

## Brief Report

# The Use of Polymerase Chain Reaction Assay versus Cell Culture in Detecting Neonatal Chlamydial Conjunctivitis

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## Abstract

One of the most common bacterial infections that causes ophthalmia neonatorum is *Chlamydia trachomatis*, (*C. trachomatis*). Very few studies have been performed in Iran using both cell culture and polymerase chain reaction (PCR) methods to determine the prevalence of *C. trachomatis* as an etiological agent of ophthalmia neonatorum. This study aimed to evaluate the prevalence of neonatal chlamydial conjunctivitis (NCC) as diagnosed by both methods in two hospitals in Tehran, Iran.

From March 2008 to May 2009, out of 2253 neonates, 241 (10.7%) with clinical findings of conjunctivitis were included in this study. A total of 241 conjunctival swabs were tested by cell culture (as the gold standard test), PCR, and Giemsa staining.

Cell cultures were positive for *C. trachomatis* in 31 (12.9%) neonates, *C. trachomatis* was positive in 40 (16.6%) neonates by PCR and 18 (7.5%) by Giemsa staining. The sensitivity of PCR was 100%, whereas Giemsa staining sensitivity was 58.1%.

High sensitivity (100%) and specificity (95.7%) of PCR as compared to culture makes it a proper diagnostic method for the detection of *C. trachomatis*.

**Keywords:** Cell culture, Chlamydia trachomatis, Ophthalmia neonatorum, PCR.

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## Introduction

Ocular infection during the neonatal period is an important health problem which may result in permanent sequelae.<sup>1</sup> Conjunctivitis occurring in infants younger than 4 weeks of age is the most common eye disease of newborns<sup>2</sup> and is caused by different agents, varying greatly in their virulence and outcome.<sup>3</sup>

The causes can be septic (bacterial or viral) or aseptic (i.e., a chemical agent such as optical silver nitrate), however the majority of infectious neonatal conjunctivitis (NC) cases are of bacterial etiology.<sup>4,5</sup> Bacterial agents that have been reported as common causes of NC are *Staphylococcus aureus*, *Hemophilus influenzae*, *Streptococcus pneumoniae*, *Gonococci*, *Pseudomonas aeruginosa*, and *Chlamydia trachomatis*, (*C. trachomatis*).<sup>5</sup> The prevalence of different microorganisms is variable among different societies due to diverse cultures, economic situations, and health care facilities.<sup>6</sup>

The occurrence of *C. trachomatis* infection in infants is directly related to the prevalence of maternal urogenital infections and vertical transmission rates.<sup>7</sup> The most frequent clinical manifestation of chlamydial infection in the newborn is conjunctivitis.<sup>8</sup> The incubation period for chlamydial conjunctivitis is 5 to 14 days. Presentation before 5 days is unusual but has been reported to occur earlier in infants born to mothers with premature rupture of the membranes (PROM).<sup>9–12</sup>

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*C. trachomatis* is the most common organism that causes NC in developed countries, with an incidence of 8.2/1,000 births in the United States during the 1990s.<sup>10,13</sup> This organism is also common in some developing countries, such as China, where it is the cause of NC in 51.2% of Chinese neonates.<sup>14</sup> However in some developing countries such as Argentina, the most frequent microorganism causing NC has been reported to be *Staphylococcus aureus*.<sup>15</sup> In a 5-year study in Iran, Amini et al. have shown that the most common microbial pathogen causing NC was *Staphylococcus aureus* (31%).<sup>16</sup> In one study in Trinidad, *Staphylococcus aureus* has been isolated in 40% and *C. trachomatis* in 11% of infants with NC; the prevalence rate of *C. trachomatis* is 3.84% per 1,000 live births.<sup>17</sup> These different results may also reflect the prevalence of sexually transmitted diseases in these communities.

Neonatal chlamydial conjunctivitis (NCC) is characterized by ocular congestion, edema and discharge. The clinical features range from an almost asymptomatic infection to severe purulent conjunctivitis.<sup>11</sup>

The “gold standard” test for diagnosis of NCC is isolation by culture. Different lab tests in use to detect *C. trachomatis* are the nucleic acid amplification test (NAAT) [i.e., polymerase chain reaction (PCR)]; transcription-mediated amplification or strand displacement amplification; and antigen detection methods that include direct fluorescent antibody (DFA) and enzyme immunoassay (EIA) tests.<sup>8</sup> Examination of Giemsa-stained cell scrapings for the presence of inclusions is the first method that has been used for the diagnosis of *C. trachomatis* infection. However, this method lacks sensitivity and is no longer recommended.<sup>18</sup>

The exact determination of the etiology in NC cases and proper antibiotic therapy are important to prevent further complications.

In Iran, few studies have been undertaken to evaluate the prevalence of NCC. This study was performed to compare different diagnostic methods (cell culture as the gold standard, PCR and Giemsa staining) to detect chlamydial infection in patients with NC.

**Table 1.** Demographic characteristic data and clinical findings of 241 neonates with conjunctivitis, March 2008- May 2009, Tehran, Iran.

Parameters	<i>C.trachomatis</i>	
	Positive Patients (n=31)	Negative Patients (n=210)
<b>Maternal characteristics</b>		
<b>Type of delivery</b>		
VD*	18 (58.1%)	89 (42.4%)
CS**	13 (41.9%)	121 (57.6%)
PROM <sup>‡</sup>	2 (6.5%)	12 (5.7%)
<b>Neonatal characteristics</b>		
<b>Gender</b>		
Male	19 (61.3%)	112 (53.3%)
Female	12 (38.7%)	98 (46.7%)
<b>Age (days)</b>		
<5	6 (19.4%)	67 (31.9%)
5-15	21 (67.7%)	76 (36.1%)
16-30	4 (12.9%)	67 (31.9%)
<b>Clinical findings</b>		
<b>Infected eye</b>		
Bilateral	19 (61.3%)	124 (59%)
Unilateral	12 (38.7%)	86 (41%)
Erythema	29 (93.5%)	187 (89%)
Discharge	28 (90.3%)	166 (79%)
Swelling	25 (80.6%)	173 (82.4%)

VD\* = Vaginal delivery; CS\*\* = Cesarean section; PROM<sup>‡</sup> = Premature rupture of membranes.

**Table 2.** Comparison of PCR and Giemsa staining tests for detection of *C. trachomatis* with cell culture in 241 neonates with conjunctivitis, March 2008-May 2009, Tehran, Iran.

Cell culture	Other tests			
	PCR		Giemsa staining	
	Positive	Negative	Positive	Negative
Positive (n=31)	31 (77.5%)	0	18 (100%)	13 (5.8%)
Negative (n=210)	9 (22.5%)	201 (100%)	0	210 (94.2%)
Total (n=241)	40	201	18	223

**Table 3.** Performance of PCR and Giemsa staining tests for detection of *C. trachomatis* compared to cell culture in 241 neonates with clinical conjunctivitis, March 2008-May 2009, Tehran, Iran.

Test	Sensitivity(95%CI)	Specificity (95%CI)	PPV*(95%CI)	NPV**(95%CI)
PCR	100 (88.8–100)	95.7 (92.0–98.0)	77.5 (61.5–89.2)	100 (98.2–100)
Giemsa staining	58.1 (39.1–75.5)	100 (98.3–100)	100 (81.5–100)	94.2 (90.2–96.9)

PPV\* = Positive predictive value; NPV\*\* = Negative predictive value.

## Materials and Methods

There were 2253 neonates hospitalized in the nursery and neonatal wards of Mahdih and Mofid hospitals in Tehran, Iran, from March 2008 to May 2009. Of these, 241 infants (10.7%) who developed clinical findings of conjunctivitis were included in our study. Conjunctivitis was diagnosed by a pediatrician in infants that presented with conjunctival erythema, swelling of the eyelids and mucopurulent discharge. All 241 neonates were of ages 1-30 days. None had received prophylactic eye drops. For each patient an information form was completed, which included data about mode of delivery, history of PROM (more than 4 – 6 hours before delivery), age at presentation, gender, and signs and symptoms of conjunctivitis. Obtained samples were taken for laboratory analysis.

Informed consent was taken from parents of the neonates. This study was performed after approval of the Ethics Committee at Shahid Beheshti University of Medical Sciences, Tehran, Iran.

### Sample collection

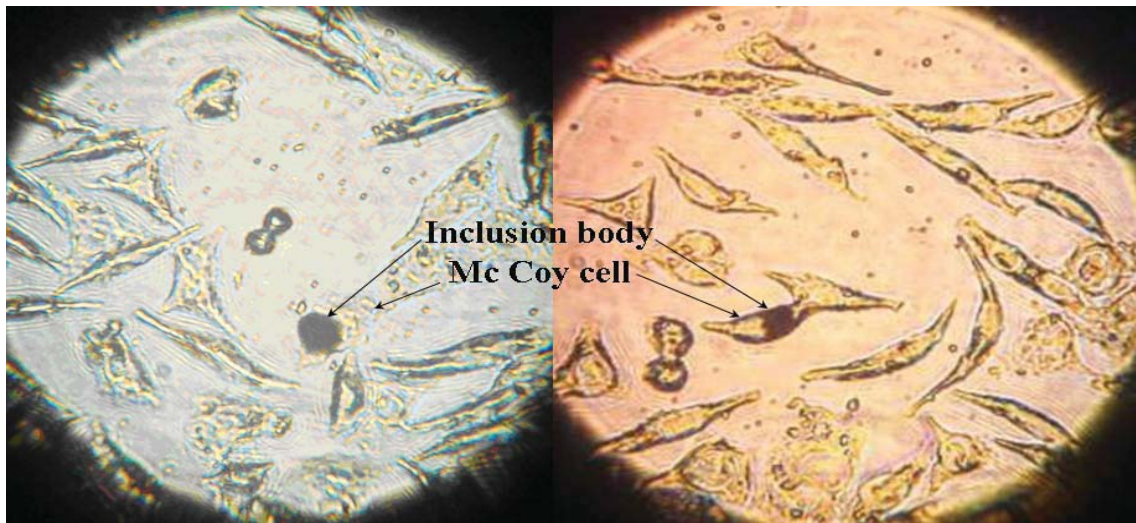
At bedside, conjunctival specimens were taken from each infant by a trained person who used two sterile swabs. Specimens were obtained from the inferior conjunctival fornix using sterile Dacron swabs (Delta Lab, Company code: 300299). The first swab was used for Giemsa staining. The second conjunctival swab was used for chlamydia detection by cell culture and PCR; this was transported via 2SP transport medium. Giemsa staining, cell culture and PCR were performed for all specimens.

### Giemsa staining

Diagnosis was based on the presence of inclusions that were basophilic and stained pinkish-blue.

### Cell culture

The presence of typical dark-brown inclusions surrounded by a halo was considered to be a positive culture. This occurs due to the consumption of glucose by *C. trachomatis* and production of glycogen which reacts with iodine.<sup>19</sup>



**Figure 1.** *C. trachomatis* inclusion bodies in McCoy cell culture (Giemsa staining, 100x).

## PCR

A gene amplification method that combines PCR with measurement of PCR products was used to detect a conserved region of the MOMP gene. The primers were obtained from MWG Company.

The sensitivity of the primers was estimated by counting the number of elementary bodies present in the culture harvest of *C. trachomatis* Ba (ATCC-VR 347). Positive control DNA was *C. trachomatis* (ATCC-R 347).

We searched for an 871 bp band on 1% agarose gel which had undergone electrophoresis for 2 h at 53 mA.

## Statistical analysis

All variables were summarized as frequency and percentage. Chi-square was used to categorize factors associated with the diagnosis of chlamydial conjunctivitis. Statistical significance was assumed at the  $P < 0.05$  level. Sensitivity, specificity, positive and negative predictive values were calculated according to routine formulas and the binomial exact method was performed to calculate related 95% confidence intervals (95% CI) by STATA-11 software.

## Results

There were 241 (10.7%) neonates out of the 2253 neonates hospitalized in nursery and neonatal wards of Mahdih and Mofid hospitals during 14 months who developed conjunctivitis. Cell cultures, as the gold standard test, were positive for *C. trachomatis* in 31 (12.9%) neonates (Figure 1). Of the 31 neonates with NCC, 6 infants (19.4%) were younger than 5 days, 21 (67.7%) were between 5-15 days, and 4 neonates (12.9%) were between 16-30 days. Nineteen patients (61.3%) were males. The average age was 10 days (range: 2-30 days). Thirteen neonates (41.9%) were delivered by cesarean section and 18 (58.1%) were vaginal deliveries. A history of PROM was positive in 2 (6.5%) mothers out of the 31 neonates with NCC. Conjunctivitis was bilateral in 19 (61.3%) neonates. Erythema was present in 29 (93.5%), purulent discharge was present in 28 (90.3%), and eye swelling in 25

(80.6%) neonates. Most signs were seen together (Table 1).

Of the 241 conjunctival specimens, *C. trachomatis* was detected in 40 specimens (16.6%) by PCR and in 18 (7.5%) by Giemsa staining for the presence of inclusions (Table 2). The sensitivities, specificities, and positive and negative predictive values (PPV and NPV) for PCR and Giemsa staining compared to cell culture for detection of *C. trachomatis* are shown in Table 3.

There were no statistically significant differences in age ( $P = 0.09$ ), gender ( $P = 0.40$ ), mode of delivery ( $P = 0.10$ ), and history of PROM ( $P = 0.69$ ) between neonates with positive or negative investigations for *C. trachomatis*.

No statistically significant correlations were found between unilateral or bilateral infections ( $P = 0.81$ ), erythema ( $P = 0.75$ ), discharge ( $P = 0.14$ ), and swelling ( $P = 0.81$ ) with chlamydial conjunctivitis.

## Discussion

NC is a common disease usually acquired from mothers' birth canals, though sometimes it may be acquired from the environment.<sup>20</sup> Approximately 30%-50% of infants born to mothers with active, untreated, chlamydial infection develop clinical conjunctivitis.<sup>12</sup>

In the present study, the prevalence of NC was 10.7%. Our results were higher than the prevalence of 5.4% previously reported in Iran by Soltanzadeh et al. in 2002<sup>6</sup> and the prevalence of 4.9% from a study by Amini.<sup>16</sup> However, the prevalence in our study corresponded to other reports in which conjunctivitis rates ranged from 1.6% to 12% of neonates.<sup>21-23</sup>

Our study demonstrated that 12.9% of the neonates had chlamydial conjunctivitis diagnosed by cell culture. In one study in 2007, the incidence of NCC in one region of Hong Kong was 12.5%.<sup>24</sup> Another study in Hong Kong showed the incidence of NCC at 21%.<sup>4</sup>

Isolation of chlamydia in tissue culture is still considered the "gold standard" for diagnosis of chlamydial infections.<sup>11,12,25</sup> In the present study, the prevalence of chlamydial conjunctivitis appeared high, but to our knowledge there was no other study in Iran



that determined the prevalence of *C. trachomatis* conjunctivitis using the culture method and PCR, or any comparison between them. The prevalence of chlamydial conjunctivitis was reported as 6% in a previous Iranian study that used Giemsa staining and 2% in a previous Iranian study that used DFA test.<sup>6,16</sup> Using cell culture as the gold standard to detect *C. trachomatis* in our study can provide an explanation for these differences.

We performed PCR for the diagnosis of chlamydial conjunctivitis and Giemsa staining to demonstrate chlamydial inclusions in comparison to cell culture. All cell culture positive cases had positive PCR results. PCR sensitivity was 100%, whereas Giemsa staining was 58.1% (Table 3). One study conducted by Hammerschlag et al. showed PCR to be equivalent to culture for eye specimens and more sensitive than culture for nasopharyngeal specimens. When compared with culture for conjunctival specimens, PCR had a sensitivity of 92.3%, specificity of 100%, PPV of 100% and NPV of 98.4%.<sup>26</sup> In 2002 Veringa et al. showed that PCR was the most sensitive and specific assay in their study. The assay is rapid, easy to perform and less expensive than culture. The researchers found PCR to be very suitable for use in a clinical diagnostic microbiology laboratory.<sup>27</sup>

Symptoms due to NCC usually develop 5-14 days after delivery.<sup>9,12</sup> Most neonates with chlamydial conjunctivitis presented between the ages of 5 and 15 days in our study.

In our study, 61.3% of neonates with chlamydial conjunctivitis were males, which was similar to the study conducted by Yescas et al. in 1993.<sup>28</sup> However, two studies in Hong Kong showed most neonates with chlamydial conjunctivitis were females.<sup>4,24</sup> We found no statistical relationship between sex and chlamydial conjunctivitis ( $P = 0.40$ ). We observed that 58.1% of neonates with chlamydial conjunctivitis were delivered vaginally. However, the incidence of NCC was not significantly different between neonates delivered vaginally or by caesarean section ( $P = 0.10$ ). In one study, Yescas et al. showed that 56.25% of NCC cases were delivered vaginally.<sup>28</sup>

PROM is a predisposing factor that can increase the chances that a newborn will acquire NC.<sup>20</sup> In Iroha's study, PROM was the most important predisposing factor in NC.<sup>29</sup> In our study, two neonates (6.5%) with chlamydial conjunctivitis had been delivered from mothers with histories of PROM, but there was no significant association between chlamydial conjunctivitis with maternal history of PROM ( $P = 0.87$ ).

Some studies indicated that in cases with PROM, the newborn can be infected even if delivered by cesarean section.<sup>30,31</sup> There is also some evidence that *C. trachomatis* can be detected by PCR in the amniotic fluid of pregnant women without PROM.<sup>32</sup> In 2003, Wu et al. have reported 4 neonate cases with *C. trachomatis* conjunctivitis born by cesarean section without PROM.<sup>14</sup> No correlation could also be observed between age and chlamydial conjunctivitis ( $P = 0.09$ ).

## Conclusion

From our study the prevalence of conjunctivitis due to *C. trachomatis* is determined to be 12.9% of all NC. Taking into consideration the high prevalence of *C. trachomatis* in our study, we suggest that clinicians should have a high index of suspicion for NCC. Cell culture as the gold standard is costly and takes at least 2 to 3 days before results are available, however because of the high sensitivity (100%) and specificity (95.7%) of PCR in

our study, PCR can be considered a proper diagnostic method for detection of *C. trachomatis*. Since Giemsa staining is simple to perform and available in most laboratories, we recommend performing Giemsa staining to diagnose NCC in areas where PCR is not readily available.

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