

Diagnosis of Chlamydial Ophthalmia in Egyptian Neonates

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Abstract: This study was performed to find out the relation between maternal genital Chlamydial infection and ocular Chlamydial infection of their neonates using two methods, Giemsa staining for detecting inclusion bodies and immunofluorescence (IF) staining for detecting elementary bodies (EBs) using conjugated monoclonal antibody and also comparing the sensitivity between the two tests. The study was performed on 50 pregnant ladies with vaginal discharge and 25 asymptomatic pregnant ladies and their neonates. It was found that immunofluorescent test is more sensitive and accurate than Giemsa staining for the detection of *C. trachomatis* both in mothers and their neonates and also in asymptomatic controls. It is advised in *Chlamydia trachomatis* (*C. trachomatis*) endemic areas like Egypt to apply antibiotic eye drops to all newly born eyes immediately after birth. It is also recommended to treat any genital tract discharge in the last month of pregnancy.

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1. Introduction

Chlamydia trachomatis has become the most common infectious cause of ophthalmia neonatorum in developed countries. Widespread use of silver nitrate drops resulted in a dramatic decline in the incidence of gonococcal ophthalmia but had much less impact on the incidence of neonatal chlamydial infection (1). *C. trachomatis* is the most common cause of neonatal conjunctivitis and one of the most common aetiologies for pneumonia in neonates (2). Nearly two thirds of infants born vaginally to infected mothers develop chlamydial infection (3, 4).

30% to 50% of neonates with proven exposure to *Chlamydia*, will develop conjunctivitis (5). The prevalence of chlamydial infections is higher in the spring and summer months (6). The incubation period is typically 1 week after delivery; however, it varies from 5 to 14 days or earlier if membranes ruptured prematurely (7). The clinical manifestations vary from mild conjunctival injection with scanty watery discharge to severe mucopurulent discharge with eyelid oedema, chemosis, and pseudo-membrane formation (8). Loss of vision is very rare. Most cases of chlamydial infections resolve spontaneously without complications, but, if left untreated, superficial corneal vascularization and conjunctival scarring can occur (5). Newborns with conjunctivitis should have specimens of their conjunctiva and pharynx sent for culture. The American Academy of Pediatrics recommends a 14-day course of systemic erythromycin (50 mg/kg/d, divided in 4 doses) (9).

Topical therapy is not indicated (5). Erythromycin has a 10% to 20% failure rate and thus some infants will require a second or occasionally a third course of erythromycin. The infant's mother and her sexual partners should be treated for *Chlamydia* (10, 11).

All pregnant women should be routinely screened for *Chlamydia trachomatis* early in pregnancy to prevent the adverse effects of *Chlamydia* during pregnancy, but supportive evidence for such screening is lacking (12, 13, and 14).

2. Materials and Methods

A total of 50 pregnant ladies with vaginal discharge and 25 asymptomatic pregnant ladies were collected from women attending in labour at the Delivery Department at Alzahraa Hospital. Gynaecological examination was performed for all patients looking for any evidence of genital tract *Chlamydial* infection. Vaginal swabs were obtained just before birth. Duplicate specimens were taken one for the monoclonal immunofluorescent test and the other for Giemsa staining. Seven of the symptomatic patients delivered by Caesarean section. The remaining 43 cases and all the asymptomatic cases delivered vaginally.

Eyes of the delivered babies were examined for any evidence of conjunctival affection. A topical anaesthetic was applied to the eyes. Two swabs were taken from the upper palpebral conjunctiva, on the 1st, 7th and 14th day of age; one for the monoclonal

immunofluorescent test and the other for Giemsa staining.

To ensure specimen adequacy, at least 10 intact columnar epithelial cells should appear on the slide.

A smear was made by rubbing the swab immediately, firmly and evenly over the whole uncoated area (well) of a multisport glass slide (Flow lab; USA). The smear was allowed to dry then fixed immediately in acetone, and stored at 2-8°C to be stained within 7 days of collection.

For the immunofluorescent staining, the commercially available *Chlamydia trachomatis* direct fluorescent monoclonal antibody reagent kit (Syva, U.K) was used. It contains fluorescein- labelled monoclonal antibodies specific to the membrane-protein of *C. trachomatis* and Evans blue counter stain in a protein stabilized buffer solution. The kit also contains positive and negative control slides prepared from *C. trachomatis* infected and non-infected tissue culture cells.

A direct immunofluorescent staining method was used as described by Thomas *et al.* (15). In brief, 25 µl of the monoclonal antibody was added to each control slide and fixed specimen, and incubated for 30 minutes at room temperature in a moist chamber, following washing, slides were allowed to air dry then mounted in a buffered glycerol and examined under fluorescent microscope using the oil immersion lens. Elementary bodies (EBs) appear as individual pin points of medium to bright apple- green fluorescence with contrasting reddish brown background of counterstained cells. At least 10 EBs should be identified on positive specimens.

For Giemsa staining, fixed specimens and control slides were floated with freshly prepared 10%Giemsa stain for 1 hour. The slides were then rinsed rapidly in 95% ethyl alcohol to remove excess dye. The slides were dried and examined for the presence of intra-cytoplasmic inclusion bodies with a light microscope at X200 magnification. The EBs stained purplish, while the initial bodies tend to stain bluish. The inclusions stand against the greyish cytoplasm in contrast with the pink nucleus of the cell (16).

3. Results

Laboratory results were correlated with the clinical findings for maternal genital tract and neonatal ocular affection. Neonatal involvement was also correlated with maternal affection and mode of delivery.

Results obtained are presented in Tables (1, 2) and Figs (1,2) demonstrate positive immunofluorescent test and Giemsa stained smears respectively.

Table (1) showed that Giemsa staining is less sensitive in detecting *C. trachomatis* than IF test. Neonatal affection is always less common than the maternal affection. Chlamydial identification in neonatal conjunctivitis is highest at the end of the first week and least immediately after birth.

C. trachomatis were detected in 27 (54%) and 38 cases (76%) of infected mothers by Giemsa and IF respectively. Rate of affection of their neonates depends upon their age. It was 4 (8%), 6 (12%) on the first day, 10 (20%), 20 (40%) on the 7th day and 13 (26%), 27 (54%) on the 14th day by Giemsa and IF staining respectively.

Table: (2) showed also less sensitivity of Giemsa staining than IF in demonstrating *C. trachomatis* in asymptomatic mothers. Although they were asymptomatic clinically, yet some genital swabs were positive for *C. trachomatis*. Conjunctival swabs of neonates of asymptomatic mothers also reported positive results. The rate of affection was less in the asymptomatic mothers and their neonates. It was 2(8%), 4 (16%) in the mothers, and 0(0%), 1 (4%) in neonates on the first day, 1 (4%), 3 (12%) on the 7th day and 1(4%), 3 (12%) on the 14th day by Giemsa and IF staining respectively.

Figure (1): showed IF staining of neonatal conjunctival swabs. EBs appeared as apple –green pin points. At least 10 EBs with contrasting reddish-brown background of counterstained cells were identified on the positive specimen.

Figure (2): showed Giemsa staining of neonatal conjunctival swabs. The EBs stained purplish whereas the initial bodies tend to stain more blue. The inclusions stand out against the cytoplasm in contrast with the pink nucleus of the cell.

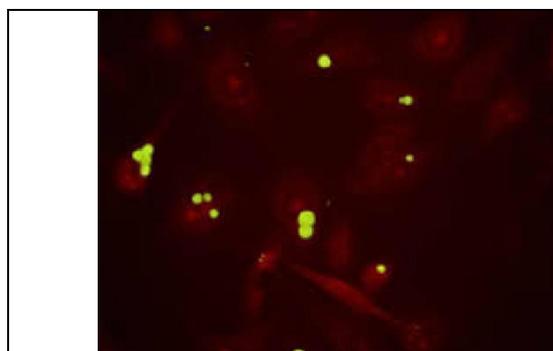


Figure (1): Positive immunofluorescent staining of conjunctival smears

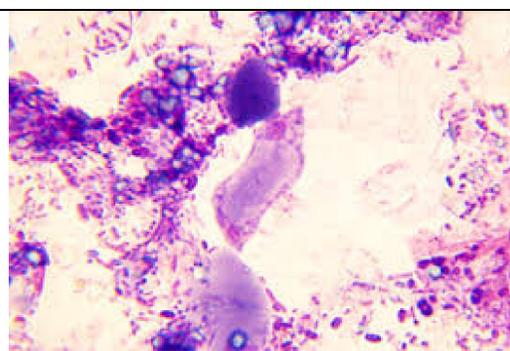


Figure (2): Positive Giemsa staining of conjunctival smears

Table: (1) Demonstration of *C. trachomatis* from genital swabs of symptomatic mothers and conjunctival swabs of their neonates:

	Mothers		Neonates					
			1 st day		7 th day		14 th	
	Giemsa	I F	Giemsa	I F	Giemsa	I F	Giemsa	I F
Positive	27	38	4	6	10	20	13	27
Negative	23	12	46	44	40	30	37	23
Total	50	50	50	50	50	50	50	50

Table: (2) Demonstration of *C. trachomatis* from genital swabs of asymptomatic mothers and conjunctival swabs of their neonates:

	Mothers		Neonates					
			1 st day		7 th day		14 th	
	Giemsa	I F	Giemsa	I F	Giemsa	I F	Giemsa	I F
Positive	2	4	0	1	1	3	1	2
Negative	23	21	25	24	24	22	24	23
Total	25	25	25	25	25	25	25	25

4. Discussion:

Ophthalmia neonatorum refers to any conjunctivitis occurring in the first 28 days of life. It is most commonly infective in origin; bacterial causes include *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and various other organisms (17).

Ophthalmia neonatorum affects 1-12% of infants in the western world. The precise figure varies according to the socio-economic status of the area. The figure goes up to 23% in developing countries. Originally, the term neonatal ophthalmia referred to conjunctivitis in the newborns caused by *N. gonorrhoeae*, but now the term is used for any conjunctivitis in this age group, irrespective of causative organism (18).

Genital infection with *Chlamydia trachomatis* is the most common sexually transmitted bacterial infection worldwide (19). *C. trachomatis* is primarily transmitted to newborns via exposure to an infected mother's genital tract during vaginal birth (20).

Although, approximately 75% of women with *C. trachomatis* infection are asymptomatic, it has a major role in neonatal infection (21).

The onset of neonatal conjunctivitis varies with different aetiological agents. Chlamydial conjunctivitis usually has a later onset than gonococcal conjunctivitis; the incubation period is 5-14 days but Gonococcal conjunctivitis tends to occur 2-7 days after birth, but could present later (22).

Demonstration of *C. trachomatis* in conjunctival and vaginal epithelium is a useful adjunct to clinical examination in studying the epidemiology of *C. trachomatis* infection, it confirms the diagnosis in doubtful cases, gives a measure of the reservoir of infection in the community and is helpful in monitoring the effect of control programs especially in endemic countries.

In the present study, EBs of *C. trachomatis* were demonstrated rapidly and easily with fluorescein conjugated- antibodies to species specific *C. trachomatis* surface antigen. This technique has been shown to be comparable to isolation in cycloheximide-treated McCoy cells for genital tract specimens (15). And for those taken from the eyes of experimentally infected monkeys (23).

In our study IF staining was more sensitive than Giemsa staining in detecting *C. trachomatis* both in

mothers and their neonates and also in asymptomatic controls Table (1, 2).

The findings in this study showed that all smears which were positive for inclusion bodies by Giemsa were also positive for EBs by IF, which confirms the specificity of the test. The IF technique however was more sensitive as compared to Giemsa staining since EBs were detected in vaginal smears of a total 38 (76%) of symptomatic mothers of whom 27 (54%) were also positive for inclusion bodies. This results are in accordance with a study done by Max (24) who stated that the direct fluorescent antibody test has approximately 75% to 85% sensitivity and 98% to 99% specificity compared with culture, and lower sensitivity (approximately 70%) compared with Nucleic acid amplification tests.

Our findings stated that more than half of neonatal Chlamydial conjunctivitis start monocularly, but after 7 days progressive involvement of the other eye occurs. Our results correlate with Eszter and Fruzsina (25) who reported that neonatal Chlamydial conjunctivitis generally starts monocularly, but after 2-7 days the progressive involvement of the other eye can be observed in 75% of the newborns.

Chen *et al*, and Rourset *al*,(26, 27) reported that vertical transmission rates are relatively high (50-75%) of infants born vaginally as they acquire *C.trachomatis* directly from their infected mothers; this is in accordance with our study which revealed neonatal Chlamydial infections reaching up to 54% at 14th day as detected by IF staining.

Conclusion:

C.trachomatis is a major cause of neonatal conjunctivitis that can be efficiently and rapidly diagnosed by direct IF monoclonal antibody staining of conjunctival smears. Screening for *C. trachomatis* is not warranted during pregnancy since it is easily treated with erythromycin. However, any vaginal discharge especially in the last month of pregnancy should be treated carefully. Eyes of neonates should be carefully observed at least 4 weeks and microbiological examinations for *C. trachomatis* is indicated if signs of infection appear. So focused screening efforts should be made to reduce the number of infected pregnant women and thereby the rate of vertical transmission.

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