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Early Onset Sepsis in South Indian Newborns

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Abstract

Background: Early onset neonatal sepsis is one of the leading causes of perinatal and neonatal mortality more so in low and moderate income Asian countries. **Objective:** The objective of the study was to identify various ante-partum, intra-partum and neonatal risk factors associated with Early Onset Sepsis (EOS) in South Indian newborns. **Method:** The study comprised of 2,750 consecutive singleton live births with 244 "EOS cases" and 2,506 "Controls". Sources of data were Labor room records, NICU register and neonatal charts. Analysis by binary logistic regression with statistical significance set at 0.05 using SPSS Version 21. **Results:** The incidence of early onset sepsis in South Indian newborns was 8.9%, high risk factors for EOS include young mothers ≤24 years, OR 1.53, (95% CI 1.2-2.0), P>|z|=0.002, primigravidae, OR 2.08, (95% CI 1.6-2.7) P>|z| <0.001, Premature Rupture of Membranes (PROM), OR 12.96, (95% CI 9.5-18.4), P>|z| <0.001, Gestational diabetes, OR 2.19, (95% CI 1-1.3), P>|z| <0.008, so also birth by emergency Lower Segment Cesarean Section (LSCS), OR 1.82, (95% CI 1.3-2.5), P>|z| <0.001. Neonatal risk factors include prematurity ≤36 weeks, OR 2.57, (95% CI 1.8-3.6), P>|z| <0.001, Low Birth Weight (LBW) ≤2 ,499g, OR 2.76, (95% CI 2.1-3.7), P>|z| <0.001 and male gender, OR 1.88, (95% CI 1.1-3.0), P>|z| <0.008 being highly statistically significant. **Conclusion:** The incidence of early onset neonatal sepsis was 8.9%. High index of clinical suspicion for early diagnosis should be based on history of maternal, obstetric and neonatal high risk factors predisposing to EOS.

Keywords

Early Onset Sepsis (EOS), High risk antepartum, intra-partum and neonatal factors

Aim

- To determine incidence of Early Onset Sepsis in South Indian newborns.
- To identify various risk antepartum, intrapartum and neonatal risk factors associated with Early Onset Sepsis.
- Comparison of various high risk factors between "EOS cases" and "Controls".
- To determine intensity levels of various high risk maternal, obstetric and neonatal factors to EOS by binary logistic regression, odds ratio (OR) in a case-control study and statistical significance set at P>.05.

1. Introduction

Neonatal infection is one of the leading single largest contributors to around 35% of neonatal mortality [1] and global incidence of neonatal sepsis being 2.8-3.9% up till 2018 [2]. The incidence of EOS in India is around 20.7-28.6 per 1,000 live births constituting 55.4% of overall neonatal sepsis with case fatality rate 19.4% [3, 4]

while the incidence in US is around 0.77-1 per 1,000 live births in developed countries with 10-25% of stillbirths due to infection [5, 6].

Mortality is high around 17.6% (95% CI 10.3% to 28.6%) more so in Low and Middle income Asian countries with high perinatal and neonatal mortality rates [7]. In fact, several studies suggest that infections may be responsible from 8% to 80% of all neonatal deaths in Asian countries with 42% of deaths occurring in the first week of life, as also majority of these deaths occur at home without coming to medical attention [8].

Clinical signs and symptoms of EOS are often non-specific and may be present in absence of positive blood culture, while a "perfect" biomarker high sensitivity, specificity and predictive value as such is not available in pediatric practice, clinicians need to balance between marker characteristics and their sensitivity and specificity, thus presence of various antenatal, intra-natal and neonatal risk factors predisposing to early onset neonatal sepsis should alert clinicians for possible infection of newborn.

Studies show certain high risk predisposing factors such as maternal infection, premature rupture of membranes (PROM), prematurity, low birth weights etc should warrant screening for neonatal sepsis, as 20.6% neonates with predisposing maternal and obstetric risk factors developed sepsis compared to 0.5% newborns who were born to healthy mothers being highly statistically significant ($P < 0.001$). In fact, risk factors such as PROM, foul smelling liquor, unclean vaginal examination and maternal urinary tract infection was significantly related to EOS ($p<0.05$) [3], as most early onset sepsis starts with etiological pathogens in intra-amniotic infections [9].

This study was undertaken to identify various maternal or antenatal, intranatal and neonatal risk factors associated with early onset neonatal sepsis, aid clinicians to make early diagnosis as it is better to over diagnose infection in newborns and treat unnecessarily rather than under-diagnose and not treat infection that will have more serious consequences, more so in many low and middle income (LMIC) Asian countries have limited resources and diagnostic facilities compounded by high prevailing perinatal and neonatal mortality rates.

Since an ideal early diagnostic test with 100% sensitivity in that all newborns with infection are always detected by the test and 100% specificity i.e. all newborns without infection, tests being negative are lacking, hence clinicians have to rely on a high index of clinical suspicion based on history of maternal and obstetric as well as neonatal high risk factors associated predisposing to early onset neonatal sepsis for diagnoses and empiric antibiotic therapy as in-utero infections are common [9, 10].

Therefore strategies to reduce morbidity and mortality of EOS diagnosis within 24 hours of birth includes identification of high risk maternal, obstetric and neonatal factors, warranting screening tests for sepsis and in combination with clinical signs, hematologic and serologic markers testing to confirm and monitor progress of course of infection or to withhold antimicrobial therapy in uninfected newborns.

2. Methods

2.1 Design

Newborns with early onset sepsis termed as "EOS cases" were compared to "controls" or healthy newborns. The assessment of various antepartum risk factors or events occurring before birth in reference to maternal age, gravida, premature rupture of membrane (PROM) of the amniotic sac and chorion prior to onset of labor, PIH, gestational diabetes as well as intrapartum risk factors associated with mode of delivery and neonatal risk factors such as prematurity, low birth weight and gender were analyzed.

2.2 Setting

A total of 2,750 consecutive live births from January 1st 2015 to 31st May 2017 were included in the study at Shifa hospital, a multispecialty center in the Metropolitan city of Bangalore, South India. Data were obtained from Labor room records, NICU register and neonatal records. Data analysis and statistical significance was done using SPSS Version 21. Mean and standard deviation were used for continuous data while frequency and percentage were calculated for categorical data. Risk factors were grouped into antepartum, intrapartum and neonatal variables.

2.3 Statistical analysis

Association between Early Onset Sepsis and various maternal, obstetric and neonatal risk factors were determined by multivariate analysis and multiple logistic regressions. All data presented as odds ratio (OR), mean difference (95% CI), number or percent (%) and the percentage of risk factors associated with early onset neonatal sepsis at different intensity levels were estimated by OR with Confidence Interval (CI of 95%) to determine the intensity levels between EOS and various risk factors that represents that the odds of that outcome will occur with

particular exposure compared to the odds of the outcome occurring in the absence of that exposure in a case-control study is used in the present study to measure association between exposure to a specific antenatal, intra-natal and neonatal risk factors that predisposes to increased outcome of EOS, or the number of times positive correlation occurs or failure.

Hence OR greater than 1 indicates that the odd of exposure is a high risk factor for developing EOS, in contrast OR < 1 or = 1 indicates lower odds of outcome with exposure i.e. when exposure does not affect odds of outcome. Confidence Interval (95% CI) is used to estimate precision of OR, 95% of times or that the estimated interval 95% will contain the true value and a small CI indicates higher precision of OR. P>|z| in linear regression indicates that a relationship exists between the two categorical variables "EOS cases" and "controls" and $P \leq 0.05$ indicates statistical significance, rejecting the null hypothesis that no relationship exists between predisposing factors and early onset neonatal sepsis. The threshold of statistical significance set at ≤0.05 using SPSS Version 21.

2.4 Results

The incidence of EOS was 8.9% with 244 " EOS cases " and 2506 "Controls" among 2,750 consecutive singleton live births during January 1st 2015 to 31st May 2017 in a hospital set up. Identification of certain high risk antepartum, intrapartum and neonatal factors predisposing to early onset sepsis such as young, primigravida mothers, obstetric complications of PROM, Gestation diabetes and obstetric intervention by emergency LSCS and neonatal factors of prematurity and low birth weight of high statistical significance are important.

2.5 Antepartum Risk Factors for Early Onset Neonatal Sepsis

2.5.1 Mother's Age

The mean maternal age among EOS cases was 24.675 years, S.D. 4.9016 compared to controls who were older at 25.246 years, S.D. 4.4123, however the difference of 0.57 years in the mean maternal ages between EOS cases and controls was statistically insignificant P=0.06. The 25th, 50th and 75th percentile for mothers among EOS cases being 20.5, 24.0 and 28.0 years and for controls 22.0, 25.0 and 28.0 years respectively.

Majority mothers 57.1% (n=136/237) (missing n=7) with EOS cases were younger ≤24 years of age, OR 1.53 (CI 95%) 1.2-2.0 times greater odds being highly statistically significant P>|z| =0.002 when compared to 46.8% (n=1142/2379) (missing n=127) controls. In fact older mothers 25-29 years comprised 35.5% (n=845/2379) for controls contrasted to lower 26.6% (n=63/237) with EOS cases.

Thus younger mothers were more prone to EOS in their newborns while older mothers above 24 years had progressively decreasing incidence of EOS, however slightly more EOS cases were noted in those above 35 years in comparison to controls. Antepartum risk factor in relation to mother's age, multiple logistic regressions to EOS cases and controls seen in Table 1 and percentage distribution of EOS cases and controls in relation to mother's age groups is seen in Figure 1.

Table 1. Antepartum Risk Factors Multiple Logistic Regressions for Early Onset Neonatal Sepsis

Category	Controls *n* (%)	Cases *n* (%)	Odds Ratio	(95% Conf. Interval)	P>|z|
Mother's Age					
≤24 years	1,114(46.8%)	136(57.4%)	1.53	(1.2-2.0)	0.002
≥25 years	1,265(53.2%)	101(42.6%)	*(Ref)*		
Gravida					
1	774(31.38%)	118(48.8%)	2.08	(1.6-2.7)	<0.001
2-5+	1,692(68.61%)	124(51.2%)	*(Ref)*		
Premature Rupture of Membranes (PROM)					
Yes	108(4.3%)	92(37.7%)	12.96	(9.5-18.4)	<0.001
No	2,398 (95.7%)	152 (62.3%)	*(Ref)*		
Pregnancy Induced Hypertension (PIH)					
Yes	140(5.6%)	15 (6.1%)	0.51	(0.6-1.8)	0.823
No	2,366(94.4%)	229(93.9%)	*(Ref)*		
Gestational Diabetes					
Yes	125 (5%)	22 (9%)	2.19	(1.1-3)	0.008
No	2,381 (95%)	222 (91%)	*(Ref)*		

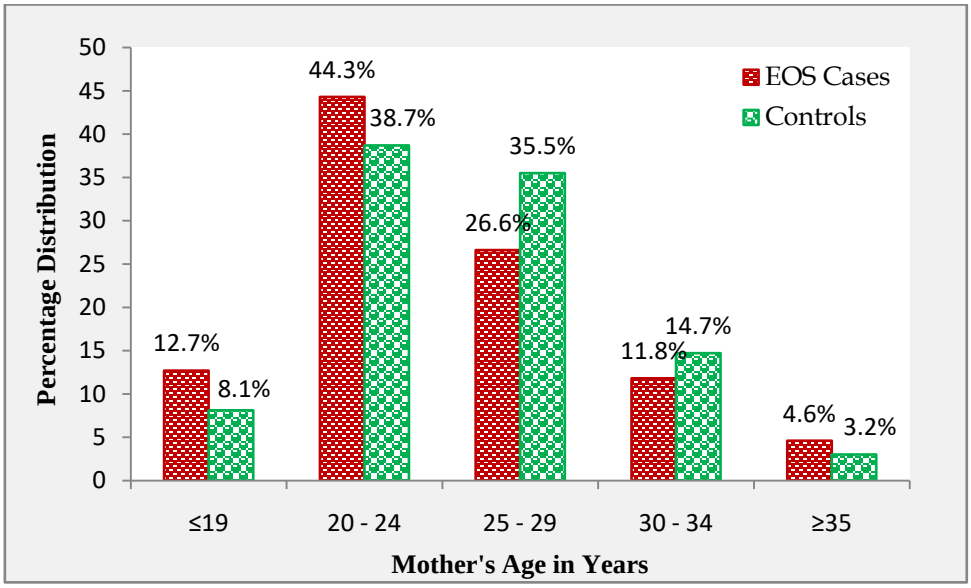

Figure 1. Percentage distribution of EOS Cases and Controls in relation to Mother's Age in years (P>|z|0.002).

2.5.2 Primigravida

Nearly half 48.8% (n=118/242), (missing n=2) primigravida mothers, newborns developed EOS, OR 2.08, (CI 95%) 1.6-2.7 times higher odds, being highly statistically significant P>|z| <0.001, when compared to a lower 31.3% (n=774/2,471) (missing n=35) controls. While among second gravida mothers EOS cases decreased to 25.6% (n=62/242) contrasted to controls 30.2% (n=747/2,471). Similarly also third gravida mothers had higher controls 22.1% (n=546/2,471) compared to a low 14.9% (n=36/242) EOS cases.

Thus increasing parity was associated with decrease in incidence of EOS and primigravida mothers had highest risk of newborns developing EOS probably due to prolonged labor. Antepartum risk factor in relation to gravida, multiple logistic regressions to EOS seen in Table 1 and percentage distribution of EOS cases and controls in relation to gravida is seen in Figure 2.

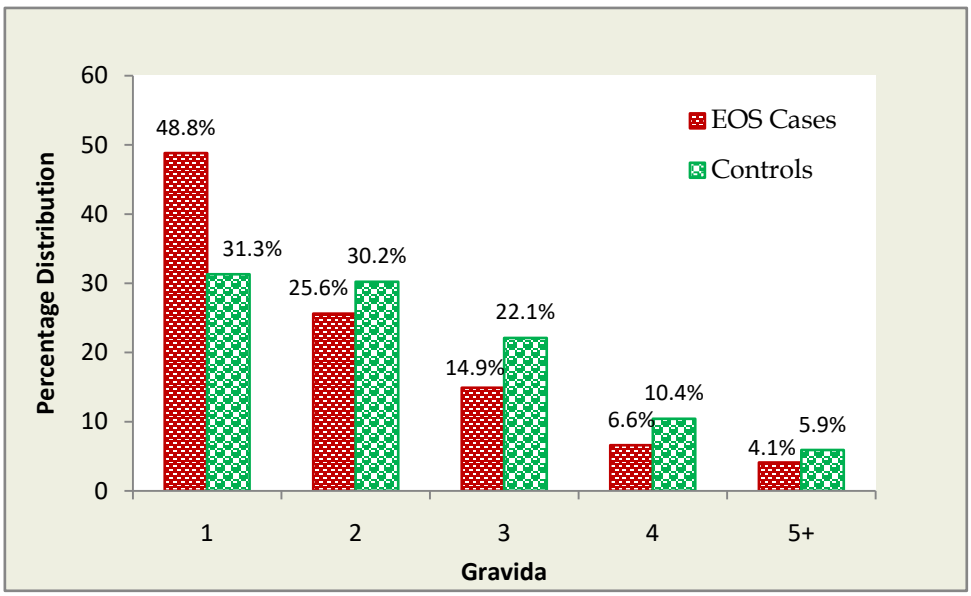

Figure 2. Percentage distributions of EOS Cases and Controls according to Gravida (P>|z|<0.001).

2.5.3 Premature Rupture of Membranes (PROM)

The incidence of Premature Rupture of Membranes (PROM) or leaking membranes of more than one hour prior to onset of labor was 7.27% among total deliveries. PROM occurred in over one-third 37.7% (n=92/244) EOS cases, OR 12.96 (CI 95%) 9.5-18.4 times greater odds being highly statistically significant P>|z| <0.001 when com-

pared to only 4.3% (n=108/2,506) healthy controls. Almost all 95.7% (n=2,398/2,506) controls had intact membrane prior to onset of labor, contrasted to 62.3% (n=152/244) EOS cases.

Thus PROM was associated with higher risk for early onset sepsis probably due to ascending infection from vaginal colonization causing chorio-aminonitis or may even be acquired by horizontal infection through passage an infected vaginal canal. Antepartum risk factor of PROM multiple logistic regressions to EOS seen in Table 1 and the percentage distribution of PROM among EOS cases and controls seen in Figure 3.

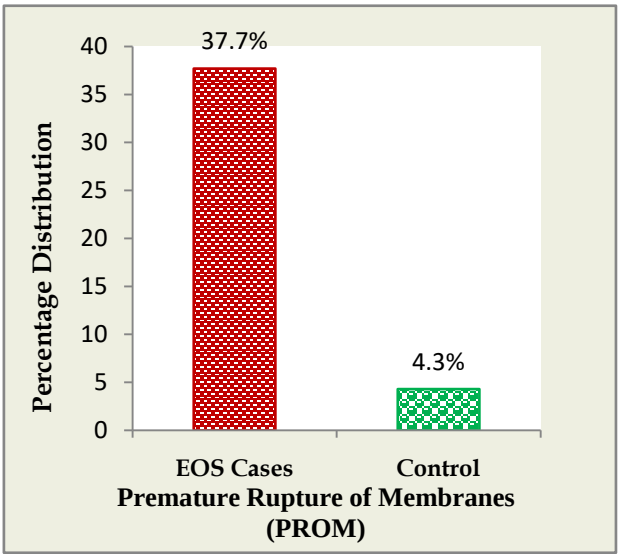

Figure 3. Percentage distributions of EOS Cases and Controls with PROM (P>|z|<0.001).

2.5.4 Gestational Diabetes

The overall incidence of Gestational Diabetes complicating pregnancy was 5.35% (n=147/2,750) among total deliveries. In contrast incidence of gestational diabetes among EOS cases was significantly higher at 9% (n=22/244), OR 2.19, (CI 95%) 1.1-3 times higher odds being highly statistically significantly P>|z| = 0.008, when compared to a lower 4.9% (n=125/2,506) controls.

Gestational diabetes predisposes to increased susceptibility to infection commonly presenting as asymptomatic urinary tract infections resulting in vaginal bacterial colonization with ascending route of infection causing in-utero infection resulting in chorioaminonitis. Antepartum risk factor of gestational diabetes, multiple logistic regressions to EOS cases and controls seen in Table 1 and the percentage distribution of gestational diabetes among EOS cases and controls is seen in Figure 4.

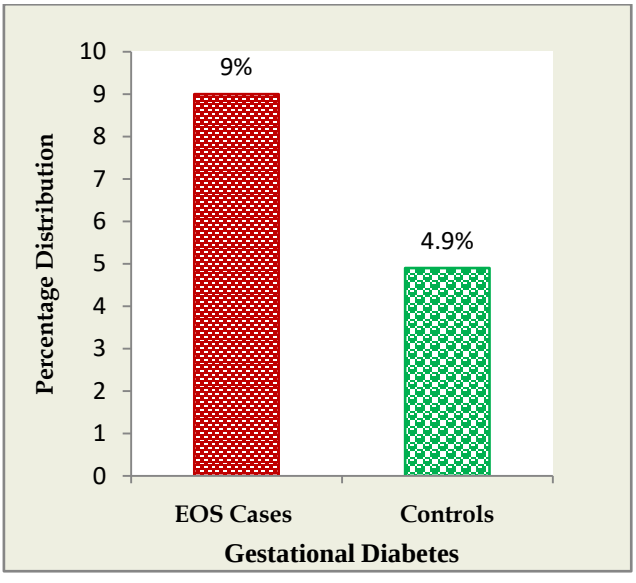

Figure 4. Percentage distributions of Gestational Diabetes in EOS Cases and Controls (P>|z|0.008).

2.5.5 Pregnancy Induced Hypertension (PIH)

The overall incidence of Pregnancy Induced Hypertension (PIH) was 5.63% (n=155/2,750) among total deliveries. However incidence was high 6.1% (n=15/244) among cases of EOS, OR 0.51, (CI 95%) 0.6-1.8 was statistically insignificant P>|z| = 0.832, with incidence of 5.6% (n=140/2,506) PIH for controls.

Thus obstetric complication of PIH was not significantly associated with increase predilection of EOS. Antepartum risk factors of PIH complicating pregnancy, multiple logistic regressions for EOS cases and controls seen in Table 1 and the percentage distribution of PIH among EOS cases and controls is seen in Figure 5.

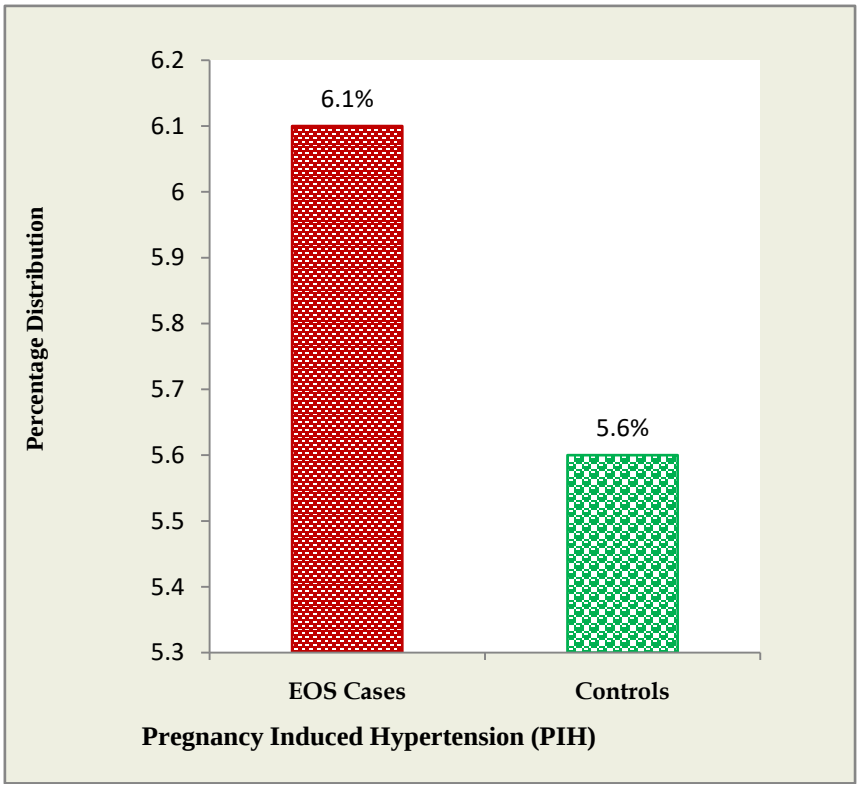

Figure 5. Percentage distributions of EOS Case and Controls with PIH (P>|z|0.823) (NS).

2.6 Intra-partum Risk Factors for Early Onset Neonatal Sepsis

2.6.1 Emergency Lower Segment Cesarean Section (LSCS)

Mode of delivery by emergency Lower Segment Cesarean Section (LSCS) was significantly associated to early onset neonatal sepsis as fetal distress was a common indication probably due to in-utero infection that often presents as fulminant sepsis in newborn. Significantly higher 39.3% (n=96/244) of EOS cases were delivered by emergency LSCS, OR 1.82, (CI 95%) 1.3-2.5 times higher odds, being highly statistically significant P>|z| <0.001. In contrast lower 26.3% (n=659/2,504) controls were born by emergency LSCS.

However normal vaginal deliveries peaked 53.6% (n=1,342/2,504) (missing n=2) for controls compared to 44.3% (108/244) EOS cases. It should be noted that LSCS per say does not cause early onset sepsis but indication for judicious surgical obstetric intervention being fetal distress due to in-utero infection causing hypoxia or metabolic acidosis. However breach of skin integrity causes neonatal infection acquired by horizontal transmission through infected vaginal canal.

The incidence of elective or planned LSCS was 9.4% (n=23/244) in EOS, OR 0.75 (CI 95%) 0.4-1.2 was statistically insignificant P=0.220, in contrast nearly twice higher 15.2% (n=381/2,504) controls were delivered by elective LSCS, frequency increased to 34.3% in third gravida mothers to only 10.3% for primigravidaes, most 43.11% of whom delivered by emergency LSCS.

Most 39% older mothers aged 25-29 years had elective LSCS, contrasted to mothers aged 20-24 years and 25-29 years with similar 34% emergency LSCS. Mode of delivery, multiple logistic regressions to EOS cases and controls seen in Table 2 and the percentage distribution of EOS cases and controls by mode of delivery is seen in Figure 6.

Table 2. Intrapartum risk factors multiple logistic regressions for Mode of Delivery

Category	Controls *n* (%)	EOS cases *n* (%)	Odds Ratio	(95% Conf. Interval)	P>|z|
Mode of Delivery					
Normal Delivery	1,342 (53.6%)	108 (44.3%)	*(Ref)*		
Emergency LSCS	659(26.3 %)	96(39.3%)	1.82	(1.3-2.5)	<0.001
Elective LSCS	381 (15.2%)	23 (9.4%)	0.75	(0.4-1.2)	0.220
Outlet Forceps	87 (3.5%)	12 (4.9%)	1.75	(0.8-3.4)	0.077
Vacuum Extraction	35 (1.4%)	5 (2.1%)	1.79	(0.5-4.7)	0.226

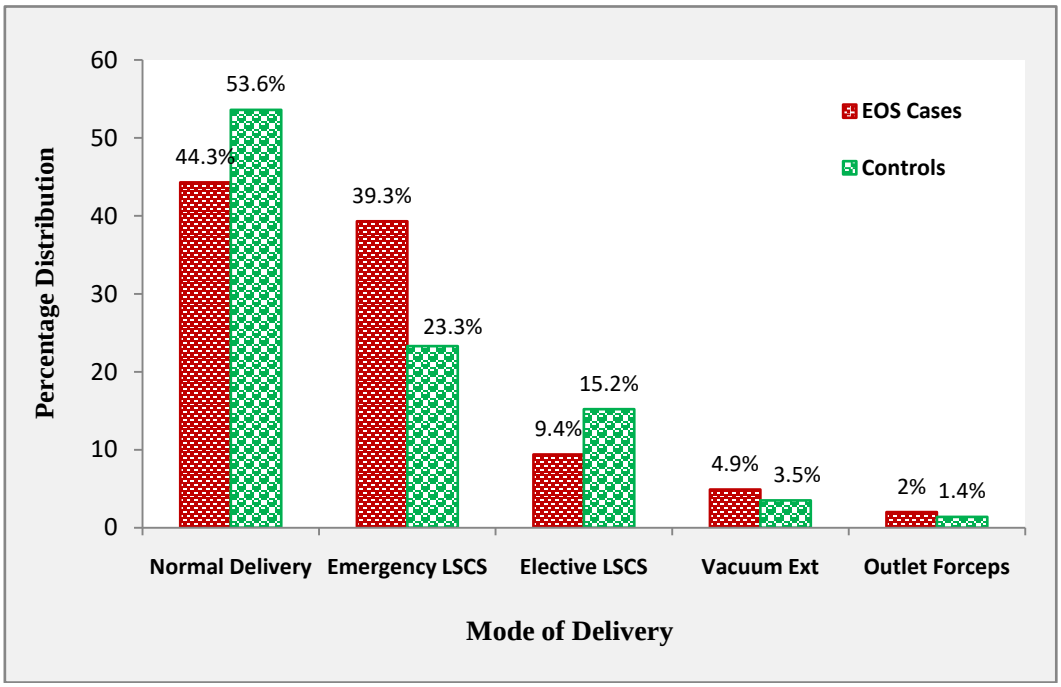

Figure 6. Percentage distributions of EOS Cases and Controls in relation to Mode of Delivery Normal delivery versus emergency LSCS (P>|z|<0.001).

2.7 Neonatal risk factors for Early Onset Neonatal Sepsis

2.7.1 Prematurity

The overall incidence of prematurity ≤36 weeks gestation was 9.68% (n=233/2,602) among total births in the present study. However the incidence of prematurity ≤36 weeks gestation among EOS cases was 21.88% (n=51/233) (missing n=11), OR 2.57 (CI 95%) 1.8-3.6, times higher odds being highly statistically significant P>|z| <0.001, compared to low 8.48% (n=201/2,369) (missing n=137) controls.

The mean gestation at birth among EOS cases was shorter at 37.68 weeks, S.D. 4.9016 compared to controls at 38.39 weeks, S.D. 1.565, the difference of 0.7 weeks being highly statistically significant P<0.001. The 25th, 50th and 75th percentile for EOS cases was 36.6 weeks, 38.1 weeks and 39.2 weeks when compared to controls with longer duration of gestation at 37.6 weeks, 38.4 weeks and 39.4 weeks respectively.

Percentage distribution of term newborns ≥37 weeks among EOS cases was lower at 78.11% (n=182/233), contrasted to controls at higher 91.26% (n=2,162/2,369). However term births at 38 weeks peaked 27.6% (n=654/2,369) among controls compared to 25.3% (n=59/233) for EOS cases. Similarly also controls figured more at 26.2% (620/2,369) at 39 weeks gestation compared to 20.2% (n=47/233) EOS cases. So also 22% (n=520/2,369) controls at 40 weeks gestation, contrasted to only 16.3% (n=38/233) EOS cases.

Thus increasing gestation at term was associated with reduced risks for early neonatal sepsis. Neonatal risk factor of prematurity, multiple logistic regressions to EOS cases and controls seen in Table 3 and the percentage distribution of EOS cases and controls by week of gestation is seen in Figure 7.

Table 3. Neonatal risk factors multiple logistic regressions for Early Onset Neonatal Sepsis

Category	Controls *n* (%)	EOS cases *n* (%)	Odds Ratio	(95% Conf. Interval)	P>|z|
		Prematurity			
≤36 weeks	201 (8.48%)	51 (21.88%)	2.57	(2.1-3.7)	<0.001
≥37 weeks	2,162 (91.26%)	182(78.11%)	*(Ref)*		
		Birth weight			
≤2499g	448(17.75 %)	90(37.34 %)	2.76	(2.1-3.7)	<0.001
≥2500g	2,076 (82.25%)	151(62.66%)	*(Ref)*		
		Sex Distribution			
Male	1,280 (50.59%)	123 (50.83%)	1.88	(1.1-3.0)	0.008
Female	1,250 (49.41%)	119(49.17%)	*(Ref)*		

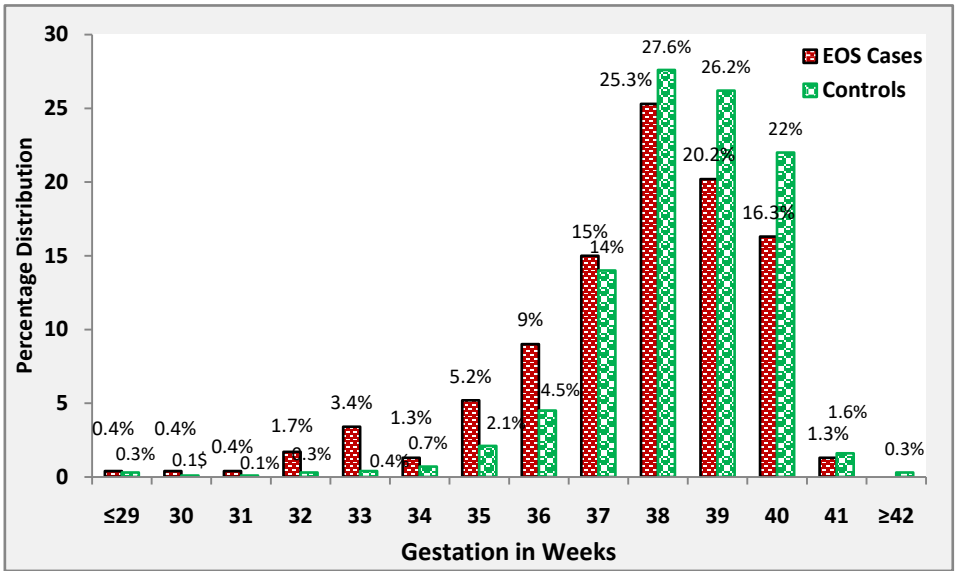

Figure 7. Percentage distributions of births by Gestation in weeks among EOS Cases and Controls (P>|z|<0.001).

2.7.2 Low Birth Weight

The overall incidence of Low Birth Weight (LBW) ≤2,499g was 19.2% (n=523/2,722) among total live births. However LBW ≤2,499g for EOS cases was much higher at 37.6% (n=91/242) (missing n=2), OR 2.76, (CI 95%) 2.1-3.7 times higher odds being highly statistically significant P>|z| <0.001. In contrast incidence of LBW ≤2,499g among controls was lower at 17.4% (n=432/2,480) (missing n=26).

Also the distribution of mean birth weight between EOS cases and controls revealed marked reduction being 2,674.75g, S.D. 607.9726 among EOS cases, compared to 2,888.34 g, S.D. 494.4190 for controls, the difference of 213.56g being highly statistically significant P<0.001. The 25th, 50th and 75th percentile among EOS cases being 2,280g, 2740g and 3,105g compared to a slightly higher 2,600g, 2,920g and 3,200g for controls respectively.

Percentage distribution of newborns by 500g birth weight category revealed that twice as many 28.1% (n=68/242) EOS cases were LBW between 2,000-2,499g compared to 13.95% (n= 346/2,480) controls. In contrast majority 38.75% (n=961/2,480) controls weighed 2500-2999g compared to only 30.16% (n= 73/242) EOS cases. Similarly also higher birth weight category 3,000-3,499g comprised 35.24% (n=874/2,480) controls compared to lower 28.09% (n=68/242) EOS cases. In addition controls 8.5% (n=213/2,480) weighing above 3,500g, constituted twice as much as 4.1% (n=10/242) EOS cases.

Thus LBW ≤2,499g neonates have increased susceptibility to infection when compared to newborns weighing ≥2,500g, while increase in birth weight ≥2,500g had higher incidence of controls. Neonatal risk factor of LBW multiple logistic regressions to EOS cases and controls seen in Table 3 and the percentage distribution of EOS cases and controls in 500g birth weight categories is seen in Figure 8.

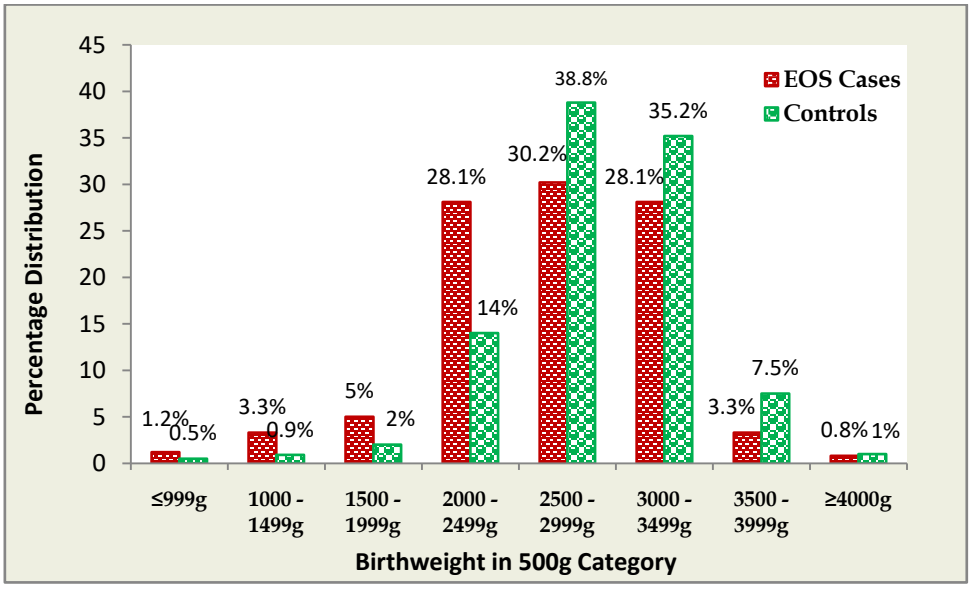

Figure 8. Percentage distributions of EOS Cases and Controls according to Birth weight categories in 500g (P>|z|<0.001).

2.7.3 Male Gender

Sex distribution revealed Male: Female ratio, M:F::1:0.9, with male predominance of 50.16% (n=1,366/2,723) compared to 49.83% (n=1,257/2,723) females. Slightly more males 50.8% (n=123/242) (missing n=2) developed early onset sepsis, OR 1.88 (CI 95%) 1.1-3.0 times higher odds being highly statistically significant P=0.008, in contrast a female predominance was noted among controls 49.9% (n=1,238/2,481) compared to slightly less 49.2% (n= 119/242) EOS cases.

Thus male newborns had increased predilection to EOS when compared to female newborns. Neonatal risk factor for male and female newborns, multiple logistic regressions for EOS cases and controls seen in Table 3 and the percentage distribution of male and female newborns among EOS cases and controls is seen in Figure 9.

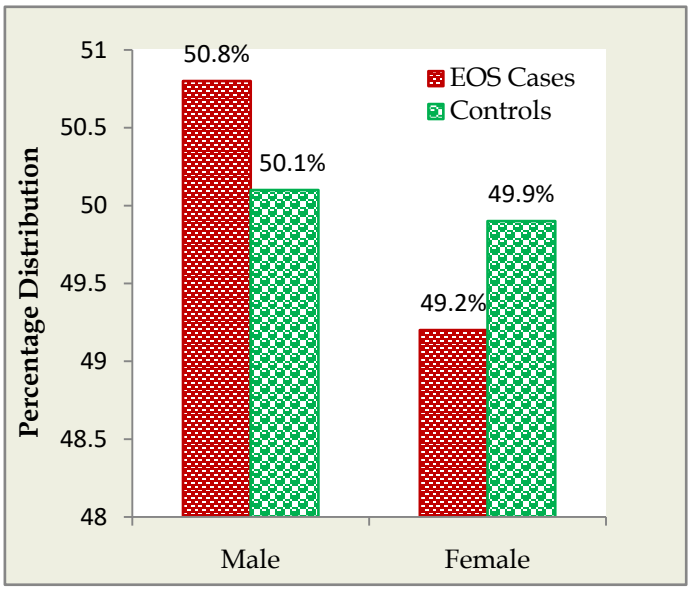

Figure 9. Percentage Sex distributions among EOS Cases and Controls (P>|z|0.008).

3. Discussion

The Agenda for Sustainable Development for child survival cannot be achieved without substantial reductions in infection-specific neonatal mortality, newborn deaths comprising 47% of all under five years deaths globally with around one-third dying on day of birth and about three-quarters within the first week of life [11-13]. India has the

highest under-5years child mortality rates and 60% of these occur during the neonatal period of which nearly 12% of whom are related to neonatal sepsis with high economic burden in Asian countries especially in sub-Saharan Africa ranging from 10 to 469 billion USD with serious consequences of neurodevelopmental impairment (NDI) in survivors [14-17]. The Early Neonatal Mortality Rate (ENMR) in India has not shown the same reduction as late neonatal mortality, infections being a major contributor of newborn deaths, as also in many Low and Middle income countries (LMIC) [14, 16, 17]. In fact, several studies suggest neonatal infections as high as 170/1,000 live births diagnosed clinically to 5.5/1,000 live births, confirmed by blood culture, responsible for 8% to 80% of all neonatal deaths with about 42% of deaths occurring in the first week of life however as most births are domiciliary, majority of these deaths occur at home without coming to medical attention [7, 8]. Hence the importance of early diagnosis of EOS and empiric antibiotic therapy within 24 hours of birth, reduces not only mortality but also morbidity with severe lifelong threatening sequelae such as seizures, mental retardation, blindness, hearing loss etc. [9, 18].

The two patterns of neonatal sepsis include early onset sepsis (EOS) and late onset sepsis (LOS). EOS occurs within 0-3 days i.e. within 72 hours of life, 85% present within 24 hours of birth, median age of onset being 6 hours, 5% at 24-48 hours and the remaining 10% in the next 48-72 hours of life. Majority EOS present as fulminant multi-system illness within first 24 hours carries a high mortality of up to 50%, although late onset sepsis is less fulminant having a lower mortality of only 10 to 20%, however meningitis is a frequent occurrence [9, 18]. Another study reported majority 36% neonatal sepsis occurred within first week, 25.9% in second week, 26.6% third and 11.4% during the fourth week of life, mortality was highest 43% within first 3 days of life [19].

A total of 369 early neonatal deaths (ENDs) among 21,585 consecutive births during the five-year period 1979 to 1983 at Christian Medical College & Hospital, Vellore, Neonatal infection ranked as the fourth cause, accounted for 14.9% (n=55/369) ENDs. Maternal and obstetric risk factors predisposing to infection were present in nearly two-thirds septic ENDs, commonest risk factor being PROM 29% (n=16/55), while PIH complicated pregnancy in 21.8% (n=12/55) and antepartum hemorrhage (APH) 5.6% (n=3/55).

Predisposing neonatal factors included very low birth weight ≤1,499g among 52% (n=192/369) EOS deaths, while 40% (n=148/369) were born to primigravida mothers. There were three second of twins. Majority 80% (n=44/55) EOS presented as fulminant septicemia and 24% (n=13/55) with pneumonia, two of whom died within 2 hours after birth and autopsy showed multiple abscesses in the lung parenchyma. Two other newborns had meningitis and three necrotizing entercolitis. Positive blood cultures were obtained in 30% (17/55) of EOS, ENDs. The commonest organism isolated were gram negative organisms in 93.7% such as klebsiella, E. coli, citrobacter, pseudomonas and salmonella typhimurium. Remaining 6.3% were gram positive organisms, staphylococcus aureus and Group I B streptococci [20].

Almost all early onset neonatal sepsis starts in-utero with fetal infections either by hematogenous spread acquired transplacentally or due to ascending infections transmitted vertically from vaginal canal before delivery with contamination of the amniotic fluid, placenta and cervix causing chorioamnionitis or during delivery often due to colonizers of the maternal genitourinary tract, presenting as fulminant multi-system illness in the newborn [9]. Bacteria causing perinatal infection usually are normal vaginal flora; however other numerous vaginal strains of bacteria frequently involved in ascending transmission of infection include Group B streptococci, E. Coli, other Gram-negative bacilli and Listeria Monocytogenes. While organisms responsible for late onset sepsis (LOS) are Staphylococcus Aureus, Staphylococcus Epidermis and Pseudomonas in addition to the above organism [9, 18, 21].

Study from India reported pathogens isolated among 64.1% of EOS cases, commonest being CONS 32.2% followed by Klebsiella 19.3%, Staph Aureus 18.1%, E. Coli 15.9%, Actinobacter 12.3% and Enterococcus spp. 1.8%. In LOS Gram negative was predominant in 59.5% of cases compared to gram positive 40.5%. However, gram negative sepsis had higher mortality with isolates being sensitive to Meropenem, Piperacillin-tazobactum, Cefepime, Ceftazidime while gram positive isolates were sensitive to Vancomycin and Linezolid [4].

Studies have reported risk factors such as maternal fever, PROM >24h, foul smelling liquor or frequent >3 unclean vaginal examination and/or severe prematurity or birth asphyxia necessitating active resuscitation predisposing to EOS [3, 4, 19]. Still another study reported 41.6% positive blood cultures, commonest pathogen being pseudomonas 60% with case fatality rate of 19.4%. Other neonatal comorbid conditions included pneumonia 66%, shock 27.7%, metabolic acidosis 19.4% and meningitis in 8.3% [3]. Culture positive rates in 26% neonatal sepsis, almost all 100% gram negative bacilli and 90% gram positive Staph. Aureus were resistant to gentamicin, 52.9% and 20% respectively to Ciprofloxacin, however 17.6% of gram negative bacilli resistant to Amikacin and 31.2% to Cephalosporins with none to 20% Staph Aureus respectively. Increasing multidrug resistant organisms cause neonatal sepsis [22].

The increased susceptibility of newborn to gram negative infection may be explained by the fact that the antibodies against these microorganisms are primarily IgM type. Unlike humoral immunity there is no passive or transplacental transfer of markers of cell mediated immunity (CMI) but a healthy term infant would have satisfactory CMI at birth, though most have decreased immunoglobulin content, decreased complement activity and deficient chemotactic and phagocytic response of the polymorphonuclear cells. Low birth weight babies whether preterm or small for dates are more handicapped and hence more vulnerable to infections. In fact there has been an actual increase in the incidence of infection as sophisticated systems for respiratory and metabolic support now permit tiny babies to survive [9, 18].

However the foetus in utero is well protected from infection by the maternal immune system and by the placenta and membranes. The newborn depend on the Humoral immune response being the main defense mechanism of the foetus. IgG antibodies are passively transferred to the foetus from the third month of gestation, thus prematurely born babies have decreased IgG antibodies the levels directly related to the gestational maturity. Thus adequate IgG levels at term offer protection against several gram positive bacteria and viruses during the first six months of life. Immunoglobulins of other classes like IgM, IgA, IgD and IgE do not cross the placenta. While feeding initiates colonization in the gut and within 2 to 7 days of birth, the baby is exposed to the environment contaminated with micro-organism, which starts colonizing in the gut or rectum and other sites such as umbilicus, nasopharynx and skin of the baby gets colonized in that order. Fetal hypoxia and acidosis may lower host defense mechanism or allow localization of organism in necrotic tissues. Hypothermia in newborn infants is associated with significant increase in the incidence of sepsis.

Accurate and early diagnosis of early onset sepsis is challenging to the clinician, hence various obstetric intrapartum risk factors and antenatal maternal antibiotic use or extremely preterm and very low birth weight newborns correlate with increased susceptibility to infections supports high index of clinical suspicion of EOS. Perinatal risk factors such as PROM, foul smelling liquor, unclean vaginal examination and maternal urinary tract infection have statistical significance to early onset sepsis ($p<0.001$) [3] warrants screening for sepsis since earliest clinical signs of sepsis are often minimal, non-specific or subtle, similar to many non-infectious processes being undistinguishable between bacterial, viral or fungal infections in absence of positive blood culture.

In fact, the baby not looking well with decreased physical activity, poor feeding may be the only evidence that infection is present. Other signs include hypo or hyperthermia, bradycardia, jaundice without Rh or ABO blood group incompatibility, vomiting, diarrhea, abdominal distension, necrotizing enterocolitis, shock, poor capillary refill, suggesting decreased peripheral perfusion has been found to be a very reliable sign of sepsis, jitteriness, seizures, and pneumonia with clinical features of tachypnea, apnea, grunting, flaring of ali nasi and intercostal retraction or cyanosis, superficial infections like conjunctivitis, staphylococcal skin lesions and umbilical sepsis or urinary tract infection. Congenital or in-utero pneumonia is usually transplacental or ascending infection or intranatal pneumonia acquired by direct contact with organism during birth through in an infected birth canal or postnatal pneumonia acquired after birth. Other infections of the newborn include bacterial infections of the bone and joints. Though bacteremia may occur without signs of sepsis, serious bacterial infection is associated with systemic inflammatory response (SIR) that presents with wide variety of systemic and metabolic responses as restoring homeostasis is poor in critically ill newborns [9, 18, 23].

Neonatal meningitis is the most feared of all bacterial infections and one-fourth of neonates with septicemia have accompanying meningitis, the etiology being the same as that for sepsis though meningitis is more common among VLBW with late onset sepsis, but it is important that early diagnosis should be made when the neonate is showing the 'softer' signs like irritability, restlessness pallor, and poor feeding. 'Classic' features like bulging AF, neck retraction and convulsions are seen in advanced disease and it carries a poor prognosis. Convulsions occur in about half the cases. Routine lumbar puncture with cerebrospinal fluid (CSF) analysis in neonates because of maternal risk factors without any clinical signs as part of sepsis work up is controversial as positivity rate is low. CSF examination of > 20 WBC/mm^3 is taken as suggestive of meningitis. Upper limit of CSF protein is 150 to 200 mg% and normal CSF sugar is 1/2 to 2/3rd the corresponding blood sugar [18, 24].

Poor prognosis neonatal meningitis with high mortality in one-third and significant sequelae in 2/5th to 3/5th of survivors such as motor and mental disabilities including convulsive disorders, hydrocephalous, hearing loss, microcephaly and abnormal speech and behavior patterns with CT scan revealing high incidence of residual defects in survivors, other poor prognostic factors detected in GBS meningitis include coma or semi-comatose states with decreased perfusion. Investigations of WBC count less than 5,000/mm^3, absolute neutrophil count less than 1,000/mm^3 and CSF protein more than 300mg% are diagnostic, also poor prognostic factors identified in gram negative meningitis are thrombocytopenia, CSF WBC more than 2,000/mm^3, CSF protein more than 200 mg%, CSF to blood glucose ratio less than 0.5 if prolonged more than 48 hours and positive CSF culture [18, 24].

Screening tests in neonates with history of high risk maternal, obstetric and neonatal factors in combination with clinical signs includes multiple hematological studies such as complete blood counts (CBC), total and differential leucocyte count, immature to total neutrophil ratio, platelet count and surface swab assessment, gram stain of tracheal aspirate in intubated newborns and respiratory problems require chest x-ray [18, 21]. Other direct methods include - gastric aspirate for gram stain and polymorphs >75% suggests infection in buffy coat smear examination. However white blood cell counts are of limited value in the diagnosis of early onset sepsis because of the wide range of normal counts from 8,000 to 20,000/mm^3, often being normal at the time of initial evaluation with proven sepsis however reduced cell counts <5,000/mm^3 is a better indicator of sepsis than elevated counts >20,000/mm^3.

An absolute neutrophil count (ANC) of <1,000/mm^3 in the first 48 hours to 72 hours is more sensitive than total leucocyte count. Association between neutropenia, respiratory distress and early onset sepsis caused by GBS is well documented. Band form neutrophil count >5% is suggestive of infection and >20% is almost definitive of infection, Immature: Total neutrophil (I/T) ratio of >0.2 has a reported sensitivity of 82-90% in diagnosis of septicemia. Morphological changes in neutrophils such as toxic granulation i.e. eosinophilic granules in the cytoplasm of neutrophils, cytoplasmic vacuolization and Dohle bodies or aggregates of rough endoplasmic reticulum which stain light blue with Geimsa stain are also suggestive of infection. Micro-Erythrocyte sedimentation rate (ESR) >10mm not a very reliable marker, normal value is up to 6 mm in the first hour during the first 3 days of life [18, 25-27].

Thus, the inability of any single lab test to provide rapid reliable and early identification of neonates with bacterial sepsis has led to the development of screening panels or "sepsis screen" as a means of increasing predictive accuracy. Most sensitive combination probably is hematologic indices and acute phase reactant—C reactive protein (CRP), abnormality in any two or more tests considered to be positive and in one or none as negative [18, 25]. Leucocytes indices and CRP are "late" markers not sensitive enough for early diagnosis of sepsis and CRP at 6-12 hours of age as routine investigations has either low sensitivity or specificity. However, CRP >10mg/l and complete blood count (CBC) examination with abnormal blood film and or immature to total neutrophil ration ≥ 0.2 or gastric aspirate ≥ 5 polymorphs/high power field or pathogen on gram-stained smear and /or culture has sensitivity of 97% and specificity of 61%, negative predictive value of 98% and likelihood ratio of 49% for EOS [18, 28-31].

An increasing CPR level, more accurately predicts sepsis than an individual one and a repeated normal CRP 8-24 hours and later after birth is evidence against bacterial sepsis and antibiotics can be discontinued is a safe and practical approach in neonates with suspected sepsis distinguishing between bacterial infection and viral or self-limiting ones [29, 30], as untreated bacterial infections could cause serious complications but treating viral infection with antibiotics may cause development of resistance with risks of adverse reactions and adds unnecessary costs.

While late-onset sepsis (LOS) after 72 hours of life is associated not only with high mortality but also significantly prolonged antibiotic exposure and hospital stay for neonates admitted to intensive care units (ICU), thus, serial CRP measurements has been found to be more helpful in clinical application as normal CRP values after 24 hours indicate that sepsis is unlikely [28-30].

Procalcitonin (PCT), another acute phase reactant is a precursor protein of the hormone calcitonin is physiologically elevated during first 3 days of life is a reliable marker of late-onset sepsis in newborn babies has a sensitivity and specificity of nearly 100% [32,33]. Thus in attempting to discover the presence of a serious illness such as neonatal septicemia which is life threatening, yet treatable, diagnostic tests with maximal sensitivity and negative predictive value are desirable [25-31].

Hence strategies to reduce morbidity and mortality early onset of neonatal sepsis within 72 hours of birth includes a combination of clinical signs with hematologic and serologic markers. The development and implementation of an early onset sepsis calculator guides antibiotic management in late preterm and term neonates [34]. Neonatal early onset sepsis calculator with multivariate risk estimate combined with newborn's clinical score within first 24 hours ensuring early diagnosis and empiric antibiotic therapy with prior blood culture is perhaps more appropriate for western countries that would result in decrease laboratory testing, without any apparent adverse effects. The on-line tool for suspected and confirmed early onset of neonatal sepsis includes Kaiser permante sepsis score which calls for more stringent diagnosis of chorio-aminionitis, however risks of delaying treatment have also been reported [34, 35].

The standard and most specific test is the isolation of bacteria from a central body fluid such as blood, urine culture and lumbar puncture for cerebrospinal fluid (CSF) cell count and cultures, with proper collection and culturing of the sample has however low positivity rates with isolation of bacteria from a blood culture ranging from 8% to 73%, usually requires 36-72 hours, hence does not aid in diagnosis of sepsis within the first 24 hours [36, 37]. Certain distinguishing criteria for differentiating bacterium from contaminants are based on clinical signs and microbiological factors to determine sepsis. Contaminants requires longer time >48 hours to detect growth in conventional

media or when only one bottle is positive of an anaerobic-aerobic set, or if different species are grown in two bottles or the organism is part of the normal skin flora such as coagulase negative staphylococci (CONS) diptheroids, non-hemolytic streptococci. Clinically if the baby is well without the use of antibiotics, a positive culture for a commensal is more likely to be a contaminant. Repeat blood culture should be obtained when the initial culture is ambiguous. However CONS infection is more likely if more than one culture or multiple site cultures are positive especially in preterm and LBW babies and in sick neonates should be considered as pathogen and managed with appropriate antibiotics [36]. Blood culture negative for pathogens and serial CRP decline to normal is indication to discontinue antibiotics if newborn is clinically well [36, 38].

Counter immuno-electrophoresis (C.I.E.) allows for rapid detection of bacterial antigen even in killed organism from blood after centrifugation and separation of plasma or even gram staining of buffy layer is positive in 57-70% EOS cases [38]. Bactec media contains resins effectively neutralizes antibiotics allowing for growth of organisms and increases chances of recovery of pathogen, that may not occur with conventional media such as aerobes, anaerobes, yeast, fungi and mycobacterium. Automated blood culture system is the primary choice that utilizes fluorescent technology in detecting growth of organism in blood, increase yield of blood cultures with reduced time for organism recovery [39].

Molecular genetics such as PCR- MicroArray chip technology identifies rapidly within 30 minutes, bacterial genomes including appropriate antimicrobial therapy based on sensitivity pattern of bacteria specific 16SrRNA gene present in all bacterial genome with antimicrobial resistance markers being highly 100% sensitive and 97.9% specific, aid in early exclusion of bacterial infection to reduce overuse of antibiotics. Hence PCR combined with DNA Microarray technology will not only allow identification of the microorganism but also the antimicrobial sensitivity pattern so critical to clinical care [40-42].

Other biomarkers include cytokines which are small proteins produced by almost all cells in response to inflammation or bacterial toxins, releases pro-inflammatory mediators causing activation of immune cells to produce interleukins (IL) such as IL-6, IL10, IL8, IL-1α or -1β, IL-33, Tumor necrosis factor-A (TNF α), or type 1 interferon (IFNs) and cell surface antigen –sICAM and CD64. Various cell surface markers in response to circulating cytokines such as Neutrophil DC11b, a subunit of b2 integrin adhesion molecule has 2-4 fold increase with positive blood culture sepsis having 86.3-100% sensitivity and 100% specificity for diagnosis of early and late onset neonatal sepsis including lymphocyte surface markers CD 25, CD 45RO and CD 64 with 97% sensitivity and 90% specificity and negative predictive value (99%) being both early as well as later than 24 hours diagnostic marker. CD4 or IL6 and CRP improve sensitivity and negative predictive value to 100% enhances diagnosis of localized infection [43-47].

Preterm newborns show increase in number of lymphocyte CD3, CD19, CD 25, CD 26, CD 71 and CD69 as well as neutrophil CD 11b, CD11c, CD13, CD15, CD33 andCD66 antigens in response to infections with increase in expression of CD19, CD33 and CD 66b are under evaluation not yet readily diagnostically available. Level of IL6 cut off at 30pg/ml has a mean sensitivity of 78% and specificity of 79% for detecting neonatal sepsis [45]. IL-6 increases 1000 fold in sepsis, correlates with gravity for predicating mortality or organ failure, however its clinical use is limited as combination of WBC, CRP levels and clinical monitoring of fever is more effective rather than expensive IL-6 determination. The binding to mbIL6R or membrane bound il6 receptor, promotes cluster of differentiation of CD4+T- cells via IL-21 production of T-helper2 cells and Th17 cells similar to CRP is used to monitor inflammation level [46, 47]. Highly sensitive markers predicative of neonatal sepsis are required such as acute phase reactants, cell surface markers, cytokines etc however are usually not routinely available in many diagnostic laboratories [25, 45-47].

Diagnostic test for intra-amniotic infections are often delayed as amniocentesis is required and non-invasive diagnostic tests have limited predictive value. The amniotic fluid tumor necrosis factor (TNF α) >41 pg/ml with preterm delivery and intact membranes has a sensitivity of 82% and specificity of 79% is a better independent indicator of early onset neonatal sepsis, rather than placental histology or amniotic fluid gram stain for septic shock and SIRS; however, its utility is lower than IL6 or IL8 or polymerase chain reaction (PCR) tests [48]. G-CSF and measurements of inflammatory cell surface markers such as CD 64 etc. Neutrophil CD -11b and CD-64 rises 2-4-fold in blood culture positive sepsis and umbilical cord IL6 increase precedes CRP in EOS [25, 43, 49]. Maternal CRP does not have a high sensitivity in predicting underlying asymptomatic intra amniotic sepsis and as such not recommended. Recent studies examine global expression of proteins or peptides in tissues and fluids especially amniotic fluid by surface-enhanced laser desorption/ionization (SELDI) in intrauterine infection at risk for impending preterm delivery with identification of a unique peptide profile within 12 hours of intra-amniotic inoculation of a microorganism before onset of labor or other clinical signs or symptoms of infection to proceed with delivery rather

than use tocolytic agents [50]. The accurate and early assessment by molecular biology techniques will allow for rapid identification of pathogens and microbial resistance [40-42].

Currently the gold standard for distinguishing between infectious, inflammatory, auto-immune diseases and malignancy in infants and children is not available in pediatric practice. Blood culture still remains the most specific standard test with isolation of bacteria from a central body fluid but usually requires 36-72 hours hence does not aid in diagnosis of sepsis within the first 24 hours [36, 37]. However a combination of biomarkers with clinical features and other diagnostic tests would help clinicians in the diagnostic process, such as screening test for sepsis, cytokines IL6, IFN-γ, TNF α, cell surface antigen –sICAM and CD64 as well as recently developed biomarkers IL-27 and IL-8, though are often inconclusive in diagnosis of early onset sepsis and by virtue of high negative predictive value screening panels would result in a significant increase in use of antimicrobial agents in Intensive Care Nursery as many asymptomatic neonates are empirically treated for extended period which drastically increases the load on neonatal units and exposes neonates to hospital-acquired infections and medication side effects [43-46]. Thus there is a lack of accuracy of biomarkers and physical examination in detecting early onset bacterial infection [10]. In many lower socio-economic Asian countries with limited resources and lack of diagnostic facilities for rapid identification of early onset sepsis, requires a high index of clinical suspicion for early diagnosis, based on history of high risk perinatal factors that is significantly associated with early onset sepsis in newborns with institution of empiric antibiotic therapy within 24 hours preferably in 6 hours of birth to prevent any adverse neonatal outcome.

Thus maternal, obstetric, neonatal risk factors remain the most practical means for alerting clinicians in creating a high index of suspicion in the detection of early onset of serious bacterial infection in newborns is key to avoid missing neonates with true sepsis. Significant criteria in present study include young mothers ≤24 years OR 1.53 (CI 95%) 1.2-2.0, being highly statistically significant P>|z| =0.002, primigravidae OR 2.08 (CI 95%) 1.6-2.7, P>|z| <0.001 probably due to prolonged labor in many young primigravidae that usually lasts up to 16-18 hours or so, compounded by repeated vaginal examinations to assess progress of labor.

Prolonged rupture of membranes (PROM), of more than one hour prior to onset of labor in 37.7% of EOS cases had OR 12.96 (CI 95%) 9.5-18.4, being highly statistically significant P<0.001, probably due to contamination of in-utero environment from infection of birth canal. Other studies have reported incidence of 8%-19% PROM, more than 18 hours prior to delivery developed EOS in 14.5%, more so a high 61.5% in preterm newborns compared to38.5% term neonates [19]. While other studies reported lower incidence 9.25% EOS among mothers with prenatal antibiotics compared to 20.65% in those who did not receive antibiotics. Respiratory distress was the commonest presenting clinical signs and CRP showing a high sensitivity of 90.90% [19, 51]. Also newborns of mothers with PROM had OR 62.04 (CI95% 0.00-22.79) times higher odds for early onset neonatal sepsis than newborns of mothers without risk factor of PROM [51] as pathogens causing intra-amniotic infections due to vertical transmission of infection from bacteria in vaginal canal are commonly involved in early onset neonatal sepsis [9,52].

Amniotic fluid has anti-inflammatory, anti-bacterial and antiviral properties that protect the fetus [53]. Ascending infection causing intra-amniotic infection and chorioamnionitis is usually from maternal endogenous vaginal flora that is preventable resulting in maternal febrile morbidity and mortality due to release of endotoxin and/or exotoxin from microorganisms that increases inflammatory cytokines, prostaglandins, metalloproteinase with maternal septicemia, post-partum endometritis, extremely preterm stillbirths or preterm delivery with higher risk of cerebral palsy, IVH, RDS and fulminant sepsis in newborns [54, 55]. Bacteria causing perinatal infection usually are normal vaginal flora including numerous vaginal strains of bacteria most frequently involved in ascending transmission are Group B streptococci, E. coli, other Gram-negative bacilli and Listeria Monocytogenes. Organism responsible for late onset sepsis includes Staphylococcus aureus, staphylococcus epidermis and pseudomonas in addition to the above organism. Overt or subclinical intra-amniotic infections are a potentially preventable cause in half of extremely preterm births periventricular leucomalacia/cerebral palsy [54-56].

The incidence of maternal sepsis 1.18 per 1,000 pregnant women is commonly due to E Coli followed by GBS, source being genital tract in 61%, urinary tract 25% associated with fetal loss preterm delivery and high perinatal mortality rates more common among primigravida. GBS is a major cause of perinatal infection like ammonites, bacteremia, endometritis and urinary tract infections in pregnancy [56]. There were three mothers HbsAg positive and one case with Group B Streptococcus (GBS) culture positive had meconium stained liquor with maternal factors such as fever indicating intrauterine infection or chorio-aminonitis, however maternal intrapartum antimicrobial prophylaxis against GBS has reduced current rates, an increase in gram negative infections noted more so in VLBW. Antibiotics typically given intravenously ≥4 hours before delivery includes high dose Penicillin or clindamycin, ceftriaxone etc, however resistance to penicillin with emerging resistant strains is increasing, Ciproflox-

acin/Ceftriaxone and Aminoglycoside are suitable. Prophylaxis against GBS includes current ongoing research on vaccine against GBS [57, 58].

Incidence of GBS vaginal colonization in Nigerian population was 3.8% had significant relationship to low socio-economic status in 57.1% of 185 pregnant women studied at 35-37 weeks gestation between December 2017 and April 2018. The vaginal and rectal swabs cultured for GBS were sensitive to Clindamycin while one case was sensitive to both Clindamycin and Ceftriaxone, none being sensitive to Penicillin [59]. However GBS rates vary up to 28% in Amman [60], while in the west about 1 in 4 pregnant women carry GBS bacteria in their body which can cause ascending infection, also crossing intact membranes without causing cervicitis resulting in in-utero infection usually manifesting as a fulminating pneumonia [61].

Gestational diabetes OR 2.19 (CI 95%) 1-1.3 times higher odds of EOS, being statistically significant P = 0.008. Similarly another study reported gestational diabetes at OR 2.19 (CI 95% .97-4.97) times higher odds when compared to newborns of mothers without the risk factor due to increased susceptibility of upper genital tract infection, usually asymptomatic but also an important cause of preterm birth [19].

Another observation noted in the present study was the quick resort to judicious obstetric surgical intervention by emergency LSCS in 44.3% of newborns with EOS, OR 1.82, (CI 95%) 1.3 - 2.5 times higher odds, being highly statistically significant P<0.001, compared to normal vaginal delivery, indicating that in most EOS cases with in-utero infections presented with signs of fetal distress indicating emergency obstetric surgical intervention, contrasted to elective LSCS having insignificant risk of EOS, OR 0.75, (CI 95%) 0.4-1.2, P=0.22.

Neonatal risk factors include prematurity ≤36 week's gestation with overall incidence of 9.68% for total births and higher incidence 21.88% in EOS cases, OR 2.57 (CI 95%) 2.1-2.57, being highly statistically significant P>|z| <0.001. In contrast incidence was only 8.48% in controls, in fact, 12% EOS cases at 36 weeks were more than twice that of 5.9% controls. Also, the mean gestation in EOS cases was shorter at 37.68 weeks compared to 38.39 weeks in controls, the difference of 0.7 weeks being highly statistically significant P<0.001. In contrast, lower term 78.11% noted in EOS cases compared to 91.26% controls.

The overall incidence Low Birth Weight (LBW) ≤2,499g was 19.2%. In contrast incidence of LBW among EOS cases was twice 37.6% with OR 2.76, (CI 95%) 2.1-3.7, being highly statistically significant P<0.001 compared to a low 17.75% for controls. The mean birth weight among EOS cases was 2,674.75g compared to controls at 2,888.34 g, a difference of 213.56g being highly statistically significant P<0.001. Another study reported 46% sepsis cases weighed below 2,000g with high mortality of 60.2% compared to 28.2% weighing above 2001g [19]. Sex distribution showed increased susceptibility in males OR 1.88 (CI 95%) 1.21-3.0 times odds compared to female newborns being highly statistically significant P=0.008. The increased susceptibility of male newborns to EOS compared to female neonates was also reported in other studies [19, 51]

Infection can be transmitted to the newborn either vertically or horizontally. Vertical transmission or materno-fetal transmission of infection through the ascending pathway, direct contact or transplacental transmission of bacterial infection which is rare as it requires maternal bacterium (Congenital infection). In ascending pathway, organism in the vaginal and cervical canal ascends into the amniotic cavity, is the most frequent route by which the foetus is infected. The usual outcome is colonization with no active disease, even in the presence of chorioamnionitis. However initial colonization of the foetus usually takes place after rupture of membranes and less frequently leads to neonatal disease.

Clinical entities of bacterial infection in the neonate may result from fetal aspiration and swallowing of organisms that have proliferated in the amniotic fluid. Vertically transmitted infections are clinically recognizable within approximately 72 hours of birth, often earlier in premature babies. The most common vertically transmitted congenital infections are septicemia and pneumonia. During the second stage of labor the fetus comes into direct contact with organisms in the cervix and vagina. Gonococcal conjunctiva is an example of infection acquired during birth by direct contact.

Horizontal or post natal transmission by direct contact occurs after the first three postnatal days. Infection is mainly due to bacteria from nursery environment. These infections are considered to be from nursery personnel or as complications of invasive procedure. In the recent years Coagulase Negative Staphylococcal (CONS) have emerged as the predominant organism involved in nonsocomial infections.

The risk of horizontal transmission is enhanced by factors such as decreased patient-nurse ratio, over-crowding, lack of hand washing, length of nursery stay, invasive procedures like arterial lines or in dwelling catheters. By far the most common modality of organism spread is unwashed hands of personnel, admission to the Neonatal Intensive Care Units including invasive procedures performed do have a predilection towards neonatal infection. Significant other diagnostic considerations include microbiological factors such as inoculum size and virulence of the

organism, environmental factors such as admission to the Neonatal Intensive Care Units, invasive procedures predisposing to neonatal infection, metabolic problems such as fetal hypoxia with acidosis and hypothermia alter host defense mechanism and allow localization of organism in necrotic tissues significantly increases incidence of sepsis [18]. Other preventive measures to reduce vertical transmission of infection includes hygienic measures to prevent nosocomial infection and improved care of the mother, while barrier nursing and the strict hand washing before and after touching a baby still remains the single most important factor in preventing nosocomial infections as the major route of horizontal transmission of bacterial pathogen to the newborn in the nursery is via the hands of personnel.

Thus, significant maternal obstetric and neonatal factors aids early diagnosis predisposing to neonatal infection, however best confirmed by isolation of pathogen with broad spectrum antibiotics based on sensitivity pattern, while blood culture negative for pathogens with serial CRP decline to normal is indication to discontinue antibiotics if newborn is clinically well. The choice of antibiotics for GBS includes Penicillin/Ampicillin, Ecoli & Gram negative bacilli:-Gentamycin/Kanamycin/Amikacin/Tobramycin/Netilmycin, Listeria - Ampicillin + Gentamicin, S. aureus - Methicillin / Cloxacillin, Vancomycin. S . Epidermis – Vancomycin. Group D Streptococci including Enterococci - Penicilin + Gentamicin. Pseudomanas - Ceftazidine/ Ticarcillin/ all Aminoglycosides except Kanamycin [25, 29, 61, 62].

The third generation Cephalosporins, Cefataxime, Ceftriaxone and Ceftazidine possess attractive features of therapy of bacterial sepsis and meningitis in newborn with excellent activity against gram negative bacteria, high concentration of drugs in the serum, good CSF penetration and less toxicity as it has no dose related toxicity. Disadvantages include - inactivity against Listeria and enterococci, variable activity staph aureus and more rapid emergence of drug resistant bacteria than that has been identified with standard regimen of Penicillin and Amino glycoside. Because of this problem, it is not advisable to use Cefotaxime alone. It is better to combine it with an aminoglycoside. Routine use or over usage of third generation Cephalosporin should be avoided and it should be reserved for serious infections such as meningitis [18, 21].

Antibiotic choice for continuation of therapy should be re-evaluated after 48-72 hours on basis of microbiological and clinical data with the aim to use a narrow spectrum antibiotic, to prevent development of resistance to reduce toxicity and to reduce costs. Antibiotic should be continued for 10-14 days in infants with culture proven sepsis. However if cultures are negative, the pediatrician must decide on the subsequent course of therapy for the infant who was treated for probable sepsis and if the baby appears well and there is reason to believe the presenting clinical symptoms are determined to be due to a noninfectious cause, antimicrobial therapy should be stopped promptly to minimize development of resistant pathogens and super-infection with other pathogenic organism. In a symptomatic baby or baby who improves, antibiotics treatment should be continued as for septicemia. However, there is insufficient evidence to recommend use of antibiotics cycling as a strategy to reduce development of antibiotic resistance and antimicrobial treatment should preferably be based on local antibiotic sensitivity pattern [18].

Empirical antifungal therapy should not be used as a routine basis in patients with severe sepsis or septic shock but it may be justified in selected subsets of septic patients at high risk for invasive candidiasis. Azoles (fluconazole) and echinicandin (caspofungin) are efficacious as and less toxic that amphotercin B deoxycholate for treatment of patients with candidemia and antimicrobial treatment should preferably be based on local antibiotic sensitivity pattern [18, 21].

Viruses should also be considered in the differential diagnosis such as enteroviruses, parechoviruses and herpes simplex virus (HSV) as well as rubella virus, cytomegalovirus, lymphocytic choriomeningitis virus or human immunodeficiency virus including seasonal virus such as influenza, respiratory syncytial virus (RSV), adenovirus, rhinovirus, Rota virus or fungal infection such as candidiasis etc should also be considered in differential diagnosis have been identified in hospitalized neonates are primarily horizontally transferred and a late onset infection [18].

Once a causative pathogen is identified there is no evidence that combination therapy is more effective than monotherapy in patients with severe sepsis or septic shock and local antibiotic sensitivity should guide antibiotic use. Newer antibiotics include Netilimycin, a newer amino glycoside effective against E-coli, Klebsiella, Enterobacter group resistant to Gentamicin, Ticarcillin, newer Penicillin specific for pseudomonas as well as Piperacillin effective against E.coli, Klebsiella, Enterobacter. Imipenam is the broadest spectrum ever documented, covers gram positive gram negative, listeria and anaerobes [18, 21, 63].

Adjunctive therapies in neonatal sepsis include—Exchange transfusion—it helps in removal of toxins and other bacterial products, improved oxygen delivery, corrects coagulation abnormalities and provides specific antibodies, complements and phagocytic cells. Simple plasma or whole blood transfusion is reported to be useful in GBS as it gives group specific opsonins against infecting organism. Intravenous Immuno-globulin (IVIG) has been used to overcome antibody deficiency in neonates. Transfusion of Fibronectins, high molecular weight glycoprotein has

been shown to be involved in homeostasis, vascular integrity, tissue repair, leucocyte migration, adhesion and phagocytosis. Infection is associated with decreased levels of fibronectin. Other supportive measures include control of seizures, fluid restriction, and anti-odema measures. Intraventricular gentamicin 0.5 mg to 1 mg daily for 3 to 4 days is indicated in cases of ventriculitis. If this fails to clear the infection, as repeated ventricular taps are not advisable, it is better to insert an Ommaya reservoir for intraventricular therapy. Antibiotics should be continued for at least 2 weeks after the clearance of ventriculitis. Intrathecal treatment has no role in neonatal meningitis [18, 21, 23].

Antibiotics should be started within the first hour of recognition of severe sepsis after appropriate cultures have been obtained; Monotherapy is as efficacious as combination therapy with a beta lactam and an aminoglycoside as empirical therapy for patients with severe sepsis or septic shock [21, 24, 63]. Antibiotic therapy should always be reassessed after 48-72 hours on basis of microbiological and clinical data with the aim to use a narrow spectrum antibiotic, to prevent development of resistance to reduce toxicity and to reduce costs. The duration of therapy should typically be 7-10 days guided by clinical response. However with emerging multidrug resistance bacterium to even penicillin, chloramphenicol etc with constantly changing antibiotic sensitivity patterns, local antibiotic sensitivity should guide antibiotic use.

In a symptomatic baby or a baby who improved with antibiotics treatment should be continued as for septicemia unless other diagnosis becomes apparent, if baby's condition worsens then antibiotics are changed depending on sensitivity report if blood culture if positive and continued up to 7 or to14 days, even upto 21 days with CNS infection and supportive management. However, safety of empiric neonatal antibiotic treatment in first week of uninfected preterm due to increased risk of fungal infection, bacterial late onset sepsis, necrotizing enterocolitis and death is questioned.

Thus, a high index of clinical suspicion for diagnosis of early onset neonatal sepsis is required and clinicians need to consider history of high risk predisposing maternal, obstetric and neonatal factors and antimicrobial therapy should be started within the first hour of recognition of severe sepsis after taking appropriate cultures and in combination with clinical signs and relevant tests confirm neonate to be septic and continued for 7-10 days guided by clinical response. Re-evaluation of choice of antibiotics for continuation of therapy is required when cultures results are available and antibiotic continued for 10-14 days in infants with culture proven sepsis, but negative cultures in infant who was treated for probable sepsis depends on early symptoms and signs and if infection is an unlikely cause, treatment can be discontinued after a period of 3 to 5 days. Symptomatic neonate who improved with antibiotics treatment should be continued as for septicemia unless other diagnosis becomes apparent. If the presenting clinical symptoms are determined to be due to a noninfectious cause, antimicrobial therapy should be stopped promptly to minimize development of resistant pathogens and super-infection with other pathogenic organism, antibiotics cycling as a strategy to reduce development of antibiotic resistance is not recommended.

In many LMIC Asian countries have poor resources with limited diagnostic laboratory facilities, hence the importance of identification of high risks maternal-antenatal intra-natal and neonatal factors predisposing to EOS, aids in early diagnoses, since almost all early onset neonatal sepsis starts in-utero with fetal infections acquired either transplacentally by hematogenous spread or vertically ascending infections from vaginal canal bacteria usually being normal vaginal flora that contaminates amniotic fluid, placenta and cervix causing chorioamnionitis or may be horizontally acquired during delivery from colonizers of the maternal genitourinary tract and often present as fulminant multi-system illness in the newborn.

The absence of a single reliable early diagnostic tools or ideal early diagnostic test with 100% sensitivity that always detects disease in all infected patients or 100% specificity with negative tests results in all patients without the disease, high sensitivity rather than high specificity becomes the main criteria in reducing antibiotic overuse or to withhold antibiotics in uninfected newborns. Sepsis screening panel tests may increase accuracy for early identification of neonates with bacterial sepsis. In view of the above, it is recommended that presence of two or more high risk obstetric or neonatal factors forms basis for high index of clinical suspicion of sepsis in newborn. For example PROM in primigravida mothers are important criteria for early diagnosis of neonatal sepsis and indication for empiric antibiotic therapy preferably monotherapy within 6 hours of birth, unless proven otherwise by subsequent testing and clinical features to exclude bacterial etiology of sepsis and antibiotics discontinued, as it is better to suspect and treat infection even if unnecessarily, rather than to miss diagnosis of sepsis and not treat infections that have disastrous consequences of either mortality and morbidity in newborns.

As predictability of laboratory investigations such as complete blood count (CBC) and C - reactive protein (CRP) is low especially at onset of illness or in asymptomatic newborn, though CRP >10mg/l with serial increasing levels more accurately predict sepsis than an individual one, while repeated normal CRP 8-24 hours after birth and later is

evidence against bacterial sepsis [25, 29-31] and antibiotics can be discontinued in well newborn since many asymptomatic neonates are empirically treated for extended period which drastically increases the load on neonatal units and exposes neonates to hospital-acquired infections and medication side effects and reduce emergence of multidrug resistant pathogens.

4. Conclusion

The awareness of the role of infection causing morbidity and mortality in the newborn has increased dramatically over the past few decades. Though incidence of early onset neonatal sepsis is low, it is associated with high mortality accounting for almost four-fifths of all neonatal deaths with nearly one-half occurring within first week of life. The lack of ideal biomarkers with high sensitivity, specificity and predictive value for distinguishing between infectious, inflammatory, auto-immune diseases and malignancy in pediatric practice requires a high degree of clinical suspicion should be based on presence of two high risk maternal-obstetric and neonatal factors that is key to avoid missing neonates with true sepsis.

Certain high risk anteparum, intrapartum and neonatal risk factors include young, primigravidae mothers, premature rupture of membranes, gestational diabetes and delivery by emergency LSCS, prematurity, low birth weight or male gender of high statistical significance in diagnosing early onset neonatal sepsis being most commonly due to in-utero infection and institution of empiric antibiotic therapy started within six hours of birth. The combination of clinical observation despite its limitation and diagnostic hematologic tests including serological markers tests remain the most practical means to confirm diagnosis and to monitor progress of disease or to withhold antibiotics in uninfected newborns thereby decreasing the emergence of multidrug resistant pathogens.

References

[1] WHO | Newborn death and illness – WHO | World Health Organization. https://www.who.int > press materials.

[2] Fleishmann, C., Reichert, F., Cassini, A., Horner, R., et al. Global incidence and mortality of neonatal sepsis: a systematic review and meta-analysis. Arch Dis Child, 2021, 06(8): 745-752. Doi: 10.1136/archdischild-2020-320217.

[3] Chacko, B., Sohi, I. (2005). Early onset neonatal sepsis. The Indian Journal of Pediatrics, 2005, 72(1): 23-26.

[4] Mistry, M., Battacharaya, A., Parmar, T. K. (2021). Neonatal septicemia and current scenario of antibiotic sensitivity pattern-A study of blood vulture isolates in a tertiary care hospital, Rajkot. International Journal of Medical Microbiology and Tropical Diseases, 2021, 7(4): 222-226. Doi:10.18231/j.ijmmtd.2021.046.

[5] Weston, E. J., Pondo, T., Lewis, M. M., et al. (2011). The burden of invasive neonatal sepsis in the United States, 2005-2008. Pediatr. Infect. Dis. J., 2011, 30: 937-941.

[6] Liu, L., et al. The Lancet Global Health. 2019 Jun 1; 7(60): e721-34.

[7] McClure, E. M., et al. (2009). Seminars in Fetal and Neonatal Medicinem 2009, Aug 1 (vol. 14, No. 4, pp. 182-189).

[8] Zaidi, A. K. M., Durrane, T., Asad, A. S., et al. (2009). Pathogens associated with sepsis in newborns and young infants in developing countries. The Pediatric Infectious Disease Journal, 2009, vol. 28(1): S10-S18. doi: 10.1097/INF.0b013e3191958769.

[9] Polin, R. A., St Geme, J. W. III Neonatal sepsis. Adv. Pediatr, Infect. Dis., 7: 25-61.

[10] Díaz, M. G., García, R. P., Gamero, D. B., González-Tomé, M. I., Romero, P. C., Ferrer, M. M., et al. (2016). Lack of accuracy of biomarkers and physical examination to detect bacterial infection in febrile infants. Pediatr Emerg Care, 2016, 32: 664-668.

[11] United Nations. (2017). Resolution adopted by the General Assembly on 6 July 2017, Work of the Statistical Commission pertaining to the 2030 Agenda for Sustainable Development (A/RES/71/313).

[12] Lawn, J. E., Cousens, S., Zupan, J. (2005). Four million neonatal deaths: where? When? Why? Neonatal survival series Paper 1. Lancet, 2005, 365: 891-900.

[13] Lawn, J. E., Wilczynska-Ketende, K., Cousens, S. N. (2006). Estimating the cause of 4 million neonatal deaths in the year 2000. Int. J Epidemiol., 2006, 35: 706-718.

[14] Sankar, M. J., Neogi, S. B., Sharma, J., Chauhan, M., Srivasta, R., et al. (2016). State of newborn health in India. Journal of Perinatology, 2016, 36: 53-58. Doi: 10.1038/jp.2016.183. https://www.ncbi.nim.nih.gov/pmc/articles/PMC5144119/.

[15] Ranjeva, S. L., Warf, B. C., Schiff, S. J. (2018). Economic burden of neonatal sepsis in Sub-Saharan Africa. BMJ Glob Health, 2018, 3: e000347. Doi: 10.1136/bmjgh-2017-000347. pmid:http://www.ncbi.nim.nih.gov/pubmed/29564153.

[16] Neonatal mortality -UNICEF DATA data.unicef.org https://data.unicef.org>child survival.

[17] Newborns: improving survival and well-being. (2020). https://www.who.int/news-room/fact-sheets/detail/newborns-reducing-mortality.

[18] Edwards, M. S., Baker, C. J. Sepsis in Newborn In Gershon AA, Hotex PJ, Katz SL. (ed.), Krugman's infectious diseases of children 11th ED. Mosby, Philadelphia, PA 2004; pp. 545-561.

[19] Mathur, N. B., Kahlil, A., Sarkar, R., Puri, R. K. (1991). Mortality in neonatal septicemia with involvement of mother in management. Indian Pediatrics, 1991, 28(11): 1259-62.

[20] Grace Lalana Christopher. (2020). 'Early Neonatal Mortality' Recent Advances in Perinatology with specific reference to ethnicity. 3rd Edition, Published by Dr. Grace Lalana Publication. 2020 https://www.newgenparenting.com.

[21] Polin, R. A. (2012). Committee on fetus and Newborn Management of neonates with suspected or proven early-onset bacterial sepsis. Pediatrics, 2012, 129: 1006-1015. Doi: 10.1542/peds.2012-0541.

[22] Pavan Kumar, D. V., Mohan, J., Rakesh, P. S., Prasad, J., Joseph, L. (2017). Bacteriological profile of neonatal sepsis in a secondary care hospital; in rural; Tamil Nadu, Southern India. J Family Med. Prim Care, 2017, 6: 735-8.

[23] Singer, M., Deutschman, C. S., Seymour, C. W., Shankar-Harris, M., Annane, D., Bauer, M., et al. (2016). The Third international consensus definitions for sepsis and septic shock. JAMA, 2016, 315: 801-10. Doi: 10.1001/jama.2016.0287.

[24] Gordon, S. M., Srinivasan, L., Harris, M. C. (2017). Neonatal meningitis: overcoming challenge in diagnosis, prognosis and treatment with omics. Front Pediatr., 2017, 5: 139. 10.3389/fped.2017.00139.

[25] Zarkesh, M., Sedaghat, F., Heidarzadeh, A., Tabrizi, M., Bolooki-Moghadam, K., Ghesmati, S. (2015). Diagnostic value of I8L-6, CRP, WBC, and absolute neutrophil count to predict serious bacterial infection in febrile infants. Acta Med Iran., 2015, 53: 408-11.

[26] Christopher, P. H., Benjamin, D. K., Becker, K. C., et al. (2012). Use of the complete blood cell count in early-onset neonatal sepsis. The Pediatric infectious disease journal, 2012, 31(8), 799.

[27] Manroe, B. L., Weinberg, A. G., Rosenfeld, C. R., Browne, R. (1979). The neonatal blood count in health and disease. 1. Reference value for neutrophilic count cells. J Pediatr, 1979, 95: 89-98.

[28] Bentitz, W. E., Han, M. Y., Madan, A., Ramachandra, P. (1998). Serial serum C- reactive protein levels in diagnosis of neonatal infection. Pediatrics, 1998, 102, E4110.1542/PEDS.102.4.E41.

[29] Garland, S. M., Bowman. (eds.) (2003). Reappraisal of C-reactive protein as a screening tool for neonatal sepsis. Pathology, 2003, 35: 240-243.

[30] Nuntnarumit, P., Pinkaew, O., Kitiwanwanich, S. (2002). Predictive values of serial C- reactive protein in neonatal sepsis. J Med Assoc Thai., 2002, (85 Suppl 4): S1151-1158.

[31] Fowlie, P. W., Schmidt, B. (1998). Diagnostic tests for bacterial infection from birth to 90 days: a systemic review. Arch Dis Fetal Neonatal Ed., 1998, 78:792-98.

[32] Guibourdenche, J., Bedu, A., Petzold, L., et al. (2002). Biochemical markers of neonatal sepsis: value of procalcitonin in the emergency setting. Ann Clin Biochem., 2002, 39: 130-135.

[33] Monneret, G., Labune, J. M., Isaac, C., Bienvenu, F., Putet, G., Bienvenu, J. (1997). Procalcitonin and C- reactive protein levels in neonatal infection. Act Pediatr., 1997, 86: 209-212. 10.1111/j.1651-2227.1997.tb08870.x.

[34] Kuzniewicz, M. W., Walsh, E. M., Li, S., Fischer, A., et al. (2016). Development and implementation of an early onset sepsis calculator to guide antibiotic management in late preterm and term neonates. JT Comm J Qual Patient Saf., 2016: 42(5): 232-9. PMID: 27066927. https://Neonatalsepsiscalculator.kaiserpermanente.org/.

[35] Rajbhandari, S., La Gamma, E. F. (2017). Early onset sepsis Calculator –risk of delaying treatment. JAMA Pediatr., 2017, 171(10): 1015. Doi:10.1001/jamapediatrics.2017.2476.

[36] Kochi-Lynch, M., et al. (2017). Positive blood culture is defined as gold standard for diagnosis of sepsis. Clinical infectious diseases, 2017, 6; 65(suppl): S190-9.

[37] Hurst, M. K., Yoder, B. A. (1995). Detection of bacteremia in young infants: Is 48 hours adequate? Pediatr. Infect. Dis. J., 1995, 14: 711-3.

[38] Sherris, John C., Ryan, Kenneth J., Ray, C. L. (2004). Sherris medical microbiology; an introduction to infectious diseases. New York: McGraw-Hill. 2004, Principles of Laboratory Diagnosis. ISBN 978-0-8385-8529-0.

[39] Rohner, P., Pepey, B., Auckenthaler, R. (1997). Advantage of combining resin with lytic BACTEC blood culture media. J Clin Microbiol., 1997, 35(10): 2634-2638. PMCID: PMC230024 | PMID:0316921,

[40] Venkatesh, M., Flores, A., Luna, R. A., Versalovic, J. (2010). Molecular microbiological methods in the diagnosis of neonatal sepsis. Expert Rev. Anti Infec. Ther., 2010, 8: 1037-1048. 10.1586/eri.10.89.

[41] Jordan, J. A., Durso, M. B. (2005). Real time polymerase chain reaction for detecting bacterial DNA directly from blood stream of neonates being evaluated for sepsis J. Mol. Diagn., 2005: 7:575-581.10.1016/S1525-1578(10)60590-9.

[42] Shang, S., Chen, G., Wu, Y., Zhao. (2005). Rapid diagnosis of bacterial sepsis with PCR amplification and Microarray Hybridization in 16S r RNA gene. Pediatr Res., 2005, 58: 143-148. 10.1203/01.PDR.0000169580.64191.8B.

[43] Mehr S, Doyle LW. Cytokines as markers of bacterial sepsis in newborn infants; a review. Pediatr Infect Dis J 200019879-887.

[44] Kuster, H., Weiss, M., Willeitner, A. E., et al. (1998). Interleukin -1 Receptor antagonist and interleukin -6 for early diagnosis of neonatal sepsis 2 days before clinical manifestation. Lancet, 1998, 352: 1271-1277.10.1016/S0140-6736 (98)08148-3.

[45] Ng, P. C. Diagnostic markers of infection in neonates. Arch Dis Fetal Neonatal Ed. 200489F229-F235.

[46] Hedegaard, S. S., Wisborg, K., Hvas, A. M. (2015). Diagnostic utility of biomarkers for neonatal sepsis-a systematic review. Infect Dis (Lond), 2015, 47(3): 117-124. Doi: 10.3109/00365548.2014.971053.

[47] Ng, P. C., Cheng, S. H., Chui, K. M., et al. Diagnosis of late onset neonatal sepsis with cytokine, adhesion molecule and C-reactive protein in preterm very low birth weight infants. Arch Dis Fetal Neonatal Ed., 199777F221-F227.

[48] Park, K. H., Yoon, B. H., Shim, S. S., et al. Amniotic fluid necrosis factor-alpha is a marker for the prediction of early onset neonatal sepsis in preterm labor. Gynecol Obstet Invest, 20045884-90.

[49] Berner, R., Tuxen, B., Clad, A., et al. Elevated gene expression of interleukin-8 in cord blood is a sensitive marker for neonatal infection. Eur J Pediatr., 2000159205-210.

[50] Tang, N., Tornatore, P., Weinberger, S. R. (2004). "Current developments in SELDI affinity technology". Mass Spectrometry Reviews, 2004, 23(1): 34-44.

[51] Thayi, S., Paramahamsa, R. (2016). Outcome of newborns born to mothers with prolonged rupture of membrane. Pediatric Review: International Journal of Pediatric Research, 2016, 3(11).

[52] Boskabadi, H., Maamouri, G., Mafinejad, S. (2011). Neonatal complications related with prolonged rupture of membranes. Macedionian Journal of Medical Sciences, 2011, 4: 93-98. doi:10.3889/MJMS.1857-5773.2011.0159.

[53] Laranjo, M., Maia, C. J., Botelho, M. F. (2012). Amniotic membrane: from structure and functions to clinical applications. Cell Tissue Res., 2012, 349(2): 447-458. Doi: 10.1007/s00441-012-1424-6.

[54] Garland, S. M., Ni Chuileannain, F., Satzke, C., et al. (2002). Mechanism, organisms and markers of infection in pregnancy. J Reprod Immunol., 2002, 57: 169-183.

[55] Simonsen, K. A., Anderson-Berry, A. L., Delair, S. F., Davies, H. D. (2014). Early onset neonatal sepsis. Clin Microbiol Rev., 2014, 27(1): 21-47. Doi:10.1128/CMR.00031-13.

[56] Knowles, S. J., O'Sullivan, N. P., A. M. Meenan, Hanniffy, R., Robson, M. (2015). Maternal sepsis incidence, etiology and outcome for mother and fetus: a prospective study. BJOG, 2015, 122(5): 663-71. PMID: 24862293 doi:10.1111/1471-0528.12892.

[57] Puopolo, K. M., Lynfield, R., Cummimgs, J. J., et al. (2019). Management of infants at risk for Group B streptococcal disease. Pediatrics, 2019, 144(2): e20191881. doi:10.1542/peds.2019-1881.

[58] Schrag, S. J., Zywicki, S., Farley, M. M., Reingold, A. L., Harrison, L. H., et al. (2000). Group B streptococcal disease in the era of intrapartum antibiotic prophylaxis. N Engl J Med., 2000, 342: 15-20.

[59] Ibrahim, U., Panti, A., Mohammed, Y., et al. (2012). Risk factors for Group B Streptococcus Colonization and Drugs sensitivity Pattern in Nigerian Obstetric Population. Open Journal of Obstetrics and Gynecology, 2012, 11: 808-814. Doi 10.4236ojog.2021.116074.

[60] Cloouse, K., Shebabi, A., Suleimat, A. M., et al. (2019). High prevalence of Group B Streptococcus colonization among pregnant women in Amman, Jordan, BMC Pregnancy and Children, 2019, 177: 19. https://doi.org/10.1186/s12884-019-2317-4.

[61] Melissa, K. V. D. (2009). Evaluation of universal antenatal screening for Group B streptococcus. The New England Journal of Medicine, 2009, 360: 2626-2636.

[62] Schuchat, A. (2000). Neonatal group B streptococcal disease-screening and prevention. N. Engl. J Med., 2000, 343: 209-210.

[63] Tewari, V. V., Jain, N. (2014). Monotherapy with amikacin or piperacillin-tazobactum empirically in neonates at risk for early-onset sepsis: a randomized controlled trial J. Trop Pediatr., 2014, 60(4): 297-302. 10.1093/tropej/fmu017.