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A microbiological study of neonatal conjunctivitis in two hospitals in Tehran, Iran

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PEER REVIEW

Peer reviewer

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Comments

This is a good study which reveals the incidence of the neonatal conjunctivitis in both hospitals. Bacterial etiologic agents were discussed. The authors have screened the etiologic agents of neonatal conjunctivitis from their geographical regions. The article creates significant outcome towards the treatment.

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ABSTRACT

Objective: To determine the prevalence of neonatal conjunctivitis and to identify the causative agents of ophthalmia neonatorum in two university affiliated hospitals from 2008 to 2009.

Methods: All neonates admitted in the neonatal department during the study period were examined for the presence of conjunctivitis. Two swab specimens containing epithelial cells of the conjunctiva were collected from newborns presenting with conjunctival inflammation. Laboratory diagnosis was based on direct smear for Gramstaining and bacterial culture. The isolated bacteria were identified using standard procedures. PCR and cell culture were used for identification.

Results: Of the 2253 neonates, (age ranged 1–30 days), clinical findings of conjunctivitis were found in 241 cases (10.7%). The most commonly isolated bacteria were *Coagulase negative staphylococci*, ($n=130$, 53.9%); *Chlamydia trachomatis* was the second most common cause of acute neonatal conjunctivitis ($n=40$, 16.6%). Bacterial cultures were negative in 47 neonates (19.5%) despite clinical signs of conjunctivitis. The median age at presentation for bacterial culture positive was day 8 of life.

Conclusions: Neonatal conjunctivitis is prevalent in newborns; Gram positive cocci and *Chlamydia trachomatis* are the most common causative organisms.

KEYWORDS

Neonate, Bacterial conjunctivitis, *Chlamydia trachomatis*

1. Introduction

Neonatal conjunctivitis (ophthalmia neonatorum), is a commonly encountered problem which presents during the first month of life^[1]. The causes can be septic (bacterial or viral) or aseptic (e.g. a chemical agent such as topical silver nitrate) and the majority of infectious neonatal conjunctivitis are of bacterial etiology^[2,3]. In spite most of these cases are benign, some of them may progress to systemic

complications or visual loss if left untreated. Often, but not invariably, bacterial conjunctivitis has a longer incubation period as compared to other infective causes^[4]. Clinical signs include redness, tearing, secretion, conjunctival and palpebral inflammation, a pseudomembrane and corneal perforation which may result in blindness^[5].

The most commonly etiology agents of conjunctivitis are Gram-positive organisms include *Staphylococcus aureus* (*S. aureus*), *Streptococcus pneumoniae*, *Streptococcus viridans*,

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and *Staphylococcus epidermidis* (*S. epidermidis*); Gram-negative organisms, such as *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Proteus*, *Enterobacter*, and *Pseudomonas* species, also have been implicated[5]. These later organisms are probably acquired after birth, as their mode of delivery has little influence on the incidence[6]. *Chlamydia trachomatis* (*C. trachomatis*) and *Neisseria gonorrhoea* (*N. gonorrhoea*) are the two well described agents associated with ophthalmia neonatorum, and are known to be associated with systemic complications and severe visual loss[7–9].

High-quality antenatal care in the West and the use of intrapartum antibiotics to treat a positive high-vaginal swab has led to a dramatic reduction in the incidence of gonococcal conjunctivitis[3]. Topical eye drops such as silver nitrate effectively prevent gonococcal neonatal conjunctivitis but it is not effective against chlamydial conjunctivitis. The exact cause of neonatal conjunctivitis cannot generally be identified on clinical grounds alone. The timing of symptom onset and a history of maternal sexually transmitted disease may give some clues to the underlying cause but do not provide a definite diagnosis[10].

In England, in the mid-1800s, there was a hospital devoted solely to the lavage of infants with gonococcal ophthalmia. Fortunately, the work of Crede, who developed prophylaxis with 2% silver nitrate in 1881, nearly eliminated visual loss from this disease in the West[11]. Unfortunately, conjunctivitis in the first month of life still remains a common threat to vision in much of the developing world. In areas without prophylaxis, the incidence of ophthalmia neonatorum is as high as 23%. In Africa, between 1000 and 4000 infants are blinded each year[12]. *S. aureus* is the most common organism causing neonatal conjunctivitis in most of the developing countries[10]. In one study in India *S. epidermidis* was the most common isolated bacterial species (28.6%) followed by *S. aureus* (10%)[13]. Amini *et al.* showed that *E. coli* was reported as the second most common cause (23%) of acute neonatal conjunctivitis after *S. aureus* (31%) in a five year study in Iran[14]. The exact determination of the etiology in neonatal conjunctivitis cases may help improve initial management and control, thereby preventing further complications.

This study was performed to determine the prevalence of conjunctivitis and its bacteriological causes in neonates of two hospitals in Tehran.

2. Materials and methods

2.1. Study population

We conducted our study at the neonatal wards of two hospitals in Tehran, over a period of 14 months (March 2008–May 2009). Of the 2253 neonates (age range 1–30 days), 241 cases who presented with clinical findings of conjunctivitis

were included in this study. After informed consent from the mothers, a questionnaire was filled for each patient by members of the study team and microbiological tests were performed. Details of mode of delivery, age at presentation, gender, signs and symptoms (conjunctival erythema, swelling of the eyelids, mucopurulent discharge, unilateral/bilateral involvement, and other problems) were documented.

2.2. Specimen collection

At the bedside, conjunctival specimens were taken by a trained person from each infant with two sterile swabs. Specimens were obtained from inferior conjunctival fornix for routine bacterial culture and PCR for *C. trachomatis*. Specimens were obtained by swab from the conjunctiva of the everted lower eyelid using a sterile swab. A smear was made from the swab taken for chlamydia detection inside 2SP transport medium and the specimens transported to the laboratory.

2.3. Specimens culture

The conjunctival scraping was inoculated at the bedside into a thioglycolate broth for detection of bacteria, the tubes were immediately transported to the laboratory and incubated at 37 °C for a minimum of 24 h and cultured immediately on blood agar and chocolate agar. The plates were incubated at 37 °C in the presence of 5% CO₂. The isolated bacteria were identified using standard procedures. Laboratory diagnosis was based on Gram staining, bacterial culture (using chocolate agar for *N. gonorrhoeae* and blood agar for other bacteria). All isolates were identified according to conventional microbiological procedures.

2.4. PCR

A gene amplification method that combines a PCR with measurement of PCR products was used to detect a conserved region of the major outer membrane protein (MOMP) gene.

These primers were made by the MWG Company: 5'-GATAGCCAGCACAAAGAGAGCTAA-3' sense and 5'-CTTTGTTTTTC GACCGTGTITTCGCAAACAGATGTGAA-3' antisense.

CinnaGen DNA Purification Kit was used for extraction of DNA and CinnaGen PCR Master Kit was used for PCR reaction. A 50 µL lysate of target cellular material was amplified through 30 cycles (1 min denaturized at 94 °C, 1 min primer annealing at 55 °C and 1 min primer extension at 72 °C) in a 100 µL master mix (PCR reaction buffer, dNTP, Tag DNA polymerase) and 0.5 µmol/L of primers that conserved MOMP nucleotide sequences of *C. trachomatis*.

The band of PCR product was showed by electrophoreses. Positive control DNA was *C. trachomatis*-ATCC-VR 347. We found an 871 bp band on 1% agarose gel which had undergone electrophoresis for 2 h at 53 mA.

3. Results

This study was done on 241 neonates, 165 infants (67.2%) were from Mahdih Hospital and 79 neonates (32.8%) from Mofid Children Hospital. One hundred and thirty one neonates (54.4%) were male, and 110 (45.6%) female. One hundred and seven babies (44.4%) had been delivered vaginally and 134 (55.6%) by cesarean section. Bilateral conjunctivitis was present in 59.3% of patients, and unilateral involvement was seen in 40.7% of the patients.

Bacterial cultures were positive in 194 neonates (80.5%), but were negative in 47 (19.5%) neonates despite presence of conjunctivitis (Table 1). Among 194 neonates with bacterial conjunctivitis, 103 (53.1%) neonates were delivered by cesarean section and 91 (46.9%) were delivered vaginally. Bacterial conjunctivitis was unilateral in 77 (39.7%) neonates of those 194 neonates and it was bilateral in 117 (60.3%) of them. Erythema, discharge and swelling were present in 177 (91.2%), 161 (83.0%) and 157 (80.9%) cases. We found relation between the presence of discharge with neonatal bacterial conjunctivitis ($P < 0.04$).

The most common organisms detected on conjunctival smears were Gram positive cocci in 56%, and coagulase-negative staphylococci were the leading bacteria grown on culture ($n=130$, 53.9%). The results of smears, bacterial cultures and PCR for chlamydia are shown in Figure 1.

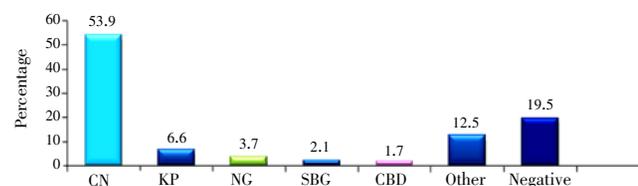


Figure 1. Results of bacterial culture in neonatal conjunctivitis ($n=241$). CN: Coagulase-negative staphylococci, KP: *Klebsiella pneumoniae*, NG: *N. gonorrhoeae*, SBG: Streptococcus B group, CBD: Coryne bacterium diphtheriae.

The most common risk factor in mothers was prolonged rupture of membranes found in 14 cases (5.8%).

There were no significant differences in gender ($P=0.14$), mode of delivery ($P=0.11$) and history of prolonged rupture

Table 1

Demographic characteristic data and clinical manifestations of 241 neonates with conjunctivitis, March 2008– May 2009, Tehran, Iran.

Patient characters	Bacterial culture			Total <i>n</i> =241 Count (percent)	<i>P</i> value
	Negative <i>n</i> =47	Positive <i>n</i> =194			
	Count (percent)	Count (percent)			
Hospital	Mofid	11 (23.4%)	68 (35.1%)	79 (32.8%)	0.12
	Mahdih	36 (76.6%)	126 (64.9%)	162 (67.2%)	
Childbirth	NVD	16 (34.0%)	91 (46.9%)	107 (44.4%)	0.11
	C/S	31 (66.0%)	103 (53.1%)	134 (55.6%)	
Sex	Male	30 (63.8%)	101 (52.1%)	131 (54.4%)	0.14
	Female	17 (36.2%)	93 (47.9%)	110 (45.6%)	
Eye involvement	Bilateral	26 (55.3%)	117 (60.3%)	143 (59.3%)	0.53
	Erythema	39 (83.0%)	177 (91.2%)	216 (89.6%)	
	Discharge	33 (70.2%)	161 (83.0%)	194 (80.5%)	
	Swelling	41 (87.2%)	157 (80.9%)	198 (82.2%)	
PROM	0 (0.0%)	14 (7.2%)	14 (5.8%)	0.05	

NVD: Normal vaginal delivery, C/S: Cesarean section, PROM: Premature rupture of membranes.

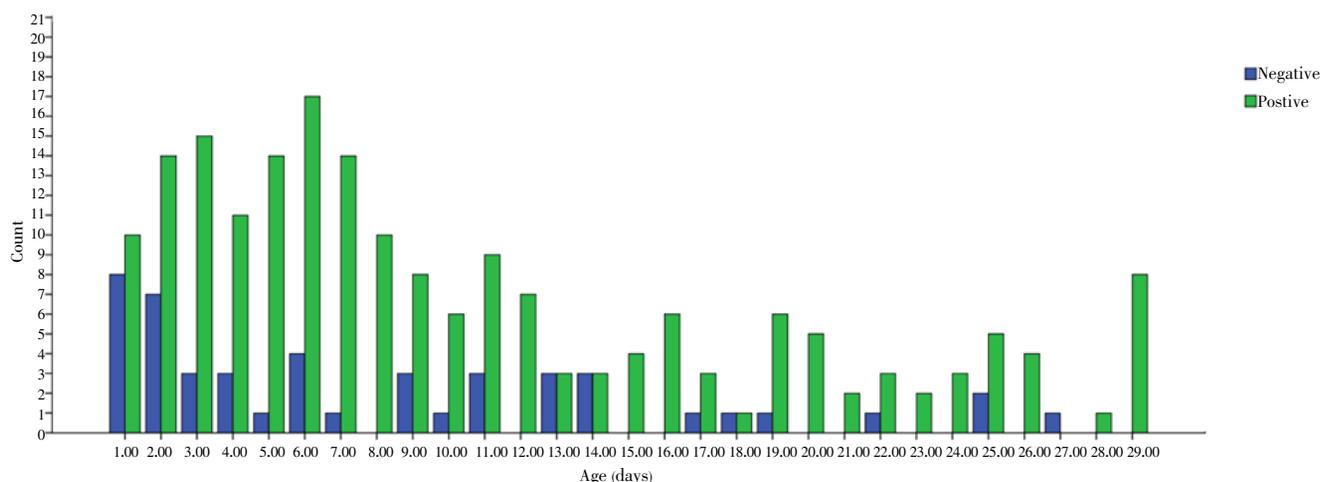


Figure 2. The relationship between age and bacterial count of neonates with neonatal conjunctivitis, March 2008– May 2009, Tehran, Iran.

of membranes ($P=0.05$) between neonates with positive or negative investigations for bacterial conjunctivitis. The median age at presentation for a culture positive conjunctivitis was day 8 of life in neonates' ages ranging from day 1 to day 30 (Figure 2).

4. Discussion

Neonatal conjunctivitis occurs in 1.6%–12.0% of newborns. Any eye infection that occurs in the first month of a baby's life is defined as ophthalmia neonatorum. The most common types of bacteria that cause infection in the infant's eye come from the mother's birth canal. The most common cause is chemical irritation from antimicrobial prophylaxis against bacterial infection. In the present study the incidence of neonatal conjunctivitis was 10.7%. The figures are similar to other reports, which quote rates from 1.6% to 12.0% of neonates^[14].

Infected newborns usually develop drainage from the eyes in the first month of life^[15].

In our study, most of the neonates with bacterial conjunctivitis were between the ages of 1 and 12 days and with all three symptoms (erythema, swelling and discharge).

In our study, the microorganisms causing neonatal conjunctivitis were coagulase–negative staphylococci, *C. trachomatis*, *Klebsiella pneumoniae*, *Neisseria mucosa*, Streptococcus B Group and *Corynebacterium diphtheriae*, respectively.

In a study by Soltanzadeh *et al.*, in which 170 of 3140 of neonates (5.4%) had conjunctivitis signs, the most common microbial organisms were coagulase negative staphylococci (15.3%), *S. epidermidis* (13.5%), *E. coli* (7.6%) and *C. trachomatis* (6.0%)^[5].

In other study by Amini *et al.* in Iran the micro–organisms causing 198 of 4021 (4.9%) neonatal conjunctivitis were *S. aureus*, (31%), *Escherichia coli* (23%), *S. epidermidis*, (22%), *Klebsiella pneumoniae* (10%), *N. gonorrhoea* (3%), *Pseudomonas aeruginosa* (2%) and *C. trachomatis* (2%) respectively^[14]. In a study by Iroha Eo and his colleagues in 1998 on 150 hospitalized neonates, the prevalence of conjunctivitis was reported as 1.8% that their microbial causes were *S. aureus* (37.4%), *Klebsiella pneumoniae* (12.9%), and *S. coagulase* negative (12.3%). The cause of lower prevalence in study of Iroha can be due to small samples or due to study of only hospitalized patients that the researchers had found lower positive cases^[16].

S. aureus was the most common organism cultured from neonates with acute conjunctivitis, but its role in neonatal

conjunctivitis remains controversial because it is frequently cultured from the eyes of asymptomatic neonates^[10]. In the study of Mohil M, the most common microbial pathogen causing neonatal conjunctivitis was *S. epidermidis* (57.14%) and corresponded to some studies which showed that *S. epidermidis* is the most common organism causing neonatal conjunctivitis. Even though *S. epidermidis* was the most commonly isolated bacterial species, the exact role of this organism in the causation of conjunctivitis in newborn babies remains unclear. These organisms might have been the part of the normal flora that is in the infants^[13]. The *Neisseria mucosa* and *Corynebacterium diphtheriae* isolates were also most likely part of the normal conjunctival flora. Gonococcal ophthalmia neonatorum has an incidence of 0.3/1000 in live births in the United States^[17]. In Dr. Amini's study of neonatal conjunctivitis in Iran the incidence of gonococcal ophthalmia was 3%^[14], however in our study, although prophylaxis treatment of gonorrhoea for newborn is not used in these two hospital, no case of gonococcal ophthalmia was detected using standard procedures for detection of *N. gonorrhoea* including Gram staining and culture.

The results of this study clearly demonstrated that the frequency of neonatal conjunctivitis is significantly high (10.7%) in Iran. Bacteria could be isolated from 80 percent of the patients with clinical signs of conjunctivitis.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

Neonatal conjunctivitis is one of the important systemic

complications. It causes visual loss if not treated promptly, most common etiologic agent is *N. gonorrhoeae*, *C. trachomatis* and herpes virus. Global prevalence of neonatal conjunctivitis is less than 0.5 percent per 1000 population, Pakistan and Africa show higher percentage of morbidity when compared to others.

Research frontiers

Incidences of neonatal conjunctivitis were 35.1% and 64.9% in both Mofid and Mahdieh hospitals from 2008–2009 in Iran.

Related reports

A recent article published similar methods in the same geographical region, it was found that 226/5206 neonates had signs of conjunctivitis. Among these, bacterial cultures were positive in 148, the major causative agents are *N. gonorrhoeae*, and *C. trachomatis*. Prophylactic measures and treatment for pregnant women were surmised.

Innovations & breakthroughs

This study revealed two aspects of infections. There is no significant difference between caesarian and normal vaginal delivery. Enteric pathogens and Gram-positive bacteria play a major role which is due to premature rupture of the membrane and skin contamination. This study reiterates the importance of screening pregnant women for *C. trachomatis* and other sexually transmitted diseases just prior to the birth of a child.

Applications

This study emphasises the importance of focusing on neonatal conjunctivitis. In addition, screening and prophylactic measures are needed for pregnant women prior to the birth of the baby.

Peer review

This is a good study which reveals the incidence of the neonatal conjunctivitis in both hospitals. Bacterial etiologic agents were discussed. The authors have screened the etiologic agents of neonatal conjunctivitis from their geographical regions. The article creates significant outcome towards the treatment.

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