Original Article

# ToRCH-screening in pediatric cataract revisited: A North Indian tertiary care centre study

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**Purpose:** To analyze and report ToRCH-serology screening profile (*Toxoplasma gondii* [TOX], rubella [RV], cytomegalovirus [CMV], and herpes simplex virus [HSV-I/II]) in pediatric cataract. **Methods:** In this prospective analytical study, 1,026 consecutive children were screened, of which 46 children with clinically diagnosed congenital ($n$ = 26) and developmental cataract ($n$ = 20) were included. Post-traumatic and familial cataracts were excluded. Sera of all children were tested both qualitatively and quantitatively for IgG/IgM-antibodies against ToRCH agents in a sequential manner. **Results:** Overall, IgM/IgG-seropositivity against ≥1 ToRCH agent was reported in 91.3% (42/46) children. IgM (±IgG) positivity against ≥1 ToRCH agent was reported in 26.08% (12/46) children (nine congenital and three developmental cataract; $P$ = 0.18), which included 8.7% (4/46) children reported positive against ≥2 agents. Finally, 13% (6/46) children were reported to be sero-clinical-positive (three were infants and three were >1 year age, $P$ = 0.55; five congenital and one developmental cataract, $P$ = 0.21). Either alone or combined, RV attributed to the majority (50%; 6/12) of the IgM (±IgG) and sero-clinical-positive (50%; 3/6) children. None of the children were HSV-II IgM-positive. Laboratory-confirmed congenital rubella syndrome was reported in 4.3% (2/46) children. One sero-clinical-positive infant with rare coexisting bilateral persistent fetal vasculature was also reported. IgG-alone positivity was reported highest with CMV in 67.4% (31/46) children, whereas 43.4% (20/46) children were found nonimmune to RV. **Conclusion:** The current study emphasizes the need to interpret ToRCH-screening in pediatric cataract with caution. Interpretation should include both serial qualitative and quantitative assays in tandem with clinical correlation to minimize the diagnostic errors. Clinicians should remain vigilant regarding sero-clinical-positivity in older children too who might pose a threat to the spread of infection.

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In India, nearly 2–3 lakh children suffer from severe visual impairment or blindness, and about 15% of childhood blindness is attributed to cataract.[1] Maternal infection in India, notably ToRCH (*Toxoplasma gondii* [TOX], rubella [RV], cytomegalovirus [CMV], and herpes simplex virus [HSV]) are recognized as potential causative agents of congenital cataract. These infections may be acquired *in-utero* or during delivery and may present clinically during the neonatal period or the adolescent years. In addition, exposure to ToRCH in any form predisposes a nonimmune child to acquire infections and poses a further threat for females of child-bearing age as it can increase the risk of fetal infections.[2]

Maternal IgM-antibody cannot cross the placenta. Hence, IgM in the fetus is specific for fetal infection, which usually persists for up to 3–4 months of age. In contrast, maternal IgG can cross the placenta and provide immunity to the immunologically immature newborn till 6 months of age or more. Thereafter, these antibodies wane over a period of 6–12 months. Therefore, a rise in titer at 2–4 months of age or persistent titer at 6–8 months of age suggests congenital infection. Although these natural infections generally confer lifelong immunity, serologically confirmed reinfections or recurrences have been reported, especially with CMV and HSV, and IgM has been observed to persist even years after primary infection as seen with TOX or RV.[3]

Despite their well-known limitations, serological tests remain the most popular frontline screening tool in India compared to other molecular tests like polymerase chain reaction (PCR) or virus isolation (VI). This is because of their rapid process, cost-effectiveness, and easy accessibility. However, the use of a single serum sample for ToRCH-screen can give a false impression. The lack of specificity from cross-reactions with other pathogens, the effect of confounding factors, such as maternal antibodies and previous vaccination, auto-antibodies, chronic persistence of IgM, and delay in IgM synthesis in the early acute phase often complicate the interpretation of test results. On the other hand, congenital infections are often mild and resemble other infections or can

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be mostly asymptomatic. Thus, optimal serological diagnosis warrants serial quantitative assays combined with a history of exposure and clinical correlation to ensure repeatability and persistent rise of titer.[3-6]

To the best of our knowledge, there is a nationwide and worldwide scarcity of data comprising complete ToRCH-serology on pediatric cataract. A few reported studies involved subjects <1 year of age, considered one or few selective pathogens, made a diagnosis based only on a single qualitative serum test or lacked a clearly defined significance of IgG/IgM.[1,7-21] This study was thus conducted to determine, analyze and report the ToRCH-serology screening profile among pediatric cataract cases.

## Methods

This prospective analytical study was undertaken from July 2017 to June 2018 at our sub-Himalayan tertiary care referral center of North India that caters to a large population of Uttarkhand and neighboring states as well. This study adhered to the tenets of the Declaration of Helsinki. Necessary approval from the institutional ethics committee was obtained at the beginning of this study.

Inclusion and exclusion criteria: A total of 1,026 children aged zero to 15 years were screened at our ophthalmology outdoor clinic. Of them, 46 consecutive children with clinically diagnosed congenital and developmental cataract with or without associated ocular and systemic manifestations were included in this study. Due consent was obtained from the parents. These patients subsequently had phaco-aspiration with or without itraocular lens implantation by an experienced surgeon. Children with post-traumatic cataract, familial cataract with clear genetic background, and secondary cataract (uveitis, glaucoma, drugs, or radiation) were excluded from our study.

### Clinical examination

A detailed history of the patients including cataract, associated ocular or systemic features, marital history, familial history, history of any gestational illness or drug ingestion, personal history, and vaccination status were obtained from the parents. A thorough and comprehensive ocular examination of both parents and the affected children included the assessment of visual acuity, fixation pattern, anterior segment evaluation comprising Hirschberg/cover test, nystagmus evaluation, corneal diameter measurement, tonometry, morphological identification of cataract by slit-lamp/distant-direct ophthalmoscopy, and fundus evaluation. In uncooperative children, the evaluation was done at the time of surgery under anesthesia. B-scan ultrasonography (USG-B) was performed in cases with dense media opacity. Children with abnormal systemic features were screened by an experienced pediatrician, and special investigations were carried out wherever required.

### Serology

ToRCH-serology was performed in all the study subjects at their first presentation by an experienced immunologist who strictly followed the manufacturer's instructions. Interpretations were based on controls provided with the kit. Sera for immediate use were stored at 4°C and those for delayed use were stored at −20°C (additional sample). Sera were subjected to both qualitative and quantitative assays against type-specific IgG/IgM-antibodies. Repeat tests were carried out in parallel for all positive samples at 2 weeks.

Method: For the qualitative assay, the serum samples were diluted with universal buffer at 1:51 for specific anti-TOX, anti-RV, anti-CMV, and anti-HSV-I/II IgG and IgM antibodies using commercial indirect ELISA kit (EUROIMMUN, Lubeck, Germany). For the quantitative assay, chemiluminescent immunoassay (CLIA) by VITROS 3600 immunodiagnostic system (Orthoclinical Diagnostics, Raritan, NJ) was performed.

The interpretation of test results is depicted in Table 1. The test sample was designated as "positive" (reactive) when its absorbance value was found higher than the absorbance cutoff value of the control kit and "significant" when the titer exceeded at least four times the normal value.

All positive cases were discussed with the pediatrician and immunologist for the need of any further preventive and curative treatment. Repeatedly equivocal or borderline positive samples were labeled as negative (nonreactive).

### Case definition

We defined "congenital cataract" as lens opacity present at birth, recognized anytime within the first year of life, and "developmental cataract" as lens opacity detected any time after the first year of life.[2,15] "Laboratory-confirmed congenital rubella syndrome (CRS)" was defined as an infant who has at least one symptom that is clinically consistent with CRS along with positive RV-specific IgM or persistently raised RV-IgG.[22] "Sero-clinical-positive" was defined as those children in whom serology was found to correlate with clinical diagnosis.

### Statistical analysis

Chi-square or Fisher's exact test was applied to categorical variables (number, percentage), while the t-test was applied to continuous variables (mean and standard deviation). $P < 0.05$ was considered to be statistically significant.

**Table 1: Interpretation of ToRCH-serology results**

| Status | IgM | IgG | IgM and IgG at 2 weeks | Interpretation |
|---|---|---|---|---|
| 1 | Positive | Negative | Both positive* | Possible recent primary infection* |
| 2 | Positive | Positive | Both positive * | Possible recent infection*/? reinfection or reactivation‡ |
| 3 | Negative | Positive | IgM negative IgG-positive | Immune (past exposure/vaccination/maternal IgG)/? reinfection or reactivation‡ |
| 4 | Positive | Negative | Both negative | Doubtful; likely false positive or nonspecific/susceptible |
| 5 | Negative | Negative | Both negative | No exposure to infection/susceptible |

ToRCH: (*Toxoplasma gondii* [TOX], rubella [RV], cytomegalovirus [CMV], and herpes simplex virus [HSV-I/II]), *When persistently raised or ≥ 4 fold raised IgG-titre. ‡When ≥ 8 fold raised IgG-titre

## Results

### Demographic and Clinical profile

A total of 26 out of 46 children had a congenital cataract and the remaining 20 had development cataract ($P = 0.29$). The male: female ratio was 1.7:1, and the mean age was 57.69 ± 40.98 months (range 3–180 months). Most of the study subjects were infants ($n = 8$) and children aged 4–5 years ($n = 9$). The mean delay in the presentation was 31.91 ± 34.82 months (range 1–166 months). Bilateral cataract was reported in 95.65% (44/46) children; lamellar cataract was the most common morphological form, and leukocoria was the most common presenting feature. Nystagmus, squint, microcornea, and microphthalmos were commonly associated with ocular features. The common systemic associations were cardiac and neurological features. The RV-vaccination status in 50% (23/46) children was unknown [Table 2].

### Serology

Overall, 91.3% (42/46) cases were found to be seropositive (IgM, IgG, or both) against any of the ToRCH-agent, and 8.7% (4/46) cases were seronegative. IgM (±IgG)-positivity against ≥1 ToRCH agent was reported in 26.08% (12/46) children, which included 8.7% (4/46) children who were seropositive against ≥ 2 ToRCH agent (mostly RV and HSV-I) [Table 3]. Among these IgM (±IgG)-positive cases, nine children were from the congenital group and three were from the developmental group (9/26 vs 3/20, $P = 0.18$), but the difference was statistically insignificant.

IgG-alone seropositive cases were mostly attributed to CMV (67.4%; 31/46), followed by RV (43.47%; 20/46), HSV-I and II (28.26%; 13/46 and 10.86%; 5/46, respectively), and TOX (8.7%; 4/46) [Table 4].

Finally, 13% (6/46) children were reported to be sero-clinical-positive. Among these, three were infants and three were aged >1 year (3/8 vs 3/38, $P = 0.055$), and five children were from the congenital group and one was from the developmental group (5/26 vs 1/20, $P = 0.21$), and the differences were statistically insignificant [Tables 5 and 6].

Either alone or combined, RV attributed to the majority (50%; 6/12) of the IgM (±IgG)-positive as well as sero-clinical-positive children (50%; 3/6), followed by HSV-I (33%; 4/12 and 33%; 2/6, respectively), TOX (25%; 3/12 and 33%; 2/6, respectively), and CMV (25%; 3/12 and 16.6%; 1/6, respectively). None of the children were HSV-II IgM-positive [Tables 5 and 6]. Laboratory-confirmed CRS cases were reported in two infants (4.34%; 2/46). One infant with a rare presentation of bilateral coexisting persistent fetal vasculature (PFV) and congenital cataract was reported to be sero-clinical-positive to CMV and HSV-I.

## Discussion

### General serology

In this study, six out of total 12 IgM (±IgG) seropositive children were reported to be sero-clinical-positive. This signifies that 50% of IgM (±IgG) positive cases might not be infected with any of the ToRCH-agents, or possibly had a latent infection without viremia, and were, thus, further subjected to confirmation by PCR and/or VI at the referral center.

Various studies have attributed the etiology of congenital cataracts to be infectious in 20–33% cases.[1]

Total IgM (±IgG) positivity rate (26%) in this study may seem slightly more than the previous related studies from India (Mahalakshmi B-20.2%, Singh MP-15.8%, Shyamala-6.0%) and neighboring countries (Bin Lu-18.84%, Saleem T-23.5%, Sharma-0.0%, Biswas SK-43.1%).[1,7,8,18-21] We presume this to be due to the marginal higher sensitivity of the EUROIMMUN kit that we used, as compared to the other validated ELISA kit.[23,24] We believe this issue was neutralized at least to some extent, by performing quantitative CLIA simultaneously.

In this study, sero-clinical-positivity rate was 13%. However, this cannot be compared with the currently available relevant studies in India or neighboring countries, primarily because of differences in methodology, inclusion criteria of age, and adoption of the entire ToRCH-profile.[1,7-21]

Among the sero-clinical-positive cases, congenital group outnumbered developmental group (5:1), but on overall extrapolation, no correlation was found ($P = 0.2$). Till date, there are no relevant Indian studies to validate this fact.

Four IgG-only positive infants showing no further rise of titer had maternal antibodies, as interpreted by pediatrician and immunologist. But, due to the lack of concurrent maternal serology, it was difficult for us to ascertain whether three sero-clinical-positive children aged >1 year contracted infection *in-utero* or had community-acquired self-infection. However, among them, two TOX-positive children had a history of positive pet exposure.

**Table 2: Demographic and clinical profile of study population ($n = 46$)**

| Variables | Congenital cataract number (%) | Developmental cataract number (%) | *P |
|---|---|---|---|
| Total patients (46) | 26 (56.5%) | 20 (43.5%) | 0.29 |
| Male: female (1.7: 1) | 1.9 : 1 | 1.5 : 1 | 0.95 |
| Bilateral cases (total=44) | 25 (56.8%) | 19 (43.2%) | 0.84 |
| Unilateral cases (total=2) | 1 | 1 | |
| Age at presentation<br>Mean 57.69±40.98 months<br>Range: 0 to 15 years | <br>43.23±42.23 months<br>3½months-15 years | <br>76.5±31.19 months<br>2½ years-14 years | <br>0.005 |
| Delay in presentation<br>Mean: 31.91±34.82 months<br>Range: 1-166 months | <br>36.19±37.40 months | <br>26.35±31.20 months | 0.34 |
| No h/o RV-vaccination | 14 children (53.8%) | 9 children (45%) | 0.77 |

RV- Rubella Virus. * Fisher's exact test

Delay in the presentation in our study possibly reflects poor socioeconomic status, inaccessibility to proper medical facilities, ignorance among parents, and gender discrimination, as supported by other studies as well.[9,25,26]

*TOX*

ELISA against TOX is a highly sensitive and specific test, but diagnosis on the basis of single-serum IgM positivity without IgG is not recommended. This is because IgM-positivity has been documented even several years after the primary infection.[27]

In this study, IgM (±IgG) antibodies against TOX were positive in 6.5% of children, and all of them were >1 year of age. Of them, 4.3% were sero-clinical-positive. In a similar study, Singh MP *et al*. from North India reported TOX to be the leading cause of pediatric cataract showing IgM-positivity of 8.3%. While Mahalakshmi B *et al*. from South India reported the same to be 1.7% in their study. Ironically, there were no IgM-positive cases reported from our neighboring countries, except Bangladesh (5.17%).[1,7,18-21]

Varying local geographical, economical, and cultural factors, food habits, inclusion age criteria and method of serological assessment might have been responsible for such varying incidences.

*RV*

In our study, 13% of children were reported IgM (±IgG) positive against RV, 6.5% were sero-clinical- positive, and 4.3% were laboratory-confirmed CRS. Within India, RV reported attributing 5–25% of pediatric cataracts. The IgM-seropositivity reported in studies from North India (Singh *et al*.-5.8%, Jain *et al*.-8.0%, Angra and Mohon -11%, Angra *et al*.-21.5%) differs from that of South India (Mahalakshmi *et al*.-8.4%, Shyamala *et al*.-14%, Chitra *et al*.-17.4%, Ballal *et al*.-28%, Ekstein *et al*.- 26.3%, Malathi *et al*.-52.7%) and West India (Johar *et al*.-11.1%, Mohon *et al*.-23%).[1,7-17] However, corresponding figures reported from neighboring countries such as Nepal (0.0%), Pakistan (0.0%), and China (1.4%) were quite surprising.[18-21]

Laboratory-confirmed CRS in pediatric cataract reported from the north (6.5–8.5%)[12,13] and West India (4.5–5%)[16,17] differs from that reported from South India (15%).[14] Marked geographical variation, variation in patient selection, type of

**Table 3: Distribution of IgM (±IgG) seropositive children (*n* = 12), either alone or in combination**

| IgM (± IgG) against single or combination of organism(s) | Seropositive children (*n*; %) |
|---|---|
| TOX alone | 3 (25) |
| RV alone | 3 (25) |
| CMV alone | 2 (16.66) |
| HSV-I alone | 0 (0.0) |
| HSV-II alone | 0 (0.0) |
| CMV + HSV-I | 1 (8.33) |
| RV + HSV-I | 3 (25) |
| Total | 12 (100%) |

**Table 4: Distribution of IgM/IgG-seropositivity against type-specific ToRCH-antibodies in study population (*n* = 46), either alone or in combination**

| ToRCH-agents | Seropositive children | | | | | Seronegative children | |
|---|---|---|---|---|---|---|---|
| | IgM-alone | IgM + IgG | IgG-alone | Total (*n*) | (%) | Total (*n*) | (%) |
| TOX | 1 | 2 | 4 | 7 | 15.21 | 39 | 84.78 |
| RV | 3 | 3 | 20 | 26 | 56.52 | 20 | 43.47 |
| CMV | 0 | 3 | 31 | 34 | 73.91 | 12 | 26.08 |
| HSV-I | 2 | 2 | 13 | 17 | 36.95 | 29 | 63.04 |
| HSV-II | 0 | 0 | 5 | 5 | 10.86 | 41 | 89.13 |

**Table 5: Distribution of ToRCH IgM/IgG-antibodies in study population according to different age groups**

| Age group (year); Total children (*n*) | IgM (±IgG) | | | | | IgG-only | | | | | Total IgM/IgG seropositive children (*n*) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | TOX | RV | CMV | HSV-I | HSV-II | TOX | RV | CMV | HSV-I | HSV-II | |
| 0-1; (8) | - | 2 C | 1 C | 1 C | - | - | 4 | 3 | 4 | 1 | 7 |
| >1-2; (4) | - | 1 C | - | 1 C | - | 1 | - | 4 | - | - | 4 |
| >2-3; (3) | - | - | 1 C | - | - | - | - | 1 | - | - | 2 |
| >3-4; (6) | - | 1 C | - | 1 C | - | 1 | 2 | 4 | 1 | 1 | 5 |
| >4-5; (9) | 1 C | - | 1 C | - | - | - | 6 | 5 | 2 | 1 | 8 |
| >5-6; (5) | 1 D | - | - | - | - | - | 3 | 5 | 3 | 2 | 5 |
| >6-7; (3) | - | - | - | - | - | - | 3 | 2 | 2 | - | 3 |
| >7-8; (1) | - | 1 D | - | 1 D | - | - | - | 1 | - | - | 1 |
| >8-9; (4) | - | 1 C | - | - | - | 2 | - | 3 | 1 | - | 4 |
| >9-10; (1) | - | - | - | - | - | - | 1 | 1 | - | - | 1 |
| >10-15;(2) | 1 C | - | - | - | - | - | 1 | 2 | - | - | 2 |
| Total (46) | 3 | 6 | 3 | 4 | Nil | 4 | 20 | 31 | 13 | 5 | 42 |

C - Congenital cataract. D - Developmental cataract

**Table 6: Sero-clinical features of IgM (±IgG)-positive children (*n* = 12)**

| | Positive test result | Abnormal test result at 2 weeks | Associated clinical features. | Action taken. |
|---|---|---|---|---|
| **Early detected group, ≤1-year age; congenital cataract (*n* = 3)** | | | | |
| Case-1 | CMV + HSV-I; IgM + IgG | IgM +ve ↑IgG | Bilateral PFV with nystagmus, Anemia, thrombocytopenia, failure to thrive, hepatomegaly, ↑liver enzymes, deafness, febrile rashes, pneumonitis. | Gancyclovir I.V Supportive Reassurance Close follow-up Adv: PCR. |
| Case-2 | RV; IgM + IgG | IgM +ve ↑IgG | CRS: microcornea/microphthalmia, dacryostenosis, PDA, hepatomegaly, low birth weight, H/O-maternal fever, and rash. | Supportive Reassurance Close follow-up. |
| Case-3 | RV; IgM + IgG | IgM +ve ↑IgG | CRS: microcornea/microphthalmos, NLD-stenosis, PDA, hepatomegaly, low birth weight, H/O-maternal fever, and rash. | Supportive Reassurance Close follow-up |
| **Late detected group, >1-year age; congenital cataract (*n* = 6)** | | | | |
| Case-1 | RV + HSV-I; IgM | IgM –ve IgG-No rise | Squint, nystagmus. | Adv: Retesting/PCR No treatment Close follow-up. |
| Case-2 | TOX; IgM + IgG | IgM +ve ↑IgG | Squint, choro-retinitis, nutritional anemia, neuro-motor delay, ↑liver enzymes, hepato-spleenomegaly, H/O-exposure +ve. | Adv: Sabin-Feldman dye test and/or PCR sulfadiazine + pyrimethamine + folinic acid. |
| Case-3 | RV + HSV-I; IgM | IgM +ve ↑IgG | Squint, nystagmus, perioral rash, fever, Jaundice, hepatomegaly, head nodding, anemia, hypotonia, neuromotor in-coordination, dev/language delay, deafness. | Gancyclovir I.V Supportive Reassurance Close follow-up Adv: PCR. |
| Case-4 | CMV; IgM + IgG | IgM –ve IgG-No rise | Squint, nystagmus. | Adv: Retesting/PCR No treatment Close follow-up. |
| Case-5 | RV; IgM | IgM –ve IgG-No rise | nil. | Adv: Retesting/PCR No treatment Close follow-up. |
| Case-6 | TOX; IgM | IgM –ve IgG-no rise | Squint, jaundice, motor/dev delay, no cerebral lesion. | Retesting/PCR No treatment Close follow-up. |
| **Late detected group, >1-year age; developmental cataract (*n* = 3)** | | | | |
| Case-1 | CMV; IgM + IgG | IgM –ve IgG-No rise | Squint, nystagmus. | Adv: Retesting No treatment Close follow-up. |
| Case-2 | TOX; IgM + IgG | IgM +ve ↑IgG | Fever with recurrent seizure, cerebral calcification, jaundice, neuro-motor delay, chorio-retinal scar, H/O- exposure and maternal jaundice +ve. | Adv: Sabin-Feldman dye test and/or PCR sulfadiazine + pyrimethamine + folinic acid. |
| Case-3 | RV + HSV-I; IgM + IgG | IgM–ve IgG-No rise | Nil. | Adv: Retesting/PCR No treatment Close follow-up. |

PFV: Persistent fetal vasculature, CRS: Congenital rubella syndrome, PDA: Patent ductus arteriosus

kit used, laboratory techniques and inferences drawn thereof have been cited as prime reasons for such different results.

RV attributed to the majority of both IgM (±IgG)-positive and sero-clinical positive children in our series. The reason could be either poor immunization status or sub-clinical infection caused by a less virulent type of strain. Increasing incidences of RV-infection at par with the progression of age has been recently cited by a few Indian authors.[9,25]

*CMV*

CMV is considered the commonest cause of intrauterine and congenital infections worldwide.[28,29] In our study, CMV attributed to the majority (73.9%) of the type-specific IgG seropositive children; 6.5% of children were IgM (±IgG) positive, and only 2.17% was sero-clinical-positive. To date, there is no relevant study reported from North India, but two separate studies from South India reported CMV-IgM and IgG-positivity rate to be 7.8% vs 66% and 0% vs 54%, respectively.[7,8] In similar studies, CMV IgM (±IgG) rate reported from neighboring countries were 15.5%, 8.8%, 0.0%, and 32.7%, respectively.[18-21]

*HSV*

In our series, 47.8% of children were HSV (mostly HSV-I) IgG-positive, 8.6% cases were HSV-I IgM (±IgG) positive and

4.3% were sero-clinical-positive. IgM and IgG-positivity reported by similar studies from North India (Singh *et al*.- 1.7% and 20.8%, respectively)[1] and South India (Mahalakshmi *et al*.-5.1% and 10%; Shyamala *et al*.-0.0% and 3.0%, respectively),[7,8] vary considerably. The corresponding value reported from three neighboring countries were 17.4% and 2.8%, 5.1% and 8.6%, and 94% and 4.4%, respectively.[18,19,21] Varying nature of sexual preferences, marital status, interpersonal or environmental contacts, and bad obstetric history may possibly have a role behind such different outcomes.[30]

HSV-serotype evaluation is recommended in view of different prognostic and counseling implications. However, serological differentiation of HSV-IgM was found conspicuously absent in previous studies because of the issues related to the kit used. In our study, this problem was resolved by adopting the EUROIMMUN ELISA kit, which can detect serotype-specific IgM, glycoprotein C1 for HSV-I and glycoprotein G2 for HSV-II.

HSV-I was reported to be the predominant serotype in our study, contrary to the common notion that congenital HSV is mostly HSV-II serotype which is transmitted through the birth canal. However, Raghu *et al.* reported four cases of congenital cataract positive to HSV-I IgM, and recent reports suggested HSV-I seroprevalence in genital herpes is on the rise.[1,5,30-32]

### Children with >1 ToRCH-agent

In this study, among the four children (8.7%) who were IgM (± IgG) seropositive against >1 agent, two (4.3%) were sero-clinically positive. A North Indian study reported coinfection in 29.1% of children with congenital cataract on the basis of both serology and PCR.[1] However, similar reports from two other South Indian studies were only 2.5% and 0.0%, respectively, in which the authors used the same kit having identical sensitivity and specificity.[7,8] From neighboring countries, the corresponding figure reported was 7–10%.[19,21] This prompted us to speculate that, apart from the kit, factors such as the method of testing, technical error, interpretation of results, the serological definition of cases, the virulence of strains, and immunization status could also be responsible for such differences.

### Infection related to persistent fetal vasculature cases

In this study, the presentation of one child with coexisting bilateral PFV and cataract, who was reported sero-clinical-positive to CMV and HSV-I, was undoubtedly a very rare finding. However, RV, HSV-I, and II IgM-positivity have been reported earlier in bilateral PFV children in some sporadic studies, as well.[33,34] Such an occurrence can possibly be explained by the developmental arrest of the lenticulo-vascular system secondary to infection *in-utero*.

The main strength of this study was performing the serological tests prospectively in a sequential manner in tandem with the clinical manifestations so as to arrive at the final sero-clinical diagnosis. Besides, the inclusion of children with a high upper age limit and developmental cataract might have added further strength to our study. Our study was possibly limited by small sample size and inability to serologically correlate mothers.

## Conclusion

In summary, as ToRCH serology is an indirect method of testing, its interpretation in children with pediatric cataracts should be made with caution. Our results showed that diagnostic error can be minimized several folds by serial qualitative and quantitative tests besides clinical correlation. The possible existence of sero-clinical-positive children above 1 year of age should be taken seriously because of their potentiality to spread infection. Larger cohort studies incorporating maternal serology would be warranted for a better understanding of sero-clinical correlation. RV still constitutes a major infective burden and nearly 50% of the children are nonimmune to RV, despite the fact that there is an effective vaccination available. Acquisition of collateral knowledge on delay in presentation, RV-immunization status, CRS-load and PFV possibly may aid clinicians and epidemiologists to identify infectious cases and formulate preventive strategies.

### Patient consent

Obtained. No identifiable patient information was used.

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### Conflicts of interest

There are no conflicts of interest.

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