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Chlamydia and ocular adnexal lymphomas: An Indian experience

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ABSTRACT

Chlamydia and ocular adnexal lymphomas: an Indian experience: Ocular adnexal lymphomas (OALs) are a heterogeneous group of malignancies, majority being extranodal mucosa-associated lymphoid tissue (MALT) type. Different geographical regions have reported association of *Chlamydia* with OALs (MALT type). In India, role of *Chlamydia* in OALs remains unexplored. The aim of this study was to detect *Chlamydia* and to correlate with clinicopathological features of OALs in India. The clinicopathological features of 41 OAL cases were studied prospectively. *Chlamydia* DNA was detected by genus specific PCR amplifying major outer membrane protein (MOMP) gene followed by DNA sequencing. *Chlamydia* immunoexpression was evaluated by immunofluorescence and immunohistochemistry. The results were correlated with clinicopathological features including follow-up and survival. *Chlamydia* genome was detected in 3/41 (7.3%) OAL cases by PCR. Direct sequencing revealed *C. trachomatis* in 3 positive cases. Immunofluorescence and immunohistochemistry showed *Chlamydia* antigen in 5/41 and 1/41 cases respectively. Immunofluorescence demonstrated higher sensitivity than immunohistochemistry. A significant association was observed between *Chlamydia* positivity and orbital location ($P = 0.05$). Follow-up revealed relapse in 2 *Chlamydia* positive cases ($P = 0.056$). Our results demonstrate for the first time presence of *C. trachomatis* genome in 7.3% OAL cases in India. As no other reports are documented, more detailed studies from different regions within India are needed to explore status of *Chlamydia* in OALs.

1. Introduction

Ocular adnexal lymphomas (OALs) are rare, distinct subgroup of lymphomas constituting about 1–2% of all non-Hodgkins lymphomas (NHL), which most commonly affects the lacrimal gland, lids, the orbit and the conjunctiva (Stefanovic and Lossos, 2009). About 95% lymphomas are B-cell type and the common subtype of OALs is extranodal marginal zone lymphoma (EMZL) of mucosa associated lymphoid tissue (MALT) type (Sen et al., 2010). Others include follicular lymphoma, diffuse large B-cell lymphoma (DLBCL) and mantle cell lymphoma (MCL) (Zucca et al., 2003; Decaudin et al., 2006).

The etiology of ocular NHL is largely unknown and is thought to be induced by chronic antigenic stimulation. Infectious agents like *Chlamydia*, Adenovirus types 8 and 19, Herpes Simplex Virus types 1 and 2 are known to cause chronic eye infections and hence may be associated with OALs (Darougar et al., 1989; Morrow and Abbott, 1998; Lee and Laibson, 1996; Elnifro et al., 1999; Lietman et al., 1998). The causative antigenic stimuli in some EMZLs, like *Helicobacter pylori* in stomach (Wotherspoon et al., 1991), Hepatitis C virus in spleen (Iannitto et al., 2004), *Borrelia burgdorferi* in skin (Cerroni et al., 1997) and *Campylobacter jejuni* in small intestine (Lecuit et al., 2004) have been well studied. Few reports from different geographical regions have documented that *Chlamydia* spp. also play an important role in the development of ocular NHL.

Chlamydiae are obligate intracellular bacteria which are well known to cause various human diseases and may also play a role in tumour development (Peeling and Brunham, 1996). *Chlamydia* spp. like *Chlamydia*

Abbreviations: CR, Complete remission; DLBCL, Diffuse large B-cell lymphoma; DNA, Deoxyribonucleic acid; EMZL, Extranodal marginal zone lymphoma; IF, Immunofluorescence; IFRT, Involved field radiotherapy; IHC, Immunohistochemistry; MALT, Mucosa associated lymphoid tissue; MCL, Mantle cell lymphoma; MOMP, Major outer membrane protein; NHL, non-Hodgkins lymphoma; OALs, Ocular adnexal lymphomas; PCR, Polymerase chain reaction; PR, Partial response.

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trachomatis and *Chlamydophila pneumoniae* have been reported to be associated with malignancies like cervical (Smith et al., 2002) and lung cancer respectively (Laurila et al., 1997). *Chlamydophila psittaci (C.psittaci),* a known etiologic agent of psittacosis in humans, has been reported to be associated with ocular adnexal lymphomas in various studies. Recent studies from Italy showed the presence of *C.psittaci* in 80% of OALs and eradication of the organism by antibiotic treatment lead to partial or complete regression of the disease in majority of the cases (Ferreri et al., 2004; Ferreri et al., 2005). The prevalence of *C.psittaci* infection in MALT lymphoma show significant variation among various geographical regions, being most frequent in Italy (Ferreri et al., 2004), Germany, the Netherlands (Chanudet et al., 2006), Austria (Aigelsreiter et al., 2008) and Korea (Yoo et al., 2007), and least common in Japan (Liu et al., 2006; Daibata et al., 2006), UK, Southern China and Africa (Carugi et al., 2010) (Table 1). Presence of *Chlamydia trachomatis* in OAL patients suggests its contribution towards the pathogenesis of OAL (Contini et al., 2013, 2009). Presence of *C. trachomatis* in OALs has also been reported from United Kingdom, the Netherlands and East Coast of USA (Chanudet et al., 2006). Reports on the possible association between *C.pneumoniae* and lymphomas are also available (Verma et al., 2008; Madico et al., 2000; Borghi et al., 2013; Shen et al., 2006; Chan et al., 2006; Zhang et al., 2009). However, status of *Chlamydia* in ocular adnexal lymphomas is not known in Indian context. This study was therefore designed to investigate the presence of *Chlamydia* in ocular adnexal lymphomas in India.

2. Materials and methods

2.1. Clinicopathological features

A total of 62 patients including 41 patients with ocular adnexal lymphomas and 21 control cases (normal orbital tissues from exenteration specimens) who attended the outpatient department of Dr. Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi, were enrolled in the study from the year 2010–14. Written consent was obtained from all the patients and the study was conducted after approval from Institute's Ethical Committee, All India Institute of Medical Sciences, New Delhi, India (ref. no. IEC/NP-341/2010). Fresh tissues from cases and controls were stored at −70 °C for molecular studies and the remaining tissues were fixed in neutral buffered formalin and embedded in paraffin wax (FFPE) sections for histopathological and immunohistochemical studies. Clinical and radiological details of all the patients were noted. Immunophenotypic characterization of all the 41 OAL cases was done using previously described panel of antibodies, which included CD20 (Clone L26; Dako), CD3 (Clone LN10; Leica Biosystems), CD5 (Clone 4C7; Dako), CD10 (Clone 56C6; Dako) and Cyclin-D1 (Clone SP4; Abcam) (Chen et al., 2000). OAL cases were classified according to WHO classification (2008) of lymphoid neoplasms, as extranodal marginal zone lymphomas (MALT lymphomas), diffuse large cell lymphomas (DLBCL) or mantle cell lymphomas (MCL) (Campo et al., 2011). In large cell lymphomas, cells were larger in size with high N/C ratio and mitosis was frequently observed. Follow up details of 32/41 (78%) patients was available. These 32 cases were also staged at the time of diagnosis based on Ann Arbor staging system (Carbone et al., 1971).

2.2. Detection of Chlamydia DNA by polymerase chain reaction

DNA from frozen or fresh specimens was extracted using the QIAmp DNA mini kit (Qiagen, Valencia, CA) following the manufacturer's instructions and quantified using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, USA). The integrity of DNA from patient samples was verified in all the 62 cases and controls by primers directed at intronic β-globin gene (*HBB*) (5′-AGGAAGGGGAGAAGTAAC-3′ and 5′-AATCCAGCCTTATCCCAA-3′), yielding a 724 bp amplicon (Sahota et al., 2000).

DNA samples positive for *HBB* were used for amplification of major outer membrane protein (MOMP) gene (*ompA*) of *Chlamydia* using a two step genus-specific polymerase chain reaction (Kaltenboeck et al., 1992). Primers 9CHOMP/CHOMP371 and 29CHOMP/CHOMP336 were used in amplification of outer and inner fragment of MOMP gene yielding products of approximately 1120 bp and 930 bp respectively. The PCR assays were performed in 25 μl reactions containing 10× PCR buffer, 200 mM each deoxynucleoside triphosphate, 4.0 mM $MgCl_2$ and 2.5 U of Ampli Taq Gold DNA polymerase provided by the manufacturer (Applied Biosystems, USA). Primers for both genus specific primary and secondary amplification were used at a concentration of 0.2 μM. For genus specific primary amplification, forty cycles of 10 min at 95 °C, followed by 1 min at 95 °C, 30 s at 56 °C, 1 min at 72 °C and 10 min at 72 °C were performed in a programmable thermal cycler (Applied

Table 1
Geographical distribution of *Chlamydia* in ocular adnexal lymphomas.

Authors	Geographical region	OAL cases analyzed	*Chlamydia* positive OAL cases	*Chlamydia* sp. involved
Ferreri et al. (2004)	Italy	40	32 (80%)	*C. psittaci*
Yoo et al. (2007)	South Korea	33	26 (79%)	*C. psittaci*
Aigelsreiter et al. (2008)	Austria	13	7 (54%)	*C. psittaci*
Gracia et al. (2007)	Cuba	46	5 (11%)	*C. psittaci*
Chanudet et al. (2006)	Germany	28	9 (32%)	*C. psittaci*
			4 (14%)	*C. pneumoniae*
			1 (4%)	*C. trachomatis*
Chanudet et al. (2006)	Netherlands	25	8 (32%)	*C. psittaci*
		15	3 (20%)	*C. trachomatis*
Chanudet et al. (2006)	Italy	21	2 (10%)	*C. psittaci*
			1 (5%)	*C. pneumoniae*
			1 (5%)	*C. trachomatis*
Chanudet et al. (2006)	United Kingdom	40	5 (13%)	*C. psittaci*
		35	5 (14%)	*C. pneumoniae*
		35	8 (23%)	*C. trachomatis*
Chanudet et al. (2006)	Southern China	52	5 (10%)	*C. psittaci*
			10 (19%)	*C. pneumoniae*
Chanudet et al. (2006)	East Coast of USA	25	6 (24%)	*C. psittaci*
Zhang et al. (2010)	Northern China	18	3 (17%)	*C. trachomatis*
Matthews et al. (2008)	Florida	38	2 (5.3%)	*C. pneumoniae*
Rosado et al. (2006)	Florida	49	0	–
Carugi et al. (2010)	Kenya	57	0	*C. psittaci*
Daibata et al. (2006)	Japan	9	0	*C. psittaci*
Vargas et al. (2006)	North Eastern USA	21	0	*C. psittaci*
Zhang et al. (2007)	North Western USA	28	0	*C. psittaci*