RESEARCH ARTICLE

Clinicopathologic Assessment of Ocular Adnexal Lymphoproliferative Lesions at a Tertiary Eye Hospital in Iran

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Abstract

    <u>Background</u>: The most common type of ocular lymphoma is non-Hodgkin lymphoma (NHL), categorized into two groups: indolent (slow growing) and aggressive (rapid growing). Differentiating benign reactive lymphoid hyperplasia (RLH) from malignant ocular adnexal lymphoma (OAL) is challenging. Histopathology, immunohistochemistry (IHC) and flow cytometry have been used as diagnostic tools in such cases. <u>Materials and Methods</u>: In this retrospective case series, from 2002 to 2013 at Farabi Eye Center, 110 patients with ocular lymphoproliferative disease were enrolled. Prevalence, anatomical locations, mean age at diagnosis and the final diagnosis of the disease with IHC were assessed. Comparison between previous pathologic diagnoses and results of IHC was made. Immunoglobulin light chains and B-cell and T-cell markers and other immuno-phenotyping markers including CD20, CD3, CD5, CD23, CD10, CYCLIND1 and BCL2 were evaluated to determine the most accurate diagnosis. The lymphomas were categorized based on revised European-American lymphoma (REAL) classification. <u>Results</u>: Mean age ± SD (years) of the patients was 55.6 ± 19.3 and 61% were male. Patients with follicular lymphoma, large B-cell lymphoma or chronic lymphocytic leukemia/small cell lymphoma (CLL/SLL) tended to be older. Nine patients with previous diagnoses of low grade B-cell lymphoma were re-evaluated by IHC and the new diagnoses were as follows: extranodal marginal zone lymphoma(EMZL) (n=1), SLL(n=1), mantle cell lymphoma (MCL) (n=3), reactive lymphoid hyperplasia RLH (n=2). Two cases were excluded due to poor blocks. Flow cytometry reports in these seven patients revealed SLL with positive CD5 and CD23, MCLwith positive CD5 and CyclinD1 and negative CD23, EMZL with negative CD5,CD23 and CD10. One RLH patient was negative for Kappa/Lambda and positive for CD3 and CD20 and the other was positive for all of the light chains, CD3 and CD20. Orbit (49.1%), conjunctiva (16.1%) and lacrimal glands (16.1%) were the most common sites of involvement. <u>Conclusions</u>: Accurate pathological classification of lesions is crucial to determine proper therapeutic approaches. This can be achieved through precise histologic and IHC analyses by expert pathologists.

Keywords: Ocular adnexal lymphoproliferative disease - immunohistochemistry - pathology - lymphoma

Introduction

    Neoplastic transformation of cells that occur mainly within lymphoid tissues result in lymphomas (Esmaeli and Sniegowski, 2015). Lymphomas are categorized in many orders, generally, into two groups of Hodgkin's lymphoma and non-Hodgkin's lymphoma (NHL). According to the revised European-American lymphoma (REAL) classification lymphomas are classified into B-cell, NK/T-cell and Hodgkin's lymphoma. In addition, based on how rapidly the cancer grows, it may be indolent (slow growing) or aggressive (rapid growing) (Harris et al, 1994). B-cell, non-Hodgkin's lymphomas (NHLs) constitute major cases of OALs which mainly include EMZL of MALT type, follicular lymphoma (FL), mantle cell lymphoma (MCL), diffuse large B-cell lymphoma

(DLBCL) and lymphoplasmacytic lymphoma (Aronow, 2015). Rare NK/T-cell lymphoma is also described at the bottom of the list of orbital lymphomas.

    Ocular adnexal lymphoproliferative disease (OALD) encompasses a wide range from benign reactive lymphoid hyperplasia (RLH) to malignant ocular adnexal lymphoma (OAL) (Gündüz and Esmaeli, 2008). None of the radiologic and clinical features of a lymphoid lesion can definitely differentiate RLH from ocular malignant lymphoma. However, they can be distinguished from each other by IHC and histopathologic analysis. In addition, routine staining is not diagnostic in some cases and therefore, immunohistochemistry (IHC) and polymerase chain reaction (PCR) investigations will come in hand. To be noted, differentiating lymphoma and RLH is still challenging (Knowles and Jakobiec, 1980).

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In the histopathologic evaluation of RLH, a variety of mature and well differentiated cell types are seen. Using light microscopic evaluation, lymphoid follicles with irregular shapes and distribution, germinal centers with tangible body macrophages and significant mitotic activity are found in RLH. IHC analysis regarding immunoglobulin profile in RLH shows both T-cell-rich subtypes (60% T-cell), predominantly helper T-cells, and B-cell-rich subtypes (Othman, 2009). OAL is rare and comprises only 1-2% of NHL of the eye (Eckardt et al., 2013). The majority of these cases are extra-nodal marginal zone B-cell lymphomas (EMZLs) of mucosa associated lymphoid tissue (MALT), also named as MALT lymphoma or MALToma (Lee et al., 2013). OALs are recognized with predominantly monoclonal B-cells (more than 60%) with only one type of light chain (mostly kappa chain) (Ioachim and Medeiros, 2009). Morphologically heterogeneous small B-cells including marginal zone (centrocyte-like) cells, small lymphocytes, monocytic-like cells and centroblastic patterns in some cases are the pathologic features of ocular lymphoma (Othman, 2009). Rare tangible macrophages and endothelial proliferation, despite RLH, are other histopathologic characteristics of OAL. Furthermore, anaplasia and Dutcher bodies are important signs of malignant lesions in OAL (Ioachim and Medeiros, 2009).

Since different types of cell behavior necessitate different treatment protocols, it is important to distinguish them from each other (Aronow and Singh, 2014). Radiotherapy alone is considered to be an effective therapy for primary orbital tumors (Bhatia et al., 2002). Treatment consists of steroid therapy in reactive lymphoid tumors and chemotherapy, radiotherapy and/ or chemo-radiotherapy in more advanced tumors (Pfeffer et al., 2004). Differentiating indolent from aggressive lymphoma is crucial; in the aggressive type treatment is started immediately, while watchful waiting may be applied for indolent tumors. Common clinical signs and symptoms of orbital lymphoma are visible or palpable mass, exophthalmos, ocular pain and eye motility/visual restrictions (Eckardt et al., 2013).

In this study we focused on ocular NHL. The main goal of the present study is to assess histopathology and IHC results of orbital lymphoid tumors based on REAL classification. Consequently, we intended to: 1) describe the prevalence of different subtypes of malignant lymphoma in our 10-year data registry, 2) extract the benign types of lymphoid hyperplasia, 3) re-evaluate the undetermined, intermediate- and high-grade malignant lymphoma cases with IHC method, 4) address the challenging debate on differentiating RLH and malignant lymphomas based on histopathologic features.

Materials and Methods

In this retrospective non-comparative case series, all cases with OALD, from 2002 to 2013 at Farabi Eye Hospital considered. We enrolled 112 patients. Definite pathological diagnoses were made accordingly, from benign RLH to malignant lymphoma subtypes (based on REAL classification). Anaplasia, mitotic activity,

tangible macrophages, endothelial proliferation, Dutcher bodies and other significant features were evaluated to distinguish between RLH and malignant OAL. Flow cytometry and IHC were applied to determine the exact type of lymphoma. Indolent lymphomas such as chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), FL and EMZL were differentiated based on both hematoxylin and eosin (H&E) staining and/ or IHC methods. RLH was the diagnosis when there were no atypia, anaplasia and Dutcher cells. In addition, well differentiated lymphocytes along with plasma cell, eosinophil and histiocyte infiltration were all markers of benign reactive hyperplasia. Indolent and aggressive lymphomas such as CLL/SLL and MCL were distinguished by flow cytometry (CD5, CD20) and also Cyclin D1.

The basic demographic features of the patients and also previous pathologic diagnoses were collected from the patients' records. We also extracted the pathologic cell blocks, H&E-stained slides, and IHC stained samples (if available) from the lab data bank registry. IHC and H&E staining were re-evaluated for each patient and the final diagnosis was made by an expert pathologist who was unaware of the previous diagnosis. The comparison between previous registered diagnoses in patients' records and results of IHC was done. Two Patients with inappropriate cell blocks for reliable IHC records were excluded. If the IHC/H&E staining was done before, we re-evaluated it. In case of low quality H&E staining, we did the H&E staining again to preclude case exclusion.

Statistical analysis

All data were analyzed by SPSS software version 14 (SPSS Inc., Chicago, IL, USA). For continuous variables, mean ± SD was calculated. We compared means of continuous variables with independent samples t-test. A p-value<0.05 was considered as statistically significant.

Results

Basic demographic features of patients with malignant lymphomas and anatomic sites intruded by different malignant lymphoma subtypes are depicted in table1. Two cases were excluded from the study due to inappropriate cell block for IHC examination. Hence, 110 patients were recruited for final analysis. The mean age±SD (years) of all patients was 55.6±19.3 (male, 60.5%) which was 58.7±19.9 in male and 50.7±17.5 in female patients (p-value=0.03).

The frequency of OAL, RLH and atypical lymphoid hyperplasia (ALH) were 63(57.8%), 44(40.4%) and 2(1.8%) respectively, based on IHC and H&E reports. The prevalence of RLH was 20(46.5%) in women and 24(36.4%) in men. Sex distribution of OAL subtypes are shown in Table 1. Patients with FL, DLBCL and CLL/ SLL were older than the others. Majority of malignant cases were EMZL and CLL/SLL, with the prevalence of 30% and 28.6%, respectively. In our study, orbit (49.6%), conjunctiva (16.5%) and lacrimal gland (16.5%) were the most involved sites with OALD's (Table2). RLH mostly affected orbit (n=14), lacrimal glands(n=13), and

Table 1. Basic Demographic Features of Patients and Involved Anatomic Sites of Malignant Tumors

Diagnosis	Number(%)	Mean Agea(Range)	Female(%)	Male(%)	Anatomic site(%)			
					Orbit	Lacrimal gland	Conjunctiva	Eye lids
EMZL*	19(30.1)	58.2(31-83)	6(31.5)	13(68.5)	13(68.4)	1(5.3)	2(10.5)	3(15.8)
CLL/SLL**	18(28.6)	59.8(35-81)	10(55.6)	8(44.4)	11(61.1)	1(5.6)	2(11.1)	4(22.2)
MCL***	13(20.6)	55.6(20-84)	3(23)	10(77)	9(62.2)	1(7.7)	1(7.7)	2(15.4)
DLBCL****	6(9.5)	68.8(65-81)	3(50)	3(50)	2(33.3)	0	1(16.7)	3(50)
FL*****	2(3.2)	70(29-82)	1(50)	1(50)	2(100)	0	0	0
Plasmacytoma	2(3.2)	58(47-69)	1(50)	1(50)	0	2(100)	0	0
T-cell lymphoma	2(3.2)	64(35-71)	2(100)	0(0)	2(100)	0	0	0
TLGL******	1(1.6)	19	0(0)	1(100)	1(100)	0	0	0
TOTAL	63	59	26(41.2)	37(58.8)	40(63.5)	5(7.9)	6(9.5)	12(19.1)

ª years old; * extra-nodal marginal zone lymphoma; ** chronic lymphocytic leukemia/small lymphocytic lymphoma; ***mantle cell lymphoma;****diffuse large B-cell lymphoma; *****follicular lymphoma; ******T-cell large granular lymphocytic leukemia

Table 2. Anatomic Sites of Ocular Adnexal Lymphoproliferative Diseases

Site	Number (Prevalence, %)
Orbit	54(49.6)
Conjuctiva	18(16.5)
Lacrimal glands	18(16.5)
Lower eyelid	10(9.2)
Upper eyelid	7(6.4)
Canthus	1(0.9)
Lacrimal sac	1(0.9)
Total	109(100)

Table 3. Chief Complaints of Patients with Ocular Adnexal Lymphoproliferative Diseases

Clinical presentation	Prevalence(%)
Eye swelling/mass	47(43.1)
Visual disturbance	2(1.8)
Lacrimal glands swelling	3(2.8)
Pain/redness	1(0.9)
undetermined	56(51.4)
Total	109(100)

conjunctiva (n=12). Tables 1 and 2 demonstrate the results after IHC re-evaluation.

We also compared the diagnoses before and after applying IHC method in order to demonstrate the importance of IHC in categorizing different lymphoma subtypes. Nine patients with the previous diagnoses of low grade B-cell lymphoma were re-assessed with IHC, and the final diagnoses were as follows: EMZL (n=1), CLL/SLL (n=1), MCL (n=3), RLH (n=2). The other two were excluded because of poor block quality. Flowcytometry reports in these seven patients revealed CLL/SLL with positive CD5 and CD23, MCL with positive CD5 and Cyclin D1 and negative CD23, EMZL with negative CD5, CD23 and CD10 and RLH. One RLH slide was negative for Kappa/Lambda and positive for CD3 and CD20, while the other was positive for all types of the light chains, CD3 and CD20. All 44 cases of RLH were re-confirmed as benign reactive hyperplasia and four patients who were previously diagnosed with ALH were re-evaluated and the following findings emerged: EMZL (n=1), MCL (n=1) and two cases were truly defined as ALH. One sample that was reported to be a malignant small round cell tumor turned

out to be a malignant melanoma. To be mentioned, among those records which included patients' chief complaints, eye swelling/mass was the most common (Table 3).

Discussion

Ocular NHL is highly prevalent among asian / pacific islanders in comparison with western populations (Moslehi et al., 2011). This study was conducted to re-evaluate previous histopathological diagnoses of OALD using IHC to precisely differentiate OAL from non-OAL types (RLH and ALH) of NHL specimens. ALH or pseudolymphoma is a morphologically intermediate type of lymphoid hyperplasia which can not fulfil all diagnostic criteria of OAL (Li et al., 2015). Our findings revealed a high prevalence of OAL (57.8%) followed by RLH (40.4%) among OALDs. Prior investistigations have reported a frequency of 68-75% and 9-28% for OAL and RLH, respectively (Knowles and Jakobiec , 1980; Mannami et al., 2001; Oh and Kim, 2007). This diversity in results can be explained by geographical differences in terms of genetics, host factors, environmental parameters and prevalence of infectious agents. As an example, Chlamydia psittaci is supposed to have a positive association with ocular adnexal NHL (Moslehi et al., 2011).

Orbital lymphoma has been shown to be the most common malignancy of the eye (Domingo et al., 2015). The greatest number of OALD cases in our results and several previous studies were seen in the orbit (Laucirica and Font, 1996; Coupland et al., 1998), in contrast to conjunctiva in other ones (Oh and Kim, 2007; Alkatan et al., 2013).

In our study when H&E staining was in favor of indolent lymphomas such as CLL/SLL, FL and EMZL, specific IHC cytometric appraisals ensued. As an instance, CD5 and CD23 markers for CLL/SLL or CD10 and BCL2 markers for FL were checked. As we wanted to draw a distinction between indolent and aggressive lymphomas (i.e., CLL/SLL vs. MCL), we tested cyclin D1 besides CD5 and CD23 to better define the subtypes. Indeed, we applied precise individualized techniques for every patient.

The present and similar researches indicate that the majority of cases of OALs are EMZLs and other indolent

lymphomas (Van der Gaag et al., 1984; Coupland et al., 1998; Oh and Kim, 2007); nevertheless, DLBCL (aggressive lymphoma) was the frequent type in a study (Giridhar et al., 2014).

RLH is a localized disease but OAL carries a high risk of systemic progression. All of the malignant cases discussed here were primary OAL and patients were free of systemic lymphoma. This may render our study prone to selection bias, as we chose the whole cases from the main referral ophthalmologic hospital in Iran, while other patients with systemic lymphoma and ocular involvement are usually admitted to other general hospitals. More comprehensive investigations regarding systemic presentations of ocular lymphoma will add further to our knowledge. This can be achievd by following up primary OAL cases in a long-term period because more than half of systemic involvements of OAL were the same type as the primary orbital lymphoma. Furthuremore, systemic involvement in those patients with orbital lymphoma is reported to be 14% at 3 years, 17% at 5 years and 33% at 10 years after the initial presentation (Li et al., 2015). Notably, EMZL has a systemic recurrence of 22.4% in 5 years (Zhu et al., 2013).

Orbital lymphoma usually occurs in the fifth to seventh decades of life and on average, OAL patients are 5 to 10 years older than RLH ones (Li et al., 2015); consistently, the mean age of our patients with OAL was 59 years, whereas, 51 years in non-OAL ones . Based on our findings, male patients were predominant (60.5% male vs. 39.5% female). This was concordant with a similar study conducted between 1994 to 2000 in Iran (Ghasemi and Gransar, 2003) and with one in Saudi Arabia (Alkatan et al., 2013). Men constituted most cases of OALs (58.8%), likewise another study in Pakistan (Bhurgri et al., 2003), and also the majority of EMZL patients were male.

The mass effect and consequent painless proptosis was the main visible clinical presentation of OALs (Ferry et al., 2007; Othman, 2009). Congruently, swelling and visible mass in the eye comprised 42% of chief complaints in our study. Visual disturbance (visual loss / diplopia) was less common probably because lymphoid tissue tends to grow around the orbit.

The crucial benefit of our investigation along with similar studies is that it paves the way of choosing the suitable therapeutic modality; corticosteroid therapy in reactive lesions and chemotherapy/chemo-radiotherapy in malignant ones (Stacy et al., 2010). Other treatment choices for OAL includes surgey, cryotherapy, immuotherapy and antibiotic therapy which may be applied when indicated (Aronow, 2015).

The importance of histopathological classification is that it empowers us to take appropriate therapeutic measures, since radiographic and clinical presentations of these tumors may be misleading. Scrutiny will be attained through histologic and IHC analysis by expert pathologists, yet, all microscopic and molecular findings should be correlated to clinical presentation.

Acknowledgements

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