the iris or angle, vitritis, vitreous hemorrhage, sub retinal or sub retinal pigment epithelium (sub-RPE) infiltrates, retinal haemorrhage or exudates, perivascular infiltration, retinitis, or optic disc edema.

**Diagnosis**

Ultrasound may also be helpful in narrowing the diagnosis. Ultrasonographic findings in 13 patients with ocular lymphoma included vitreous debris (77\%), choroidal-scleral thickening (46\%), widening of the optic nerve (31\%), elevated chorioretinal lesions (23\%), and retinal detachment (15\%).\textsuperscript{31} Given the nonspecific nature of eye findings in PIOL, patients being considered for this diagnosis should be examined for other causes of uveitis, including sarcoidosis, intermediate uveitis, multifocal choroiditis, acute posterior multifocal placoid pigment epitheliopathy, birdshot chorioretinopathy, toxoplasmosis, ocular tuberculosis, and acute retinal necrosis.
Neurological and Medical Diagnosis

A thorough medical and neurological examination is important, including a chest radiograph, complete blood cell count, erythrocyte sedimentation rate, routine blood chemistries, and other laboratory studies to exclude the fore mentioned causes of uveitis. Because PIOL is closely related to PCNSL and seldom involves other organs, neuroimaging of the brain and orbits and a lumbar puncture are required. Computed tomography (CT) scans usually reveal isodense or hyperdense lesions. Magnetic resonance imaging studies usually reveal lesions that are hypodense on T1-weighted and hyperdense on T2-weighted images.

Lesions are single at diagnosis in up to 70% of cases but are usually multifocal in late stages. The site of origin usually lies in the basal ganglia, corpus callosum, or periventricular subependymal regions. While imaging studies play an important role in the diagnosis of PCNSL, their role in the diagnosis of PIOL is more limited. Ocular imaging studies of 7 patients with biopsy-proven PIOL revealed ocular findings in only 4 of the patients on T1-weighted CT and magnetic resonance images after contrast injection.

Furthermore, these imaging modalities were not able to distinguish ocular lymphoma from other diseases such as uveitis or ocular melanoma.
Cerebrospinal fluid (CSF) should be sent for routine cytologic, chemical, and cytokine analysis. Lymphoma cells can be identified in the CSF of 25% of patients with known lesions on magnetic resonance imaging.\textsuperscript{[35]} If lymphoma cells are found in the CSF, then a diagnosis of PCNSL can be made and no further diagnostic procedures are necessary to obtain an intraocular biopsy in a suspected eye. However, even if lymphoma cells are not found in the CSF, a stereotactic brain biopsy should be considered for patients who have suspicious brain lesions on neuroimaging studies.\textsuperscript{[37]} For patients with no evidence of disease by neuroimaging or CSF, a diagnostic vitrectomy should be performed on the eye with more severe vitreitis or worse visual acuity.

**Ocular Tissue Diagnosis**

**Cytology and Pathology.**

Diagnostic vitrectomy is the most common surgical procedure used to confirm a clinical impression of PIOL. Vitreous specimens should be handled with care to protect the often fragile lymphoma cells. The undiluted sample should be placed in 3 to 5 ml of cell culture medium and immediately brought to the cytology laboratory for rapid processing.\textsuperscript{39,40}
Vitreous aspiration needle tap may also be used for diagnostic purposes. An advantage in this technique is that is can be completed in an outpatient setting without the need for monitored anesthesia. Lobo and Lightman \cite{41} recently reported 26 patients with suspected PIOL, 8 of whom were confirmed to have primary B- or T-cell intraocular lymphoma by this procedure.

Two of these 8 patients required retinal biopsies in addition to the vitreous tap to confirm diagnosis, 1 of whom had been treated with cyclosporine and oral prednisolone for 2 months prior to the vitreous tap. The remaining 18 patients were found to have infectious uveitis or nonspecific chronic inflammatory cells. The only complication noted in this procedure was a retinal detachment in one eye that developed 3 months after the procedure. Thus, this procedure may serve as a simpler mechanism to differentiate between infectious, malignant, and inflammatory causes of uveitis. Malignant lymphoma cells found in vitreous fluid and CSF are usually large and pleomorphic with scanty basophilic cytoplasm \cite{42, 43} Other typical findings include hypersegmented round, oval, bean, or clover shaped nuclei with prominent nucleoli and multiple mitoses \cite{39}. However, the identification of malignant cells in vitrectomy samples is on founded by the
presence of reactive immune cells, necrotic cells, debris, and fibrin. While CSF samples have less necrotic debris, they usually have few malignant cells.\textsuperscript{30, 44}

When attempts to obtain a vitrectomy specimen have been unsuccessful or no cells are evident in the specimen, chorioretinal biopsies may be considered.\textsuperscript{45} Chorioretinal biopsies can reveal a tumour cell infiltrate between the RPE and Bruch's membrane (perivascular clumps of tumor cells in the retina and optic nerve head, diffuse infiltration in the vitreous, and hemorrhagic retinal necrosis. Chorioretinal biopsies may also reveal areas of retinal depigmentation, atrophy, and scarring due to RPE detachment and choroidal reactive lymphocytic infiltration.\textsuperscript{32, 44} Fine-needle aspiration biopsy has been used in lieu of full thickness chorioretinal biopsy when vitrectomy was non-diagnostic. Lesions selected for this technique should be 1.5 mm or greater in height in order to prevent choroidal penetration.

This technique has an advantage over vitrectomy by providing increased concentration of viable cells for cytopathologic diagnosis and molecular studies.\textsuperscript{33, 34}
It is difficult to arrive at a pathologic diagnosis. Thus, research has been focused on developing other methods to assist in the diagnosis of PIOL. These methods include Immunohistochemistry, Flow cytometry, molecular analysis, and cytokine evaluation.\textsuperscript{48, 49}

\section*{IMMUNO HISTOCHEMISTRY}

\section*{INTRODUCTION}

Immunohistochemistry is the localization of antigens in tissue sections by the use of labelled antibody as specific reagents through antigen-antibody interactions that are visualized by a marker such as fluorescent dye, enzyme, radioactive element or colloidal gold.\textsuperscript{39}
Albert H. Coons and his colleagues (Coons et al. 1941, 1955; Coons and Kaplan 1950) were the first to label antibodies with a fluorescent dye, and use it to identify antigens in tissue sections. With the expansion and development of Immunohistochemistry technique, enzyme labels have been introduced such as Peroxidase (Nakane and Pierce 1966; Avrameas and Uriel 1966) and alkaline phosphatase (Mason and Sammons 1978). Colloidal gold (Faulk and Taylor 1971) label has also been discovered and used to identify immunohistochemical reactions at both light and electron microscopy level. Other labels include radioactive elements, and the immunoreactions can be visualized by autoradiography.

Since Immunohistochemistry involves specific antigen-antibody reaction, it has apparent advantage over traditionally used special enzyme staining techniques that identify only a limited number of proteins, enzymes and tissue structures. Therefore, Immunohistochemistry has become a crucial technique and widely used in many medical research laboratories as well as clinical diagnostics.

There are numerous Immunohistochemistry methods that may be used to localize antigens. The selection of a suitable method should be based on
parameters such as the type of specimen under investigation and the degree of sensitivity required.\textsuperscript{53}

**Tissue Preparation**

**Fixation:**

Tissue preparation is the cornerstone of Immunohistochemistry. To ensure the preservation of tissue architecture and cell morphology, prompt and adequate fixation is essential. However, inappropriate or prolonged fixation may significantly diminish the antibody binding capability\textsuperscript{54}.

There is no one universal fixative that is ideal for the demonstration of all antigens. However, in general, many antigens can be successfully demonstrated in formalin-fixed paraffin-embedded tissue sections. The discovery and development of antigen retrieval techniques further enhanced the use of formalin as routine fixative for Immunohistochemistry in many research laboratories.

The most common fixatives used for Immunohistochemistry are the followings:\textsuperscript{54}

- a. 4% paraformaldehyde in 0.1M phosphate buffer
- b. 2% paraformaldehyde with 0.2% picric acid in 0.1M phosphate buffer
c. PLP fixative: 4% paraformaldehyde, 0.2% periodate and 1.2% lysine in 0.1M phosphate buffer

d. 4% paraformaldehyde with 0.05% glutaraldehyde (TEM Immunohistochemistry)

Some antigens will not survive even moderate amounts of aldehyde fixation. Under this condition, tissues should be rapidly fresh frozen in liquid nitrogen and cut with a cryostat without infiltrating with sucrose. The sections should be kept frozen at -20 C or lower until fixation with cold acetone or alcohol. After fixation, the sections can be processed using standard immunohistochemical staining protocols

**Sectioning:**

Since its introduction, paraffin wax has remained the most widely used embedding medium for diagnostic histopathology in routine histological laboratories. Accordingly, the largest proportion of material for Immunohistochemistry is formalin-fixed, paraffin-embedded. Paraffin sections produce satisfactory results for the demonstration of majority of tissue antigens with the use of antigen retrieval techniques.\cite{55}
Certain cell antigens do not survive routine fixation and paraffin embedding. So the use of frozen sections still remains essential for the demonstration of many antigens. However, the disadvantage of frozen sections includes poor morphology, poor resolution at higher magnifications, special storage needed, limited retrospective studies and cutting difficulty over paraffin sections.

Microtome sections have some advantages when doing Immunohistochemistry since the tissue is not processed through organic solvents or high heat, which can destroy the antigenicity. In addition, the morphology of tissue sections is not disrupted due to a freezing and thawing needed.

**Whole Mount Preparation:**

Small blocks of tissue (less than 5 mm thick) can be processed as whole mounts. The advantage of whole mount preparations is that the results provide three dimensional information about the location of antigens without the need for reconstruction from sections. However, the major limitation of using whole mounts is antibody penetration may not be complete in the tissue, resulting in uneven staining or false negative staining. So Triton X-
100 or saponin treatment are used routinely for whole mount Immunohistochemistry to enhance penetration of the antibody.  

**Antigen Retrieval**

The demonstration of many antigens can be significantly improved by the pre-treatment with the antigen retrieval reagent that break the protein cross-links formed by formalin fixation and thereby uncover hidden antigenic sites. The techniques involved the application of heat for varying lengths of time to formalin-fixed, paraffin-embedded tissue sections in an aqueous solution (commonly referred to as the retrieval solution). This is called "Heat Induced Epitome Retrieval (HIER)". Another method uses enzyme digestion and is called "Proteolysis Induced Epitome Retrieval (PIER)".  

Microwave Oven, Pressure Cooker and Steamer are the most commonly used heating devices. Other devices also include the use of autoclave and water bath. The heating length of 20 minutes appears to be the most satisfactory and the cooling usually takes about 20 Minutes antibody applications. The TRIS-EDTA of pH9.0 and EDTA of pH8.0 are second most used retrieval solutions. Proteinase K is effective enzyme digestion reagent for membrane antigens such as Integrins, CD31, VWF, etc. PIER
methods (such as proteinase k, trypsin, chymotrypsin, pepsin, pronase and various other proteases) has also been reported for restoring immunoreactivity to tissue antigens with different degrees of success. However, the use of enzyme digestion method may destroy some epitopes and tissue morphology. Therefore the optimal enzyme concentration and incubation time need to be tested.

Combination of Heat Mediated and Proteolytic Enzyme Method is an alternative approach to unmask antigens if other methods did not work. It is especially useful when performing double or triple labelling of two or more antigens simultaneously.

Improving antibody penetration is also important for immunohistochemical staining of frozen and microtome sections. Triton X-100 is by far the most popular detergent for improving antibody penetration for Immunohistochemistry. However, it is not appropriate for the use of membrane antigens since triton X-100 destroy membranes. Some researchers prefer the freeze and thaw method for the improvement of antibody penetration. Sodium borohydride (1% in phosphate buffer) treatment is also widely used to unmask antigens, particularly in glutaraldehyde fixed tissue to reduce the glutaraldehyde linkages. 

58
IHC Methods

Blocking:

Background staining may be specific or non-specific. Inadequate or delayed fixation may give rise to false positive results due to the passive uptake of serum protein and diffusion of the antigen. Such false positives are common in the centre of large tissue blocks or throughout tissues in which fixation was delayed.

Antibodies, especially polyclonal antibodies, are sometimes contaminated with other antibodies due to impure antigen used to immunize the host animal.

The main cause of non-specific background staining is non-immunological binding of the specific immune sera by hydrophobic and electrostatic forces to certain sites within tissue sections. This form of background staining is usually uniform and can be reduced by blocking those sites with normal serum.

Endogenous peroxidise activity is found in many tissues and can be detected by reacting fixed tissue sections with DAB substrate. The solution for eliminating endogenous peroxidises activity is by the pre-treatment of the tissue section with hydrogen peroxide prior to incubation of primary antibody\(^{59}\).
Many tissues also contain endogenous alkaline phosphatase (AP) activity and should be blocked by the pre-treatment of the tissue section with levamisole if using AP as a label. Some tissues such as liver and kidney have endogenous biotin. To avoid unwanted avidin binding to endogenous biotin if using biotin-avidin detection system, a step is necessary for these tissues by the pretreatment of unconjugated avidin which is then saturated with biotin. Auto fluorescence or natural fluorescence exists in some tissues and can cause background problems when fluorescent dyes are used in the experiments. The simplest test is to view the tissue sections with a fluorescence microscope before any antibody incubation. If auto fluorescence is detected in the tissue sections, the best solution is to avoid use of fluorescent method but choose enzyme or other labelling methods.

**Controls:**

Special controls must be run in order to test the protocol and for the specificity of the antibody being used. Positive control is to test a protocol or procedure and make sure it works. It will be ideal to use the tissue of known positive as a control. If the positive control tissue showed negative staining, the protocol or procedure needs to be checked until a good positive staining is obtained.
Negative control is to test for the specificity of an antibody involved. First, no staining must be shown when omitting primary antibody or replacing a specific primary antibody with normal serum (must be the same species as primary antibody). This control is easy to achieve and can be used routinely in immunohistochemical staining.

Second, the staining must be inhibited by adsorption of a primary antibody with the purified antigen prior to its use, but not by adsorption with other related or unrelated antigens. This type of negative control is ideal and necessary in the characterization and evaluation of new antibodies but it is sometimes difficult to obtain the purified antigen, therefore it is rarely used routinely in immunohistochemical staining. 60

**Direct Method:**

Direct method is one step staining method, and involves a labelled antibody (i.e. FITC conjugated antiserum) reacting directly with the antigen in tissue sections. This technique utilizes only one antibody and the procedure is short and quick. However, it is insensitive due to little signal amplification and rarely used since the introduction of indirect method.
**Indirect Method:**

Indirect method involves an unlabeled primary antibody (first layer) which reacts with tissue antigen, and a labeled secondary antibody (second layer) reacts with primary. This method is more sensitive due to signal amplification through several secondary antibody reactions with different antigenic sites on the primary antibody.

In addition, it is also economy since one labeled second layer antibody can be used with many first layer antibodies (raised from the same animal species) to different antigens. The second layer antibody can be labeled with a fluorescent dye such as FITC, Rhodamine or Texas red, and this is called indirect immunofluorescence method. The second layer antibody may be labeled with an enzyme such as peroxidase, alkaline phosphatase or glucose oxidase, and this is called indirect immunoenzyme method $^{50,51}$.

**PAP Method (peroxidase anti-peroxidase method):**

PAP method is a further development of the indirect technique and it involves a third layer which is a rabbit antibody to peroxidase, coupled with peroxidase to make a very stable peroxidase anti-peroxidase complex. The complex, composed of rabbit gaba-globulin and peroxidase, acts as a third
layer antigen and becomes bound to the unconjugated goat anti-rabbit gaba-globulin of the second layer. The sensitivity is about 100 to 1000 times higher since the peroxidase molecule is not chemically conjugated to the anti-IgG but immunologically bound, and loses none of its enzyme activity. It also allows for much higher dilution of the primary antibody, thus eliminating many of the unwanted antibodies and reducing non-specific background staining.

**Avidin-Biotin Complex (ABC) Method:**

ABC method is standard IHC method and one of widely used technique for immunohistochemical staining. Avidin, a large glycoprotein, can be labelled with peroxidase or fluoresce in and has a very high affinity for biotin. Biotin, a low molecular weight vitamin, can be conjugated to a variety of biological molecules such as antibodies. The technique involves three layers. The first layer is unlabeled primary antibody. The second layer is biotinylated secondary antibody. The third layer is a complex of avidin-biotin peroxidase\(^5\). The peroxidase is then developed by the DAB or other substrate to produce different colorimetric end products.

**Labeled StreptAvidin Biotin (LSAB) Method:**
Streptavidin, derived from streptococcus avidini, is a recent innovation for substitution of avidin. The streptavidin molecule is uncharged relative to animal tissue, unlike avidin which has an isoelectric point of 10, and therefore electrostatic binding to tissue is eliminated. In addition, streptavidin does not contain carbohydrate groups which might bind to tissue lectins, resulting in some background staining.

LSAB is technically similar to standard ABC method. The first layer is unlabeled primary antibody. The second layer is biotinylated secondary antibody. The third layer is Enzyme-Streptavidin conjugates (HRP-Streptavidin or AP-Streptavidin) to replace the complex of avidin-biotin peroxidase. The enzyme is then visualized by application of the substrate chromogen solutions to produce different colorimetric end products. The third layer can also be Fluorescent dye-Streptavidin such as FITC-Streptavidin if fluorescence labeling is preferred. A recent report suggests that LSAB method is about 5 to 10 times more sensitive than standard ABC method.

Polymeric Methods:

1. EnVision Systems are based on dextran polymer technology. This unique chemistry permits binding of a large number of enzyme molecules (horseradish peroxidase or alkaline phosphatase) to a
secondary antibody via the dextran backbone. The benefits are many, including increased sensitivity, minimized non-specific background staining and a reduction in the total number of assay steps as compared to conventional techniques. The simple protocol is i) Application of primary antibody; ii) Application of enzyme labelled polymer; iii) Application of the substrate chromogen. EnVision+ was developed after EnVision to provide increased sensitivity.

2. Impress polymerized reporter enzyme staining system is based on a new method of polymerizing enzymes and attaching these polymers to antibodies. The novel approach employed to form enzyme "micropolymers" avoids the intrinsic shortcomings of using large Dextrans or other macromolecules as backbones. Attaching a unique "micropolymer with a high density of very active enzyme to a secondary antibody generates a reagent that overcomes interference and provides enhanced accessibility to its target. The result is outstanding sensitivity, signal intensity, low background staining, and reduced non-specific binding. The simple protocol is i) Application of primary antibody; ii) Application of enzyme labelled polymer; iii) Application of the substrate chromogen.
**CSA Methods From Dako:**

CSA Systems use Tyramide Signal Amplification. It is ideal for the following applications:

i. Detecting small quantities of antigen;

ii. Enhancing performance of low affinity mouse and rabbit antibodies;

iii. Enabling compatibility of certain "tough" mouse and rabbit antibodies with paraffin embedded tissue sections.

The simple protocol is as follows:

i. Application of primary antibody.

ii. Application of biotinylated linking antibody.

iii. Application of the Tyramide Amplification Reagent.

iv. Application of Streptavidin-HRP.

v. Application of the substrate chromogen

2) CSA II - Biotin-free Tyramide Signal Amplification System is a highly sensitive immunohistochemical (IHC) staining procedure incorporating a signal amplification method based on the peroxidase-catalyzed deposition of a fluorescein-labelled phenolic compound, followed by a secondary reaction with a peroxidase-conjugated anti-fluorescein. In the procedure, a mouse primary antibody is first detected with a peroxidase-conjugated secondary
antibody. The next step utilizes the bound peroxidase to catalyze oxidation of a fluorescein-conjugated phenol (fluorescyl-tyramide) which then precipitates onto the specimen. The procedure is continued with detection of the bound fluorescein by a peroxidase-conjugated anti-fluorescein. Staining is completed using diaminobenzidine/hydrogen peroxide as chromogen/substrate, and can be observed with a light microscope. In comparison to standard immunohistochemical methods, such as labelled streptavidin biotin (LSAB) or avidin-biotin complexes (ABC), tyramide amplification methods have been reported to be many fold more sensitive.

The CSA II System is a simplified version of the extremely sensitive Catalyzed Signal Amplification System (code K1500) that utilizes biotinyl-tyramide. The highly sensitive CSA II System allows for the detection of very small quantities of target protein, as well as for the use of low affinity antibodies. This reagent system utilizes fluorescyl-tyramide, rather than biotinyl-tyramide, and does not contain avidin/ biotin reagents, thus eliminating potential background staining due to reactivity with endogenous biotin.

Principles of Procedure: The specimens are first incubated with Peroxidase Block for five minutes to quench endogenous peroxidase activity. The specimens are then incubated for five minutes with a protein
block to suppress nonspecific binding of subsequent reagents, followed by a 15-minute incubation with an appropriately characterized and diluted mouse primary antibody or negative control reagent (user provided). This is followed by sequential 15-minute incubations with anti-mouse immunoglobulins-HRP, fluorescyl-tyramide hydrogen peroxide (amplification reagent) and anti-fluorescein-HRP. Staining is completed by a five-minute incubation with 3,3' diaminobenzidine tetrahydrochloride (DAB)/hydrogen peroxide, which results in a brown precipitate at the antigen site.

**Immunohistochemistry Sensitivity Chart:**

<table>
<thead>
<tr>
<th>Standard Sensitivity</th>
<th>Moderate Sensitivity</th>
<th>Most Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABC Method, PAP Method, LSAB Method</td>
<td>EnVision, EnVision+, Immpress</td>
<td>CSA Method, CSA II Method</td>
</tr>
</tbody>
</table>

**Multiple Labeling**
It is often useful to be able to stain for two or more antigens in one common tissue section.

This can be achieved by immunofluorescence method using different fluorescent dyes.

Multiple staining can also be done with peroxidase conjugated antibodies developed with different chromogen substrates to produce the end products of different colours. There are three basic approaches in planning multiple staining: parallel, sequential and adjacent. In addition, the antibody dilution and condition [http://www.ihcworld.com/WebOriginal/epitope_retrieval.htm](http://www.ihcworld.com/WebOriginal/epitope_retrieval.htm) are also important factors to be considered. Finally, appropriate colour combination is also crucial since improper colour combination may produce poor result and fail to demonstrate multiple antigens in the same section. For best result, the careful design and test of multiple staining protocols are necessary.
AIMS AND OBJECTIVES

1. To study the demographic distribution of orbital lymphoma – Age wise
   Sex wise
2. To study the presenting complaints , and the duration of the symptoms
3. To study the common site of orbital lesions .
4. To study the confirmatory role of Immunohistochemistry in giving a confirmatory diagnosis of orbital lymphoma

**METHODOLOGY**

This prospective study has enrolled patients with orbital lymphoma coming to the orbit clinic of a tertiary eye care centre from September 2009 to November 2011.
Any case of orbital lymphoma that presented for the first time to the hospital was included in the study.

Follow up cases were excluded from the study there by avoiding repetition.

The orbital lymphomas were diagnosed based on the clinical history, eye examination, laboratory investigations, as well as radiological investigation like X ray, USG, CT scan were undergone by the patient as required. Histopathological examination using the light microscopic examination of H and E stained slides after fixation, paraffin embedding and section were done. Samples were also subjected to immunohistochemical analysis to have a confirmatory diagnosis.

This study includes orbital lymphoma confirmed by radiological and clinical evaluation being subjected to immunohistochemical analysis for confirmation.
RESULTS

AGE:

In total 39 patients presented to us, the number of patients at the age group of less than 40 were around 6 at an average of 15.4%, the number of patients at the age group between 41 - 50 were around 8 at an average of 20.5%, the number of patients at the age group between 51 – 60 were 16 at an average of 41.0%, the number of patients at the age group between 61
– 70 were 4 at an average of 10.3 %, the number of patients beyond the age of 70 were 5 at an average of 12.8%.

Finally, the mean average age at presentation was around 51 – 60.

<table>
<thead>
<tr>
<th>Age category</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;=40</td>
<td>6</td>
<td>15.4</td>
</tr>
<tr>
<td>41 – 50</td>
<td>8</td>
<td>20.5</td>
</tr>
<tr>
<td>51 – 60</td>
<td>16</td>
<td>41.0</td>
</tr>
<tr>
<td>61 – 70</td>
<td>4</td>
<td>10.3</td>
</tr>
</tbody>
</table>
In the total of 39 patients presented to us the sex wise distribution showed a increase in the Total number of male sex when compared to female sex.

A total of 22 males at an average of 56.4% and a total of 17 females at an average of 43.6% reported.

Sex
### DURATION OF SYMPTOMS

In the total of 39 cases presented to us the duration of symptoms more than 6 months were reported in 25 patients with an average of around 64.1%. and the duration of symptoms less than 6 months were reported in 14 patients at an average of around 35.9%.
<table>
<thead>
<tr>
<th>Duration</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;=6months</td>
<td>25</td>
<td>64.1</td>
</tr>
<tr>
<td>&gt;6months</td>
<td>14</td>
<td>35.9</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>100</td>
</tr>
</tbody>
</table>

**PRESENTING COMPLAINTS**

In the total of 39 patients, the number of patients who complained of swelling were 19 at an average of 48.7%, the number of patients who complained of prominence and protrusion of the eye ball were 9 and 3 with an average of 23.1% and 7.7%, the number of patients who complained of drooping of eye lid, mass in the eye, defective vision and proptosis were 1...
at an average of 2.6 %, the number of patients who complained of pain and redness were 4 and 2 at an average of 10.3 % and 5.1 %, the number of patients who complained of growth in the eye were around 3 at an average of 7.7 %

<table>
<thead>
<tr>
<th>Complaints</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swelling</td>
<td>19</td>
<td>48.7</td>
</tr>
<tr>
<td>Prominence</td>
<td>9</td>
<td>23.1</td>
</tr>
<tr>
<td>Protrusion</td>
<td>3</td>
<td>7.7</td>
</tr>
<tr>
<td>Drooping of eye lids</td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td>Pain</td>
<td>4</td>
<td>10.3</td>
</tr>
<tr>
<td>Redness</td>
<td>2</td>
<td>5.1</td>
</tr>
<tr>
<td>Fullness</td>
<td>3</td>
<td>7.7</td>
</tr>
</tbody>
</table>
In the total of 39 patients who presented to us, the number of patients who had the tumour incised from the conjunctiva were around 6 at an average of 15.4%, the number of patients who had tumour incised from the lacrimal sac area were 1 at an average of 2.6%, the number of patients who had tumor incised from the inferior quadrant of the orbit were 11 at an
average of around 28.2 %, the number of patients who had tumour incised from the superior quadrant were 17 at an average of around 43.6 %, the number of patients who had tumour incised from the lateral quadrant were 3 at an average of around 7.7 %, the number of who had the biopsy from an enucleated eye were 1 at an average of 2.6 %

<table>
<thead>
<tr>
<th>SITE OF BIOPSY</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjunctival mass</td>
<td>6</td>
<td>15.4</td>
</tr>
<tr>
<td>Incision made over lacrimal sac</td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td>Incision made from lower quadrant</td>
<td>11</td>
<td>28.2</td>
</tr>
<tr>
<td>Incision made from superior quadrant</td>
<td>17</td>
<td>43.6</td>
</tr>
<tr>
<td>Incision made from lateral quadrant</td>
<td>3</td>
<td>7.7</td>
</tr>
<tr>
<td>Enucleation done</td>
<td>1</td>
<td>2.6</td>
</tr>
</tbody>
</table>
DISCUSSION

Knowles DM et al. in his study has stated that lymphomas belonged to the Non Hodgkin’s lymphoma B cell type. Medrios LJ et al in his study of 99 cases also stated that most of the orbital lymphomas belonged to the Non Hodgkin’s lymphoma B cell type. Watkins LM et al., Keith D. Carter et al and Jeffrey A. Nerad, et al in their study of 57 cases stated
that Most lymphomas found in this area of the orbit are subtypes of B-cell lymphoma. Shields JA et al, 67 et al in their Survey of 1264 of varying orbital tumour patients, 111 of them were orbital lymphomas and all 111 were found to be Non Hodgkin’s lymphoma. Sullivan TJ al 69 in their study of 69 patients stated that most of the primary ocular and adnexal lymphomas were of Non Hodgkins B cell lymphoma type.

In our study of 39 cases we also have confirmed that most orbital lymphoma are of the Non Hodgkins B cell type Knowles DM 64 has stated that the average age at presentation is 50 to 70 years with a median age in the 60’s , M.C. prada et al also stated that the average age at presentation is 50 to 70.

Henderson et al 73 also states that almost all lymphomas with orbital involvement occur after the forth decade. Jack root man et al states that the lymphomas are usually characterized by an onset in the sixth and seventh decade. Shields JA et al, 67 et al in their Survey of 1264 patients with varying orbital tumours have found out that of the 111 patients with orbital lymphoma were of the age group between 60 to 70 years.
Johnson TE \(^{68}\) in their study of 77 cases have found out that the average age of presentation was 50 to 90 years with the average age being 66 years. Sullivan TJ et al \(^{69}\) in their study of 69 patients found out that the average age at presentation was between 50 to 70 years and the median age of presentation was 66 years. Sullivan TJ et al \(^{69}\) in their study of 69 patients stated that the average age at presentation was between 60 to 80 years and an average age at presentation being 60 years.

Shields, CL et al \(^{70}\) in their Clinical Analysis of 117 Cases have stated that the mean age at ocular presentation was 61 years. Fung CY et al \(^{71}\) in the study of 98 patients stated that the average age at presentation was 40 to 60 years and mean average age being 63 years. In our study of 39 cases also the average age at presentation was between 50 to 60 years. Knowles DM et al et al \(^{64}\) in their study of 108 cases has found out that there was a female dominance over male. Sharara N et al, \(^{72}\) in their study of 43 cases stated that there were 26 females and 17 males in the study. Johnson, TE et al \(^{68}\) in their study of 77 cases 36 patients were men and 41 were female. Sullivan TJ et al \(^{69}\) in their study of 69 patients there were 39 women and 30 men. Shields CL \(^{70}\) in their Clinical Analysis of 117 Cases there were 55 males and 62 females. Fung CY \(^{71}\) in the study Of the 98 patients, 56 were
females and 42 were males. Henderson et al 73 in a study of 175 cases, 94 were males and 81 were females.

In our case study of 39 cases, there is a slight preponderance of male 22 cases and female 17 cases.

Henderson et al 73 in his study of 175 cases has reported that the mean duration of presenting complaints was between 5 to 10 months. Funng CY 71, in their study of 117 cases has reported that the mean duration of symptoms was 11 months. Sullivan TJ et al 69 in their study of 69 patients has reported that the average duration of symptoms were around 7 to 8 months. Thomas E Johnson, David T Tse and Gerald E Burne at al in their study of 77 cases reported that the average duration of symptoms were between 8 to 10 months.

In our study of 39 patients, the duration of symptoms were less than 6 months.

Sharara N et al, 72 in their study of 17 in cases stated that the site of lesion was in orbit 10 conjunctiva 4, eyelid 2 and lacrimal sac 1. Sullivan TJ et al 69 in their study of 69 patients has reported that Fifty-three patients had orbital involvement, 8 had conjunctival, 4 had lacrimal sac, and 4 had lymphoma involving the eyelids Shields CL Smith et al 70 in their Clinical Analysis of 117 Cases have stated that the site of lesion was eye lids in 3
cases, orbit in 18, ocular involvement in 27 cases, the lesion in limbal area 8, mid bulbar area 48, fornix 52 and caruncle 8. Fung CY 71, et al in their study of 117 cases has reported that the two most commonly involved sub sites were the conjunctiva (55%) and nonspecific intraorbital tissue (50%). The lacrimal apparatus was involved in 24% of cases, extraocular muscle in 11%, and soft tissues of the eyelid in 9%. Extraorbital extension, such as into the bone or ethmoid sinus, was noted in 6%, primarily in high-grade lesions. Involvement of the periorbital skin was rare (1%), as was extrinsic compression of the optic nerve (1%).

In our case study of 39 cases the site of lesion in In the total of 39 patients who presented to us, the number of patients who had the tumour incised from the conjunctiva were around 6 at an average of 15.4%, the number of patients who had tumour incised from the lacrimal sac area were 1 at an average of 2.6%, the number of patients who had tumor incised from the inferior quadrant of the orbit were 11 at an average of around 28.2%, the number of patients who had tumour incised from the superior quadrant were 17 at an average of around 43.6%, the number of patients who had tumour incised from the lateral quadrant were 3 at an average of around 7.7%, the number of who had the biopsy from an enucleated eye were 1 at an average of 2.6%. Thus the majority of patient presented to us
with the site of lesion being more common at the superior quadrant of the orbit. Shields C et al. 70 in their Clinical Analysis of 117 Cases the patients presenting with complaints of mass were 35, irritation- 34, ptosis - 9, epiphora- 8, blurred vision - 6 proptosis- 4 diplopia- 3. Thomas E Johnson, David T Tse and Gerald E Burne at al in their study of 77 cases the presenting complaints of mass – 52, ptosis – 7, diplopia - 8, pain – 7. Sullivan TJ 69, in their study of 69 patients Proptosis 48 (70% ), Swelling 41 (59%), Mass 38 (55%), Inflammation 21 (30%), Pain 13 (19%), Diplopia 10 (14%), Visual loss 8 (12%), epiphora 3 (4%), Ptosis 5 (7%), Dacryocystitis 4 (6%).

In our study of 39 cases the number of patients who complained of swelling were 19 at an average of 48.7%, the number of patients who complained of prominence and protrusion of the eye ball were 9 and 3 with an average of 23.1% and 7.7%, the number of patients who complained of drooping of eye lid, mass in the eye, defective vision and proptosis were 1 at an average of 2.6%, the number of patients who complained of pain and redness were 4 and 2 at an average of 10.3% and 5.1%, the number of patients who complained of growth in the eye were around 3 at an average of 7.7%
CONCLUSION

- In our study the age wise distribution showed that the common age group at the time of presentation was between 51-60.
- In our study males were more than females.
- In our study the most common presenting symptom was proptosis followed by the presence of a mass.

- In our study the average duration of the symptoms was around 6 months.

- In our study the common site of lesion was superior orbital quadrant.

- In our study of 39 patients, we have stressed as well as confirmed the confirmatory role played by Immunohistochemistry in diagnosis of Non Hodgkin’s lymphoma B cell type.

- In our study of 39 patients, all the orbital lymphomas were of Non Hodgkin’s lymphoma B cell Type
ATYPICAL LYMPHOID CELLS WITH INTERSPERSED SMALL LYMPHOCYTES
NON HODGKINS LYMPHOMA (HAEMATOXILIN & EOSIN STAIN)
LEUCOCYTE COMMON ANTIGEN POSITIVE
CD 20
CD-3 POSITIVE CELLS


61. Immunochestochemical Staining Methods. 4 Ed Dako Guide


74. Garrity, James A Henderson's Orbital Tumors Ed. 4th, Philidelberg: LWW, 2007. ix, 393

75. Moreiras, Jose V P Orbit: examination, diagnosis, microsurgery and pathology / Vol: 2 - Highlights of Ophthalmology, 2004