Lack of association of conjunctival MALT lymphoma with Chlamydiae or Helicobacter pylori in a cohort of Chinese patients

Ji-Ping Cai*, Jin-Wei Cheng, Xiao-Ye Ma, Yu-Zhen Li, You Li, Xiao Huang, Rui-Li Wei

* Ji-Ping Cai and Jin-Wei Cheng contributed equally to this work

Department of Ophthalmology, Shanghai Changzheng Hospital, 2nd Military Medical University, Shanghai, China

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Summary

Background: This study was conducted to detect microbial pathogens in conjunctival mucosa-associated lymphoid tissue (MALT) lymphoma specimens in an attempt to determine possible associations between conjunctival MALT lymphoma and microbial infections.

Material/Methods: Using PCR technique, freshly obtained tumor specimens from 16 cases of conjunctival MALT lymphoma, as confirmed by postoperative pathology, were analyzed for DNA of Chlamydia psittaci (C. psittaci), Chlamydia trachomatis (C. trachomatis), Chlamydia pneumoniae (C. pneumoniae) and Helicobacter pylori (H. pylori). Synthetic C. psittaci, C. trachomatis, C. pneumoniae and H. pylori DNA were used as positive control, and blank plasmid DNA as negative control.

Results: Electrophoresis showed that no bands corresponding to the positive control were observed in the specimens, indicating that no DNA of the 4 microorganisms was detected in the specimens of the 16 cases of conjunctival MALT lymphoma.

Conclusions: The PCR technique was able to detect the positive control quickly and accurately, but the results of PCR in analyzing the 16 specimens were negative, indicating that there is no association between conjunctival MALT lymphoma and the 4 microorganisms in Chinese patients.

key words: ocular adnexal MALT lymphoma • Chlamydia • Helicobacter pylori

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Author’s address: Rui-Li Wei, Department of Ophthalmology, Shanghai Changzheng Hospital, #415 Fengyang Road, Shanghai 200003, China, e-mail: rulwei@gmail.com
BACKGROUND

Chlamydiae are prokaryotic organisms that differ from both bacteria and viruses [1]. There are 3 known types of chlamydiae associated with human diseases: Chlamydia psittaci (C. psittaci), Chlamydia trachomatis (C. trachomatis) and Chlamydia pneumoniae (C. pneumoniae). They may cause multiple diseases, including pneumonia, trachoma, cervicitis, pelvic inflammatory disease, urethritis and epididymitis [2]. Recent studies in other countries revealed a close association between C. psittaci and ocular adnexal MALT lymphoma [3–6], but no such association has been reported in China.

MALT lymphoma is an extra-nodal marginal zone B cell lymphoma, and is the second most common inert tumor originating from mucosa-associated lymphoid tissue. There is definite evidence that occurrence of some B-cell lymphomas is associated with long-term chronic stimulation of microbi- als or autologous pathogens, which is most clearly exemplified by H. pylori-associated gastric MALT lymphoma [7–9].

Similar to the association of gastric MALT lymphoma with chronic stimulation of H. pylori, many pathogenic microbi- als, especially Chlamydiae, are also reported to be associated with the formation of MALT lymphoma. Italian researchers were the first to study ocular MALT lymphoma, and detected C. psittaci DNA in 80% of their ocular adnexal lymphoma specimens. They found that the tumor subsided after C. psittaci was eliminated with medical treatment, thus confirming a close correlation [3,4]. However, the results of subsequent similar studies in different parts of the world were controversial – there were great differences in the detection rate of C. psittaci DNA from ocular adnexal lymphoma specimens, seemingly suggesting a regional correlation [10–14].

The present study used the PCR method to detect C. psittaci, C. trachomatis, C. pneumoniae and H. pylori DNA in specimens of conjunctival MALT lymphoma freshly obtained from Chinese patients in our hospital to determine if these microorganisms were present Chinese patients with conjunctival MALT lymphoma.

MATERIAL AND METHODS

Material

Genomic DNA Mini Preparation Kit was purchased from MN NucleoSpin®, Germany. Tissue 740952 and Hotstart Taq were the products of TaKaRa DR028 (Dalian, China). All materials used, including tubes and pipette tips, were imported from AXYGEN. C. psittaci, C. trachomatis, C. pneumoniae and H. pylori DNA positive controls were synthesized in our laboratory.

Methods

Patient and sample collection: The study was approved by the local Ethics Review Board. Conjunctival MALT lymphoma specimens in the present study were from 14 patients (16 eyes) who were admitted at Shanghai Changzheng Hospital of the Second Military Medical University (Shanghai, China) between January 2008 and December 2009, all of whom were Han ethnicity and resided mainly in East China, without histories of keeping birds or having close contacts with birds. The patients included 9 males and 5 females who ranged in aged from 14 to 83 years, with a mean age of 55±16.76 years. Of the 2 patients with both eyes infected, both were female. The common presentation of the condition included the presence of subconjunctival (especially suborninal) pink tumors (salmon patch), whose course ranged from 3 months to 2 years. No history of lymphoma was elicited, nor was any tumor detected in another part of the body on physical examination. All patients received surgical treatment, and postoperative pathology and immunohistochemistry confirmed the diagnosis of MALT lymphoma in all patients. The tumor tissues, about 5mm in diameter, were obtained surgically, immediately washed with normal saline, stored in tubes and plunged directly in liquid nitrogen. The whole procedure was completed within 5 min. After 2–4 h preservation in liquid nitrogen, the 16 specimens were transferred to a –80°C freezer for preservation until later use.

Genome extraction

Genome extraction of the specimens was performed according to the manufacturer’s instructions. In brief, about 25 mg of freshly obtained MALT lymphoma tissue was cut off, digested in 180 µl T1 and 25 µl proteinase K at 56°C for 3 h, boiled in 200 µl B5 at 70°C for 10 min, and added to 210 µl ethanol. The mixture was put into the column, centrifuged at 11000g for 1 min, washed with 500 µl BW once, and again with 600 µl B5. The column was spun without loading for 1 min to dry the membrane, and was put into BE which had been preheated up to 70°C, and then centrifuged at 11,000g for 1 min. Purity of the extracted DNA was measured by spectrophotometry, and integrity was measured by electrophoresis.

Primer design and synthesis

The 16S rRNA sequences of C. psittaci, C. trachomatis, C. pneumoniae and H. pylori were from NCBI DataBank and the previous literature [16]. Primer design was according to microbial 16S rRNA gene sequences.

Expected length of C. trachomatis product: 315 bp
C. trachomatis p5 5’ GGCTCATTTTGCGCATGAGTAAG 3’
C. trachomatis p3 5’ TCAATTCCAGGGTATTAACGC 3’

Expected length of C. pneumoniae product: 197 bp
C. pneumoniae p5 5’ GGTCTCAACCGGCTGCTGCGTGGG 3’
C. pneumoniae p3 5’ TGGCGAAAGCTGTCGTTACGGCTT 3’

Expected length of C. psittaci product: 111 bp
C. psittaci p5 5’ CCGAGGTTAGGCTGATGACG 3’
C. psittaci p3 5’ CCAACCTGCTTACAGTATTA 3’

Expected length of H. pylori product: 305bp
H. pylori p5 5’ TGGCGGTGCTCATTGAGTGACGGA 3’
H. pylori p3 5’ CCTCTGGGGCATATTCACCA 3’

The above primers were synthesized by Shanghai Bioengineering Co., Shanghai, China.

Detection of the specimens

We used 100 ng of tissue from each specimen as the template, and C. psittaci as touchdown of each 0.25°C cycle from 62°C to 50°C, pre-degeneration at 95°C for 3 min, and 45 cycles.

BR85
cer cells due to chronic microbial stimulation is of intense
discussion
were also negative. Appearance of such bands might be re
tected bands and indicated that the results of these specimens
that there was no target product detected in these suspect
trachomatis
sistent with the 16S rRNA sequences of
after gel cutting and recovery revealed that they were not
control (plasmid DNA); and 18 is the negative control. The
target band is 197 bp. The results of electrophoresis after
PCR indicate that no C. pneumoniae DNA was detected in
the 16 tumor tissue specimens.

C. psittaci

The present study searched for the presence of
DNA was detected in their 13 specimens of ocular adnexal lymphoma. Lee et al. [17] re
ported a 100% detection rate of
specimens of ocular adnexal lymphoma. Chan et al. [16] detected positive
the research on ocular adnexal lymphoma. In 2004, Chan
pylori
is the causative agent of gastric MALT lymphoma [7,8].
But there have been controversies over the conclusions in
the research on ocular adnexal lymphoma. In 2004, Chan
et al. [16] detected positive H. pylori in 4 (80%) of their 5
specimens of ocular adnexal lymphoma. Lee et al. [17] re
ported a 100% detection rate of H. pylori (15/15). Both stud
ies pointed out that there might be a correlation between
this agent and
H. pylori. However, Sjo et al. [18] reported a conflicting result, finding that no H. pylori DNA was de	ected in their 13 specimens of ocular adnexal lymphoma. The present study searched for the presence of H. pylori in

PCR cycling of C. pneumoniae, C. trachomatis and H. pylori: annealing at 55°C for 20 s, degeneration for 20 s, extension for 30 s, and 45 cycles. Electrophoresis was performed after PCR.

RESULTS

No C. psittaci, C. trachomatis and C. pneumoniae DNA of the
16 MALT lymphoma specimens from our patients were de	ected by PCR assay. The results are shown in Figures 1–4.

We observed that the electrophoretic bands of PCR amplifi
cation of specimens No. 8 and 16 C. trachomatis were similar
to those of H. pylori, and there was a suspected band at the
place corresponding to the positive control. There was also
a suspected band in specimen No. 13 C. psittaci. Sequencing
after gel cutting and recovery revealed that they were not
significantly correlated with the 16S rRNA sequences of C.
trachomatis, H. pylori and C. psittaci reported. This meant
that there was no target product detected in these suspect
bands and indicated that the results of these specimens
were also negative. Appearance of such bands might be re
lated to the environment of the templates themselves.

DISCUSSION

Research on the transformation of lymphocytes to can
cer cells due to chronic microbial stimulation is of intense
our MALT lymphoma specimens, and the results were negative in all specimens. Based on other related studies and reports [19–21], our conclusion seems to support that *H. pylori* infection is unlikely to be associated with the occurrence of ocular adnexal MALT lymphoma.

MALT lymphoma is the most common pathology in ocular adnexal lymphoma, accounting for 50–78% in developed countries [22–26], 80–90% in South Korea and Japan [27,28], and more than 80% in China [29–31]. Ocular adnexa include the eyelid, conjunctiva, orbit and lacrimal apparatus, of which the conjunctiva is most frequently exposed to the external environment directly, and therefore most susceptible to infection by microbial pathogens. If microbial infection is truly associated with ocular adnexal MALT lymphoma, the occurrence of the tumor is most likely to be related to chronic stimulation by exogenous microbes. Knowing that the incidence of conjunctival MALT lymphoma has been rising annually in recent years, we selected it as the subject of research in the present study, hoping to discover pathologic factors related to the etiology of the tumor in a limited number of cases. To our knowledge, this is the first study in China using fresh specimens of conjunctival MALT lymphoma from a cohort of Chinese patients to explore possible correlations between MALT lymphoma and microbial infections.

Italian researchers reported the presence of *C. psittaci* in 32 (80%) of their 40 specimens of ocular adnexal lymphoma [3], which aroused much attention at the time of the discovery. Later, South Korean [5] and Austrian [6] researchers confirmed this correlation. However, American [32], Dutch [10] and Japanese [11] research groups did not find any evidence to confirm the correlation between ocular adnexal lymphoma and *C. psittaci*. Husain et al. [33] published an overview of related studies and concluded that there were serious controversies over *C. psittaci* DNA detection in ocular adnexal lymphoma specimens. Out of 458 cases of ocular adnexal lymphoma that they reviewed, positive *C. psittaci* was detected in 104 cases (23%), but 90% of the 104 cases were from 5 of the 12 reports reviewed. A recent study [14] indicates that the *C. psittaci* detection rate in ocular adnexal lymphoma specimens varied from place to place: 17% in Italy and 0% in Kenya. The positive rate is geographically biased: it is relatively high in Italy and South Korea, relatively low in the United States and Japan, and 0% in our study. Interestingly, the prevalent rate of *C. psittaci* infection in MALT lymphoma was 11% in specimens from Southern China in a study whose results showed that *C. psittaci* was associated with ocular adnexal MALT lymphoma and that this association was variable in 6 different geographical areas [13]. The glaring difference between the only 2 studies in Chinese patients is that the cases in this present study resided mainly in East China, while most of the samples in the other study were collected from Southern China. A similar situation occurred in the studies of Italian patients. The *C. psittaci* detection rate in ocular adnexal lymphoma specimens varied from study to study (from 13% to 80%) [3,13,14].

Although we cannot deny possible errors and differences arising from specimen selection and detection methodology, the main problem is that we cannot either rule out nor confirm the cause-effect relationship between them. In addition, *C. psittaci* infection is most likely to occur in luminal mucosa that has the contact with the external environment, such as the respiratory, reproductive and urinary tracts. It is therefore difficult to explain how MALT lymphoma in the deep orbit is caused by *C. psittaci* infection. The correlation between gastric MALT lymphoma and *H. pylori* infection has been confirmed [7,8]. This is understandable, because *H. pylori* exists extensively in normal human gastric mucosa, while *C. psittaci* is not commonly parasitic in human ocular adnexa. However, positive detection of *C. psittaci* is an objective finding, suggesting that *C. psittaci* infection may be one of the pathogenetic factors contributing to ocular adnexal MALT lymphoma. Whether it is an initiating factor or a predisposing factor needs further study.

**Conclusions**

No *C. psittaci*, *C. trachomatis*, *C. pneumoniae* or *H. pylori* DNA was detectable in the 16 freshly obtained specimens of conjunctival MALT lymphoma in the present study. There is no evidence to confirm the correlation between the above 4 microorganisms and conjunctival MALT lymphoma in patients from the East China area.

**References:**

1. Wyrick PB: Intracellular survival by *Chlamydia*. Cell Microbiol, 2000; 2: 275–82
2. Byrne GL, O cuis DM: *Chlamydia* and apoptotic life and death decisions of an intracellular pathogen. Nat Rev Microbiol, 2004; 2: 920–82
3. Ferreri AJ, Guidoboni M, Ponzo M et al: Evidence for an association between *Chlamydia psittaci* and ocular adnexal lymphomas. J Natl Cancer Inst, 2004; 96: 586–94
4. Ferreri AJ, Ponzo M, Guidoboni M et al: Regression of ocular adnexal lymphoma after *Chlamydia psittaci*-eradicating antibiotic therapy. J Clin Oncol, 2005; 23: 5067–73
5. Yoo C, Ryu MH, Huh J et al: *Chlamydia psittaci* infection and clinicopathologic analysis of ocular adnexal lymphomas in Korea. Am J Hematol, 2007; 8: 821–23
6. Aigelsreiter A, Leitner E, Deutsch AJ et al: *Chlamydia psittaci* in MALT lymphomas of ocular adnexals: The Austrian experience. Leuk Res, 2008; 32: 1292–94
7. Zullo A, Hassan C, Cristofori F et al: Gastric low-grade mucosal-associated lymphoid tissue-lymphoma: Helicobacter pylori and beyond. World J Gastrointest Oncol, 2010; 2: 181–86
8. Stolte M: Helicobacter pylori gastritis and gastric MALT-lymphoma. Lancet, 1992; 339: 745–46
9. Berton F, Zucca E: State-of-the-art therapeutic: marginal-zone lymphoma. J Clin Oncol, 2005; 23: 6145–20
10. Mulder MM, Hedebram ER, Pannekoek Y et al: No evidence for a association of ocular adnexal lymphoma with *Chlamydia psittaci* in a cohort of patients from the Netherlands. Leuk Res, 2006; 30: 1305–7
11. Daibata M, Nemoto Y, Togitani K et al: Absence of *Chlamydia psittaci* in ocularadnexal lymphoma from Japanese patients. Br J Haematol, 2006; 132: 651–52
12. Matthies JM, Moreno LL, Dennis J et al: Ocular adnexal lymphoma: no evidence for bacterial DNA associated with lymphoma pathogenesis. Br J Haematol, 2006; 132: 2158–68
13. Chanudet E, Zhou Y, Bacon CM et al: A *Chlamydia psittaci* variant is associated with ocular adnexal MALT lymphoma in different geographical regions. J Pathol, 2006; 209: 544–51
14. Carugi A, Orzan A, Antonielli G et al: Geographic variation and environmental conditions as cofactors in *Chlamydia psittaci* association with ocular adnexal lymphomas: a comparison between Italian and African samples. Hematol Oncol, 2010; 28: 20–26
15. Madico G, Quinn TC et al: Touchdown Enzyme Time-Release-PCR for Detection and Identification of *Chlamydia trachomatis*, *C. pneumoniae*, and *C. psittaci* Using the 16S and 16S-23S Spacer DNA Genes. J Clin Microbiol, 1990; 28: 1085–93
16. Chan CC, Smith JA, Shen DF et al: *Helicobacter pylori* (H. pylori) molecular signature in conjunctival mucosa-associated lymphoid tissue (MALT) lymphoma. Histol Histopathol, 2004; 19: 1219–26
17. Lee SB, Yang JW, Kim CS: The association between conjunctival MALT lymphoma and *Helicobacter pylori*. Br J Ophthalmol, 2008; 92: 534–36
18. Sjo NC, Forgh P, Juhl BR et al: Role of *Helicobacter pylori* in conjunctival mucosa-associated lymphoid tissue lymphoma. Ophthalmology, 2007; 114: 182–86
19. Ferreri AJ, Ponzone M, Viale E et al: Association between *Helicobacter pylori* infection and MALT-type lymphoma of the ocular adnexa: clinical and therapeutic implications. Hematol Oncol, 2006; 24: 33–37
20. Gruenberger B, Woehrer S, Troch M et al: Assessment of the role of hepatitis C, *Helicobacter pylori* and autoimmunity in MALT lymphoma of the ocular adnexa in 45 Austrian patients. Acta Oncol, 2008; 47: 355–59
21. Chan CC, Shen D, Mochizuki M et al: Detection of *Helicobacter pylori* and *Chlamydia pneumoniae* genes in primary orbital lymphoma. Trans Am Ophthalmol Soc, 2006; 104: 62–70
22. Mannami T, Yoshino T, Oshima K et al: Clinical, histopathological, and immunogenetic analysis of ocular adnexal lymphoproliferative disorders: characterization of malf lymphoma and reactive lymphoid hyperplasia. Mod Pathol, 2001; 14: 641–49
23. White WL, Ferry JA, Harris NL, Grove AS Jr: Ocular adnexal lymphoma. A clinicopathologic study with identification of lymphomas of mucosa-associated lymphoid tissue type. Ophthalmology, 1995; 102: 1994–2006
24. Sjo LD: Ophthalmic lymphoma: epidemiology and pathogenesis. Acta Ophthalmol, 2009; 87: 1–20
25. Auw-Haedrich C, Coupland SE, Kapp A et al: Long-term outcome of ocular adnexal lymphoma subtyped according to the REAL classification. Revised European and American Lymphoma. Br J Ophthalmol, 2001; 85: 63–69
26. Jakobiec FA: Ocular Adnexal Lymphoid Tumors: Progress in Need of Clarification. Am J Ophthalmol, 2008; 145: 941–50
27. Nakata M, Matsuno Y, Katsumata N et al: Histology according to the Revised European-American Lymphoma Classification significantly predicts the prognosis of ocular adnexal lymphoma. Leuk Lymphoma, 1999; 32: 533–43
28. Cho EY, Han JJ, Ree HJ et al: Clinicopathologic Analysis of Ocular Adnexal Lymphomas: Extranodal Marginal Zone B-Cell Lymphoma Constitutes the Vast Majority of Ocular Lymphomas Among Koreans and Affects Younger Patients. Am J Hematol, 2003; 75: 87–86
29. You QS, Li B, Zhou XG et al: Clinical and pathological features of 112 cases with ocular adnexal lymphoproliferative lesions. Chin J Ophthalmol, 2005; 41: 871–76
30. Bi YW, Chen RJ, Hou YY et al: Clinicopathologic Analysis of Primary Ocular MALT Extranodal Marginal Zone B-Cell Lymphoma. Chin J Pathol, 2007; 36: 414–15
31. He WM, Luo QL, Xia RN: Histopathological studies on 114 cases of ocular adnexal lymphoid hyperplasia. Chin J Prac Ophthalmol, 2001; 19: 68–70
32. Rosado MF, Byrne GE Jr, Ding F et al: Ocular adnexal lymphoma: A clinicopathologic study of a large cohort of patients with no evidence for an association with Chlamydia psittaci. Blood, 2006; 107: 467–72
33. Husain A, Roberts D, Pro B et al: Meta-analyses of the Association Between *Chlamydia psittaci* and Ocular Adnexal Lymphoma and the Response of Ocular Adnexal Lymphoma to Antibiotics. Cancer, 2007; 110: 809–15