Incidence of neonatal conjunctivitis at Mbarara Regional Referral Hospital, South Western Uganda

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ABSTRACT

Objective: To determine cumulative incidence of neonatal conjunctivitis (NC) in babies delivered at Mbarara Regional Referral Hospital (MRRH), establish the responsible microorganisms and their sensitivity to available antibiotics and determine factors associated with neonatal conjunctivitis among these babies.
***Methods*:** A prospective cohort study where babies delivered at MRRH whose mothers stayed in Mbarara municipality were recruited within 24 hours after birth and weekly follow up was done until the occurrence of NC, or lost to follow up or administrative censoring at 28 days. Neonates who developed conjunctivitis had a conjunctival swab taken for microbiology.
Results: Out of 438 babies recruited, 45 (10.3%) were lost to follow up while 49 (11.2%, 95% CI 8.4 – 14.5) developed neonatal conjunctivitis by 28 days. Isolated pathogens were *Staphylococcus aureus* 23 (67.65%), *Klebsiella pneumoniae* 7 (20.59%), *Neisseria gonorrhoeae* 3 (8.82%) and *Streptococcus pyogenes* 1(2.94%). In 15 (30.6%) of 49 cultures done there was no growth. Isolates tested had only 18% sensitivity to tetracycline. However there was 100% sensitivity to gentamycin and oxacillin, while slightly lower sensitivity was achieved for ceftriaxone (76%), ciprofloxacin (68%) and chloramphenical (61%). Not receiving prophylaxis within 24 hours after birth [adjusted OR 4.85 CI (1.17 – 20.19)] and neonatal admission to Neonatal Intensive Care Unit (NICU) after delivery [adjusted OR 6.03 CI (1.09 – 33.32)] were independently associated with higher odds of developing NC.
Conclusions: The incidence of NC was unexpectedly high. Admission to NICU and lack of prophylaxis within 24 hours were risk factors for developing NC.

Key words: Neonatal, Conjunctivitis, Cohort, Incidence

INTRODUCTION

Neonatal conjunctivitis (NC) is one of the causes of avoidable childhood visual impairment and blindness, a priority in the Vision 2020 program. Global incidence and prevalence of NC has markedly reduced since the introduction of Crede method of prophylaxis in 1881 and control of sexually transmitted infections (STI). In developed countries the incidence of NC has reduced to about 0.04%[1] however, in developing countries incidence of NC is as high as 16%. Several organisms have been isolated from newborns with NC, all which show different antibiotic susceptibility patterns according to the geographical regions and whether community or hospital acquired conjunctivitis[2,3]. Some of the factors associated with NC include maternal factors like poor antenatal care hence poor screening and treatment for STI including maternal HIV/AIDS[4] and prolonged labor[5,6]. Neonatal factors like prematurity[8] and low birth weight, ocular trauma during delivery, congenital dacryostenosis, and congenital nasal lacrimal duct obstruction are frequently associated with NC as well as inadequate use of ocular prophylaxis immediately at birth[4,8,9] and hospital/NICU admission[3,10].

At Ruharo Eye Centre (REC) and Mbarara Regional Referral Hospital (MRRH) eye clinic in South Western Uganda, NC accounts for about 80% of neonatal consultations and 90% of neonates admitted for ocular conditions and often with late presentation to hospital (Hospital Records, 2011). The resulting childhood blindness is associated with high early childhood morbidity and mortality[8,11]. Again associated with loss of educational opportunities for the children and socioeconomic strain on family while taking care of the sick and visually impaired children in the already resource limited setting. Therefore our objectives were to determine cumulative incidence of neonatal conjunctivitis in babies delivered at MRRH, establish

the responsible microorganisms and their sensitivity to available antibiotics and determine factors associated with neonatal conjunctivitis among these babies so as to implement suitable interventions.

MATERIALS AND METHODS

Study design: This prospective cohort study was performed at the maternity ward of MRRH, a Tertiary and Teaching Hospital for MUST. The maternity service is equipped to carry out deliveries and has a neonatal intensive care unit (NICU) staffed by a paediatrician. The protocol for this project was approved by the university Institutional Ethics Review Committee (MUST-IRC No: 03/03/13) and the Uganda National Council of Science and Technology (UNCST; HS-1458).

We enrolled healthy babies delivered at MRRH during the study period, whose mothers resided in Mbarara Municipality and consented to the study while all neonates that had any ocular malformation at birth were excluded. Recruitment was done every day between 7:00am and 10:00am on the maternity ward (postnatal and post operation ward). The birth registry was used to identify all mothers who had delivered within the last 24 hours. The structured questionnaire was then administered to the mother and the newborn's eyes examined. Mothers' contact addresses (physical and telephone numbers) were noted and first follow up visit/ examination of all neonates carried out between Day 5 and Day 8 irrespective of notice/call from the mother. Subsequently, follow-up phone calls were made on day 14, day 21 and day 28 to all mothers whose babies had not developed any of the three signs (eye redness, eye discharge or lid swelling). For any baby with one or more of the signs found during the follow up visit or phone call or upon alert from the mother, ocular examination was done and conjunctival swab was taken off. Empirical treatment with 1% Tetracycline eye ointment (TEO) and erythromycin syrup was initiated for all symptomatic babies[12] and for the cases where *N. gonorrhoeae* was suspected and/or isolated further treatment was initiated.

Microbiological procedures: After taking conjunctival swabs, spot inoculation was made on chocolate agar plates that were transferred in an anaerobic jar /carbon dioxide chamber to the university microbiology laboratory for continued culturing in 5% carbon dioxide at 35^{o}C and examined daily. They were discarded after 7 days if no growth noted.

From the conjunctival swab specimen, instant gram staining and Giemsa staining were done. Sensitivity testing was performed using the conventional agar disk diffusion method in accordance with the Clinical and Laboratory Standards Institute[13]. The antibiotics tested included; Gentamycin (10µg), tetracycline (30µg), chloramphenical (30µg), ciprofloxacin (1µg), ceftriaxone (30µg), erythromycin (15µg), penicillin G (20µg), ampicillin (10µg), amoxicillin (20µg), and oxacillin (1µg). Bacitracin was used for *Streptococcus pyogenes* only.

Statistical analysis: The questionnaire derived data was entered into Microsoft Excel software package (Excel Version 2007) and missing entries were corrected daily. Using the same Excel spread sheet, we generated the follow up dates and follow-up due date reminders. Data from the Microsoft Excel spread sheet were exported to STATA software package (Version 11.0) for further analysis. Descriptive and analytical statistics was performed on the variables of interest. Data were tabulated and presented with odds ratio, p-values and confidence intervals.

Tabulation was used and the proportion of children who developed NC during the follow up period reported. We summarized the microbiology information to present the most common causes of NC that were isolated and sensitivity patterns of the most common organism determined. Tabulation of frequencies and the proportions for the different organisms isolated and their susceptibility to antibiotics have been reported. The factors associated with NC were assessed with logistic regression. We performed univariate analyses first to assess the unadjusted association of various predictors. Predictors that had p-values <0.2 and those with known association from literature reviews, were identified and for inclusion into the final multivariable adjusted model whether their p-value were significant or not. The p-value of 0.2 was chosen because it was more inclusive and took care of possible negative confounders. The regression diagnostics (Link test and logistic goodness of fit test) were performed. From the regression diagnostics, there was no evidence of lack of fit (p=0.177). The associated factors are reported in the table with the respective odds ratio (OR), 95% confidence interval (CI) and the P-values.

RESULTS

Descriptive data (baseline characteristics, Table 1)

Neonate: Of 438 babies enrolled, 230 (52.5%) were males. The median birth weight was 3.2 kg (minimum 1.4 – maximum 4.8) and only 59 (13.5%) received tetracycline prophylaxis within 24 hours of birth. Thirty five (8.0%) of neonates were less than 37 weeks of gestation. At the time of recruitment, 4 (0.9%) of babies had lid swelling, 2 (0.5%) had eyelid discharge and 1 (0.2%) had conjunctival redness. Nine babies (2.0%) were admitted to NICU before mothers were discharged from post-natal ward.

Mother: The mother's median age was 24 years (minimum 14 – maximum 41) years and 164 (37.4%) were para-1 mothers. Also, 99 (22.6%) had at least one episode of urogenital infection (Table 1). Seventy two (16.4%) mothers had premature rupture of membranes and 66 (15.1%) had prolonged labor. Overall, 338 (77.2%) mothers had vaginal delivery.

Table 1: Antenatal predictors (n=438)

Variable assessed	Frequency (n)	(%)
Marital status		
Single	12	2.7
Married	420	95.9
Windowed	2	0.5
Separated or divorced	4	0.9
Mother's education		
No formal	22	5.0
Primary	162	37.0
Secondary	159	36.3
Tertiary	95	12.7
Distance from hospital		
Less than 2 km	65	14.8
2 km to 4 km	299	68.3
More than 4 km	74	16.9
Gestation age(weeks of amenorrhea WOA)		
Less than 37	35	8.0
37 to 42	368	84.0
More than 42	35	8.0
Ante natal care attendance		
Never attended	3	0.7
Once	27	6.2
2 to 4 visits	281	64.2
More than 4	127	29.0
HIV status of mother		
Unknown	6	1.4
Negative	363	82.9
Positive not on HAART	15	3.4
Positive on HAART	54	12.3

HAART =Highly Active Anti-Retro-Viral Therapy

Cumulative incidence: A total of 438 babies were recruited. Forty five (10.3%) were lost to follow up while 49 [11.2%, 95% CI (8.4 – 14.5)] neonates developed clinical NC by day 28.

Microorganism and the sensitivity patterns: Of the 49 babies with clinical NC whose culture results were available, 34 (69.4%) had positive cultures, 14 (28.6%) had no growth and one (2.0%) had contamination. Of those with positive cultures, 23 (67.7%) were *Staphylococcus aureus*, 7 (20.6%) *Klebsiella pneumonia*, 3 (8.8%) *Neisseria gonorrhoea* and 1 (2.9%) *Streptococcus pyogenes*.

Sensitivity patterns of microorganisms to the antibiotics: Sensitivity test was performed in all the 34 isolates. There was 100% sensitivity to gentamycin and oxacillin. Slightly lower sensitivity was recorded for ceftriaxone (76%), ciprofloxacin (68%) and chloramphenical (61%). All the bacteria were resistant to penicillin G. *Staphylococcus aureus* the most isolated organism was less susceptible to the commonly used topical antibiotics chloramphenical (52%) and erythromycin (45%) yet resistant to tetracycline (91%). *Neisseria gonorrhoeae* (3 isolates) and *Streptococcus pyogenes* were all 100% sensitive to ciprofloxacin, chloramphenical, and gentamycin. Bacitracin was used for *Streptococcus pyogenes* only and was 100% sensitive. *Klebsiella pneumoniae* was sensitive to chloramphenical (100%), tetracycline (71%) but resistant to erythromycin (67%).

Associated factors: Univariate associations with developing of NC were determined (Table 2) and all predictors that had p-values less than 0.2 were included in the multivariate model (Table 3).

Table 2: Univariate associations with NC

Variables	Categories of variables	Crude OR (odds ratio)	95% CI (confidence intervals)	P-value
Mother's age		1.03	0.97 – 1.10	0.343
Marital status	Single	1.00	0.18 –11.18	0.740
	Married	1.42		
	Separated/divorced	Omitted		
	Widowed	Omitted		
Mother's formal education status	Never	1.00		
	Primary	0.94	0.26 – 3.46	0.930
	Secondary	0.61	0.16 – 2.32	0.470
	Tertiary	0.83	0.21 – 3.26	0.789
Distance from hospital	Less than 2km	1.00		
	2km to 4km	1.75	0.66 – 4.63	0.261
	More than 4km	1.06	0.31 – 3.65	0.928
Parity	Para l	1.00		
	Multi para	1.27	0.67 – 2.38	0.463
Gestation age	37 to 42 WOA	1.00		
(weeks of amenorrhea)	Less than 37	1.33	0.49 – 3.62	0.577
	More than 42	0.75	0.22 – 2.55	0.642
Antenatal care attendance	Never	1.00		
	Once	0.16	0.01 – 2.63	0.199
	2 to 4 times	0.20	0.12 – 2.33	0.200
	More than 4 times	0.37	0.03 – 4.32	0.431
Urogenital infection	Yes	1.34	0.63 – 2.86	0.453
Mother' s HIV status	Negative	1.00		
	Unknown	1.62	0.18 – 14.2	0.665
	HIV positive not on HAART	2.02	0.55 – 7.46	0.292
	HIV positive on HAART	0.82	0.31 – 2.19	0.698
Mode of delivery	Vaginal delivery	0.72	0.37 – 1.38	0.312
Prolonged labor	Yes	1.31	0.60 – 2.85	0.494
PROM	Yes	1.56	0.75 – 3.21	0.231
Prophylaxis to baby's eyes	No	2.58	0.78 – 8.58	0.122
Sex of baby	Female	1.41	0.78 – 2.56	0.259
Baby's birth weight		0.59	0.34 – 1.03	0.063
Baby admission in NICU	Yes	4.16	1.01 –17.21	0.049

ANC =Ante Natal Care, HIV=Human Immune deficiency Virus, HAART =Highly Active Anti-Retro-Viral Therapy, NICU =Neonatal Intensive Care Unit

Table 3: Multivariate associations

Variables	Categories of variables	Adjusted OR (odds ratio)	95% CI (confidence intervals)	P-value
Distance from hospital	Less than 2km	1.00		
	2 to 4km	2.28	0.77 – 6.74	0.136
	More than 4km	1.46	0.38 – 5.58	0.582
Antenatal care attendance	Never	1.00		
	Once	0.04	0.01 – 0.82	0.037
	2 to 4 times	0.05	0.01 - 0.78	0.034
	More than 4 times	0.12	0.01- 1.75	0.120
Mother' s HIV status	Negative	1.00		
	Unknown	2.84	0.29– 27.82	0.369
	HIV positive NOT on HAART	1.59	0.40 – 6.29	0.506
	HIV positive on HAART	0.67	0.23 – 1.91	0.453
Mode of delivery	Vaginal delivery	0.70	0.34 – 1.43	0.331
Prophylaxis to baby's eyes	No	4.85	1.17– 20.19	0.030
Baby birth weight		0.56	0.31 – 1.01	0.053
Admission in NICU	Yes	6.03	1.09– 33.32	0.039

HIV = Human Immune deficiency Virus, HAART = Highly Active Anti-Retro-Viral Therapy, NICU = Neonatal Intensive Care Unit

DISCUSSION

Cumulative incidence: The incidence was unexpectedly high with 49 [11.2%, 95% CI (8.4 – 14.5)] of babies in this study developing NC compared to the study in neighboring Kenya which reported 2.1%[14] yet in industrialized countries this proportion is as low as 0.04%[1]. This variation could be attributed to poor attendance of ANC, non-routine application of topical prophylaxis at birth[9] and the type of prophylaxis offered perinatal. As observed in this study, tetracycline was routinely used at MRRH while povidone iodine was used in the Kenyan study. Studies have shown 2.5% povidone iodine solution to have no resistance across all spectrum of bacteria as well as viruses compared to the topical antibiotics[9,15].

Microorganisms and susceptibility pattern: In this study the most common microorganisms isolated were; *Staphylococcus aureus* (67.7%), *Klebsiella pneumonia (*20.6%) and *Neisseria gonorrhoea* (8.8%). This pattern was observed in the study done in Kenya[9] which found 39.7% of all infected babies had staphylococcus, 5% had *Neisseria gonorrhoea* and majority 50.5% had *Chlamydia trachomatis*. Also, *Neisseria gonorrhoea* must always be suspected clinically when a lot of discharge is present and treated immediately pending microbiology results since it is associated with rapid progression to ocular complications.

Also, from our study, we found 28.6% negative cultures and this could possibly have been sterile (non-infectious) or due to the effect of prophylaxis or chlamydial conjunctivitis which could not be confirmed on Giemsa staining microscopy (which has very low sensitivity) hence it needed either direct immune florescent (DIF) stain or special tissue media (McCoy cell cultures) or DNA-PCR assays all of which were too expensive to be done during this study[8,16]. However to cover for possible chlamydial conjunctivitis oral erythromycin syrup was empirically given to all babies with clinical NC.

Klebsiella pneumoniae was isolated among the neonates admitted in NICU. Chen and Starr[3] study found the Gram negative conjunctivitis the commonest among babies that were admitted in NICU. *Staphylococcus aureus* was the most isolated bacteria was resistant to tetracycline (91%) the routinely used drug for prophylaxis but sensitive to the oxacillin (100%), ceftriaxone (74%) and ciprofloxacin (74%) both of which are relatively expensive. However *Klebsiella pneumoniae* was sensitive

to tetracycline (71%) and chloramphenical (100%), this trend was also observed by Chen and Starr[3] study in New York, among neonates admitted in NICU.

Associated factors (Tables 1, 2 and 3): A number of predisposing factors that could be associated with the observed high incidence of NC at MRRH were studied. Mothers attending ANC at least once were found to be protective while not applying prophylaxis to babies' eyes within 24 hours of birth and neonatal admissions in NICU were independently associated with increased odds of developing NC. Other factors included low birth weight, unknown maternal HIV status, not being on HAART for known HIV positive mothers and staying more that 2 km from hospital had more odds of developing NC though were not statistically significant.

From this study babies that did not get prophylaxis had about 5 fold odds of developing NC compared to those that got tetracycline prophylaxis (p-value = 0.030). This finding is not surprising considering the introduction of Crede method of silver nitrate prophylaxis demonstrated a marked reduction in global incidence of NC[1,8,11,15]. The routine wiping of baby's eyes lids soon after delivery and prophylaxis reduces the chances of developing NC. Considering that 3 (6.1%) of the 49 babies that developed NC had got the prophylaxis shows that some prophylaxis are better than others demonstrated by the spectrum of microbial susceptibility to the drugs. In Kenya, the use of 2.5% povidone iodine even resulted in very low NC incidence[9,15].

Attending ANC is associated with urogenital/ STI screening and treatment as well as HIV testing and HAART initiation which contributes to primary prevention of NC[4,17] which explains the study findings that babies of mothers who attended ANC at least once had less odds (adjusted OR 0.04, p-value 0.037) of developing NC compared to those who did not attend at all. Babies admitted in NICU were 6 times more odds of developing NC compared to those not admitted [adjusted OR 6.03, p-value 0.039, 35% CI (1.09 – 33.32)]. A study done in New York among babies admitted in NICU found that these babies had low birth weight and were more prone to gram negative conjunctivitis including *Klebsiella pneumoniae*[3]. Low birth weight is frequently associated with prematurity and often need admission in NICU resulting in prolonged hospital stay. Also these babies' ocular defence mechanisms are not fully developed hence at more odds of developing hospital NC than those not admitted. Surprising results from the study found that gestation age, mode of delivery; PROM and prolonged labor that are some of the known maternal predisposing factors were not statistically significant[1,5,6] and this could be attributed to the small sample size.

This study did have limitations**.** Only MRRH was selected for the study amongst all health units offering ante natal, natal and post natal care services because it is the biggest tertiary hospital in South Western Uganda yet isolates may differ from place to place. We recommend further studies to establish whether isolates from other centres are similar to what we isolated at MRRH. Also, only Gram staining, Giemsa staining microscopy and culture and sensitivity methods were used because the other diagnostic techniques that are either faster or have higher sensitivity and specificity were very expensive. This was a diagnostic limitation specifically for *Chlamydia trachomatis* which required direct immune fluorescent (DIF) stain, special McCoy cell culture or DNA PCR for its identification, growth, and confirmation.

CONCLUSIONS

The incidence of NC was unexpectedly high. While the commonest causative organism was *Staphylococcus aureus,* clinicians must be alert of *Neisseria gonorrhoea* that is associated with rapid progression to severe ocular complications hence the need for immediate intervention to save the babies eyes. Sensitivity test demonstrated that ciprofloxacin, ceftriaxone and gentamycin could be used as empirical monotherapy for treating NC at MRRH. Attending ANC more than once was protective against NC. However, admission to NICU and not receiving prophylaxis within 24 hours were independently associated with developing NC.

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