High frequency of latent Chlamydia trachomatis infection in patients with rhegmatogenous retinal detachment

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Abstract

- AIM: To determine the frequency of detection of ocular and extraocular Chlamydia trachomatis (CT) infection in non–high myopes with rhegmatogenous retinal detachment (RRD).
- METHODS: This was a single–center, nonrandomized, prospective, case–control study. One hundred and four patients were divided into a study group with RRD (n = 63) and a control group with traumatic retinal detachment (n = 41). Samples of subretinal fluid (SRF), conjunctival, urethral/cervical swabs, and blood were collected. The frequency of detection of CT infection in SRF samples was determined by polymerase chain reaction (PCR), direct fluorescence assay (DFA) and cell culture, whereas that in conjunctival swabs was determined by PCR and DFA, and those in urethral/cervical swabs and blood were determined by DFA. Yates Chi–square test (with Bonferroni correction) and two–tailed Student’s t-test were used for statistical analysis.
- RESULTS: SRF CT infection was detected more frequently in the study group (50.8%–71.4%) than in the control group (9.8%–12.2%) by all the methods used (P < 0.01). The frequency of detection of conjunctival CT infection by DFA was higher in the RRD patients compared with the controls (81.0% vs 24.4%, P = 0.004). The PCR detected conjunctival CT infection more often in the study group than in the controls (46.0% vs 9.8%, P = 0.007). The DFA detected CT in blood specimens almost as frequently as in urogenital specimens, for the RRD patients (61.2% vs 63.5%) and the controls (7.3% vs 9.8%).
- CONCLUSION: CT infection is detected with high frequency in non–high myopes with RRD.

KEYWORDS: rhegmatogenous retinal detachment; Chlamydia trachomatis; myopia; latent infection

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INTRODUCTION

Rhegmatogenous retinal detachment (RRD) is a vision-threatening disease characterized by the separation of the inner neurosensory retina and the retinal pigment epithelium [1]. According to various sources [2-4], its annual incidence is estimated to be 6.9 to 18.2 per 100 000. Retinal tear is the most important risk factor and a cause of RRD. Additionally, retinal thinning, posterior vitreous detachment (PVD), and vitreous tractions are widely considered to be essential for the formation of a retinal tear. Although the clinical picture of RRD is well known, the most fundamental causes of the development both of retinal tears and the preceding retinal and vitreous alterations remain poorly understood.

Retinal thinning, PVD and vitreous tractions may develop in the presence of a posterior-segment clinical inflammatory disease such as infectious uveitis resulting in retinal tears and RRD [5]. Moreover, it is known that inflammation in general is critical in the pathogenesis of different vitreoretinal diseases [6]. We theorized that chronic low-grade inflammation directly or indirectly caused by latent infection may have similar mechanism related to the development of retinal thinning, PVD and vitreous tractions resulting in retinal tears and detachment.

One of the most well-known causes of chronic low-grade inflammation is persistent Chlamydia trachomatis (CT) infection which can result in asymptomatic damage to the urogenital tract [7]. Furthermore, CT is a causative agent of trachoma, which is manifested by chronic conjunctival inflammation [8]. In recent years, evidence has accumulated regarding the ability of various CT serovars to induce damage not only to epithelial, but also to other structures and tissues of the body [9], including those of the ocular posterior segment [10].
In our previous work \cite{1}, CT elementary bodies were identified in 74% of the subretinal fluid (SRF) specimens of 50 patients with RRD. Ocular CT infection in such cases may be explained by evidence of the high incidence of bacteremia in urogenital CT infection \cite{13}. Therefore, the purpose of this study was to determine the frequency of detection of both ocular (namely, SRF-related and conjunctival) and extracocular (namely, urogenital tract and blood) CT infection in non-high myopes with RRD.

**SUBJECTS AND METHODS**

**Subjects** This single-center, nonrandomized, prospective, case-control study was conducted at our Ophthalmology Clinic in 2010-2012, and involved 104 patients divided into a study group and a control group. The study group included 63 patients with clinical signs of typical RRD evaluated by experts in retinal detachment surgery. These patients had confirmed idiopathic retinal tears, i.e., they had no known ocular diseases other than RRD. The control group included 41 patients with traumatic retinal detachment (TRD) resulting usually from a documented episode of open- or closed-globe injury with retinal involvement (direct retinal damage, tear or dialysis). Exclusion criteria for both groups included current and/or recent (within a year) history of uveitis, manifested or acute urogenital tract diseases, any other systemic or organ specific signs/symptoms of CT infection. Moreover, they included diabetes, hypermetropia of more than 2.0 D, and high myopia (more than 6.0 D). Additional exclusion criterion for the control group alone was the absence of reliable signs of direct traumatic damage to the retina. All the patients had retinal re-attachment surgery including extrascleral buckling or pars plana vitrectomy, either alone or in combination, depending on indications. They were informed about the purpose of the study and signed an informed written consent. The study was approved by local Research Ethics Committee and was consistent with the tenets of Declaration of Helsinki.

**Samples Collection and Identification of Chlamydia Trachomatis**

Conjunctival, urethral (for men) and cervical (for women) swabs, and blood were collected preoperatively; additionally, samples of SRF were collected intraoperatively. All of them were investigated for the presence of CT infection.

At the time of retinal re-attachment surgery, SRF was aspirated with a 27-gauge cannula attached to a sterilized syringe and inserted either through a scleral puncture for SRF drainage in extrascleral buckling (n = 65), or through a retinal tear (retinotomy hole) for transretinal drainage in vitrectomy (n = 39). Sample contamination with blood was occasional; heavily contaminated samples were discarded. Each sample was centrifuged for 20 min at 2000 rpm, and a portion of the centrifuged deposit was placed into transport medium for culture and polymerase chain reaction (PCR) testing. Another portion was used to prepare a smear that was air dried and fixed 10 min in 70% cold methanol before being subjected to direct fluorescence assay (DFA). After anesthetization of the conjunctiva with proxymetacaine 0.5% eye drops (Alcaine, Alcon-Couvreur, Belgium), conjunctival swabs were taken in a standardized manner (passed firmly four times across the conjunctiva with a quarter turn between each pass). Conjunctival smears were prepared by rolling half the swab on a glass slide. Immediately after smearing, the swab was placed into transport medium for PCR and stored at +4°C up to 3 d before being used in the study. Smears were fixed as described above.

Samples of conjunctival smears and SRF were placed into 3 mL of modified viral transport medium with Charcoal (HiMedia Laboratories Pvt. Ltd., Mumbai, India) before being subjected to PCR and cell culture. Totally 5 mL venous blood samples were collected from all patients. After clotting, the sample was centrifuged at 2000 g for 15 min at 4°C, and the serum was separated. Serum smears were prepared and subjected to DFA.

Urethral (for men) and cervical (for women) specimens were collected with a unisex swab (HiMedia Laboratories Pvt. Ltd.). The swab was rolled on a slide and fixed as described above.

A 0.2-mL portion of each transport medium specimen or each serum sample was used to detect the pathogen by culture. The portions were placed into individual wells of 24-well culture plates containing McCoy cells grown to confluence in 10% fetal bovine serum-supplemented Eagle's Minimum Essential Medium (MEM) (Biolog, Moscow, Russia) and 1 mg/L cycloheximide (Acros Organics, Geel, Belgium). The plates were then centrifuged at 2400 rpm for 60 min and incubated at 37°C in 5% CO2. After 48 h incubation, the culture media was removed from the wells, and the plates were twice washed with phosphate-buffered saline (PBS, pH 7.2) and fixed in 96% ethanol for 5 min.

Slide smears and cell cultures were incubated in the presence of ChlamyScan enzyme immunoassay system (LABDiagnostics, Moscow, Russia) fluorescein-conjugated monoclonal antibodies to a CT-specific trisaccharide epitope αKdo (2→8)αKdo (2→4)αKdo for 20 min at 37°C in a moisture chamber. The slides were then incubated in PBS for 10 min, washed twice in PBS and mounted in 90% PBS/10% glycerol.

Microscopic examination was performed with a Leica DM2500 fluorescence microscope (Leica Microsystems, Wetzlar, Germany). A sample was considered positive for CT if at least 10 loci of specific fluorescence (elementary bodies) were detected.

Real-time PCR was performed using the Rotor-Gene 6000 (Corbett Research, Sydney, Australia). Extraction of total...
nucleic acids was conducted with DNA-sorb-B kit (AmpliSens, Moscow, Russia). Specimens were tested for the presence of DNA from CT using CT-screen-tier-titer-FRT (AmpliSens), according to the manufacturer's instructions.

**Statistical Analysis**  
Yates Chi-square test (with Bonferroni correction) and two-tailed Student's *t*-test were used to evaluate the significance of differences between the RRD and control groups in the amount of positive results and average patient age, respectively. Bonferroni correction for multiple comparisons was applied where multiple tests were performed, thereby reducing the nominal *P* value for statistical significance to 0.016. Otherwise, the nominal *P* value for statistical significance was 0.05.

**RESULTS**

The frequency of detection of CT was significantly higher in the patients with RRD than in the controls, whereas no statistically significant differences were found in the average patient age and sex distribution between the groups. The percentage of patients with myopia was not statistically significantly higher in the RRD group than in the control group (55.6% ± 26.8%, *P*=0.07) (Table 1).

In the RRD group, 43 (68.3%) SRF specimens were positive by DFA, and 32 (50.8%) were positive by PCR; additionally, PCR provided the least number of positive results, whereas the numbers provided by DFA and culture were comparable. In the control group, no statistically significant differences were revealed between the methods (Table 2). The frequency of detection of CT conjunctival infection was higher in the RRD patients than in the controls. In the RRD group, 51 (81.0%) conjunctival swab specimens were positive by DFA, and 29 (46.0%) were positive by PCR (Table 2). Within each group, the PCR detected the pathogen in subretinal fluids as frequently as in conjunctival swab specimens (*P*>0.05). The frequency of detection of the pathogen in all types of specimens (SRF, conjunctival swab, urogenital swab or blood) by DFA was the same. Blood and urogenital-swab specimens yielded a statistically significantly greater percentage of positive results in the RRD group vs the control group. Within each group, the frequency of detection of the pathogen in these two types of specimens was approximately the same (*P*>0.05). In 90.5% of the RRD group patients, at least one of the four (i.e. SRF, conjunctival swab, urogenital swab or blood) specimens was found to be DFA positive for CT. Moreover, within any type of specimens, the percentage of culture-positive specimens was close to that of DFA-positive and PCR-positive specimens (*P*=0.016) (Table 3).

**DISCUSSION**

The association of CT infection with RRD has been postulated by us[10]. This was supported in a single-case study by Ghaffariyeh et al.[11]. In the present work, we confirmed this association and also found that of RRD with extraocular infection, in particular, the presence of the pathogen in the

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### Table 1 Demographic and refection data  

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1RRD (n=63)</th>
<th>1TRD (n=41)</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (a), $\bar{x} \pm s$</td>
<td>47.2±17.2</td>
<td>45.4±16.6</td>
<td>0.64</td>
</tr>
<tr>
<td>Sex</td>
<td>M</td>
<td>41 (65.1)</td>
<td>29 (70.7)</td>
</tr>
<tr>
<td>F</td>
<td>22 (34.9)</td>
<td>12 (29.3)</td>
<td>0.55</td>
</tr>
<tr>
<td>Refraction data</td>
<td>Myopia up to 6 D</td>
<td>35 (55.6)</td>
<td>11 (26.8)</td>
</tr>
<tr>
<td></td>
<td>Emmetropia and hypermetropia up to 2 D</td>
<td>28 (44.4)</td>
<td>30 (73.2)</td>
</tr>
</tbody>
</table>

**RRD:** Rhegmatogenous retinal detachment; **TRD:** Traumatic retinal detachment.

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### Table 2 Rates of positivity for CT among different types of specimens in cases (RRD group) and controls (TRD group)  

<table>
<thead>
<tr>
<th>Type of specimen/method</th>
<th>1RRD group</th>
<th>1TRD group</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Subretinal fluid</td>
<td>DFA</td>
<td>43 (68.3)</td>
<td>5 (12.2)</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>32 (50.8)</td>
<td>4 (9.8)</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>45 (71.4)</td>
<td>5 (12.2)</td>
</tr>
<tr>
<td></td>
<td>Conjunctival swab</td>
<td>DFA</td>
<td>51 (81.0)</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>29 (46.0)</td>
<td>4 (9.8)</td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td>DFA</td>
<td>40 (63.5)</td>
</tr>
<tr>
<td></td>
<td>Urogenital swab</td>
<td>DFA</td>
<td>39 (61.9)</td>
</tr>
</tbody>
</table>

**DFA:** Direct fluorescent assay; **PCR:** Polymerase chain reaction; **RRD:** Rhegmatogenous retinal detachment; **TRD:** Traumatic retinal detachment.

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### Table 3 Comparison of DFA, PCR and culture results in SRF specimens  

<table>
<thead>
<tr>
<th>Group/method</th>
<th>1Negative</th>
<th>2Positive</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>RRD</td>
<td>DFA</td>
<td>20 (31.7)</td>
<td>43 (68.3)</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>31 (49.2)</td>
<td>32 (50.8)</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>18 (28.6)</td>
<td>45 (71.4)</td>
</tr>
<tr>
<td>TRD</td>
<td>DFA</td>
<td>36 (87.8)</td>
<td>5 (12.2)</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>37 (90.2)</td>
<td>4 (9.8)</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>36 (87.8)</td>
<td>5 (12.2)</td>
</tr>
</tbody>
</table>

**DFA:** Direct fluorescent assay; **PCR:** Polymerase chain reaction; **SRF:** Subretinal fluid; **RRD:** Rhegmatogenous retinal detachment; **TRD:** Traumatic retinal detachment.
Frequent Chlamydia trachomatis infections found in RRD

urogenital tract and blood. Demonstration of the presence of CT in SRF specimens of a significant percentage of patients with RRD (50.8%-71.4%) vs controls (9.8%-12.2%) makes us suggest that the pathogen may play a role of in the etiology and pathogenesis of this vision-threatening disease. However, this issue requires further investigation.

It has been generally accepted that CT infection most commonly affects the urogenital tract [7] and conjunctiva [8]. However, recently, evidence has accumulated that, in a high percent of patients with urogenital CT infection, the latter is accompanied by bacteremia [22], and if so, the pathogen can get to other organs and tissues. Our results confirm that, in RRD, CT tends not only to induce local damage to the urogenital tissues (61.9%), but also to cause bacteremia and infect the eye (63.5% and 46.0%-81.0%, respectively). Furthermore, we do not exclude that dissemination of the agent to the posterior segment or extraocular sites might take place after primary infection of the conjunctiva, since, previously, we have detected intraocular CT infection following a subconjunctival inoculation with CT in rabbits [19]. CT can infect different types of cells, including macrophages [15], neuroglial [18], and synovial [9] cells. The pathogen initiates the inflammatory process, triggering the cytokine production by CT-infected and adjacent cells both through direct damage and antigenic stimulation [30]. Here the profile of the cytokines produced is pro-inflammatory and includes IL-10, TNF, IFN-γ, IL-6 [9], IL-12, and IL-8 [16], resulting in generation and maintenance of inflammation. Regarding the posterior segment, this explanation is supported by findings from other investigators that the immunoglobulin composition [27] and pH level [18] of SRF are close to those of inflammatory exudates (in particular, infectious exudates). Moreover, the inflammatory nature of the process in RRD is underscored by the SRF interleukin (IL-10 and IL-12) levels [10]. The pathogen is characterized by a long-term inflammatory process that involves dystrophic and proliferative histological changes similar to those observed in trachoma and urogenital chlamydial infection. By analogy, intraocular infection with CT and the ensuing chronic inflammatory process produce dystrophic and proliferative vitreoretinal changes, aggravate them, and, therefore, represent risk factors for the development of RRD.

Because high myopia has been found to be associated with significant alterations in the vitreous and retina, and to increase the risk for RRD forty-fold [29], it is an important risk factor for the disease. Only non-highly myopic patients were included in the study; in such patients, unlike highly myopic patients, the significant alterations mentioned above (as well as the development of RRD) have not been clearly explained so far.

However, because posterior vitreous detachment occurs significantly more often in individuals (including young people) with a history of uveitis [21], it might be caused by intraocular inflammation. Atrophic retinal changes may be also caused by inflammation, which is observed in herpetic infection (e.g., Cytomegalovirus retinitis) [22]. In these diseases, in the course of acute high-grade inflammation, atrophic retinal changes contribute to the development of tears and detachment (another contributor are post-inflammatory changes in the vitreous). We theorize, that similar, but in some other way manifested changes might develop in the course of intraocular low-grade inflammation induced by CT. Because the pathogenesis of RRD is closely associated with inflammatory process, we find it reasonable to believe that infectious agents might generate and maintain this disease through chronic low-grade inflammation. Therefore, we do not exclude that other bacterial or viral agents of so-called latent infections also may have association with RRD. Since the involvement of several organs in the course of chlamydial infection occurs rather often, the association of CT with RRD may be considered not only in terms of direct infection of intraocular tissues, but also in terms of the production of proinflammatory mediators at the extraocular sites of infection. The prevalence of urogenital CT infection in different populations (3%-12% in different foreign countries [23-25] and 4.9%-14% in the Russian Federation [24-25]) is higher than that of retinal detachment (0.01%-0.02%); hence, infection of urogenital tract with CT is not always associated with the development of RRD, and this can be explained by the following reasons.

First, the development of RRD requires bacteremia, which is not always observed in urogenital chlamydial infection (61% of patients with chronic pelvic inflammatory diseases and a history of chlamydial infection have been found positive for the presence of CT DNA in the serum [9]). On the other hand, chronic chlamydial infection in genetically predisposed individuals may result in the development of some specific damage to the eye, which is observed, e.g., in Reiter's disease [20]. It is still poorly understood why this chronic infection manifests itself as an ocular complication (namely, as a retinal detachment), and not as a clinically apparent conjunctivitis, urogenital or articular pathology. This is a promising area for investigation of association of chlamydial infection with RRD.

Second, the absence of RRD in most of the cases of chlamydial infection may be connected with the genetic variability in the pathogen. The CT species is divided into a number of serovars, A to K, and serovar-specific infections differ in prognosis and pathologic consequences [25-28]. However, investigation of this important issue requires serotyping, and was not the aim of the present study. Because, in prenatal infection, disseminated damage to the organs is observed, one cannot also exclude the inherent nature of chlamydial infection in some patients [26].
In the present work, to increase reliability of the results, we confirmed the presence of CT infection in patients with RRD by different methods. There are no reasons to consider clinical urogenital CT infection as a risk factor for RRD, because our study, and, to the best of our knowledge, other studies provide no basis for such conclusions. Although we may hypothesize that CT infection in any part of the body (including urogenital tract) which is usually chronic and asymptomatic may be a risk factor for RRD, additional studies are needed to establish whether this hypothesis is true.

In the present study, the reference method (cell culture) demonstrated the percentage of positive specimens similar to those of DFA and PCR. This corresponds to the report of others that the sensitivity of PCR is usually considered close to that of cell culture [30]. Moreover, the association of CT infection with RRD was found to be significant, even when based on the lowest estimates of the percentage of positive specimens (46% and 51% of conjunctival swabs and SRF specimens, respectively, by PCR). Blood and urogenital-swab specimens were not subjected to PCR, and this is a limitation of the study. Since it is well known that latent chlamydial infection provides weak stimuli for immune response and is accompanied by a low level of antibodies, we did not determine the antibody levels in sera. Additionally, because, in our study, vitrectomy for RRD was performed only in a small percentage of patients, the data related to the vitreous could not be used in full-fledged statistical analysis. The lack of this data is another limitation of the study.

We speculate that, apart from dystrophic intracocular changes and the development of RRD, the presence of CT in intraocular structures may result in aggravation of the course of RRD (potentiation of the development of PVR, in particular) due to chronic inflammation. This hypothesis does not contradict an important role for inflammation processes in the pathogenesis of vitreoretinal pathology (in particular, PVR)[2-10].

In conclusion, we demonstrated the high frequency of detection of CT in non-high myopes with RRD (which may be associated with the potential involvement of CT in the pathogenesis of RRD), with a step-by-step development of typical changes in the vitreous due to a chronic low-grade inflammation. Further investigation of the role of CT and other infections in the development of RRD may be aimed at 1) clarification of the role of the pathogen in the pathogenesis of RRD and 2) reasoning related to the use of systemic and local antimicrobial therapies in RRD.

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Conflicts of Interest: Boiko EV, None; Pozniak AL, None; Maltsve DS, None; Suetov AA, None; Nuralova IV, None.

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