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Early Onset Sepsis Risk Calculator and Vitamin D Level in Newborn Exposed to Premature Rupture of Membranes

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ABSTRACT

Background: Neonatal sepsis is an important cause of morbidity and mortality.
Aim of work: Study the diagnostic value of early-onset sepsis risk calculator (EOSR) and vitamin D as markers for early diagnosis of sepsis among the newborn infants > 34 weeks who were exposed to premature rupture of membrane (PROM).
Patients and Methods: This observational case-control study included 100 newborn infants > 34 weeks of gestation. Newborn infants were assigned into two groups according to the development of sepsis and exposure to PROM: septic and control groups. Kaiser EOSR calculator was used. Vitamin D was estimated by ELISA.
Results: There was a significant increase of EOSR calculator mean values in the septic group than the control group at birth and after clinical examination. The median ± IQR was 10.26 (5.3-24) per 1000/birth at birth in the sepsis group and 0.47(0.39 – 0.67) in the control group. EOSR calculator was 63.2 (25.5 – 142.6) per 1000/births after clinical examination in the septic group and 0.19(0.16 – 0.27) in the control group, P-value was < 0. 001. There was a significant decrease in vitamin D in the septic group (8.57 ± 9.14 ng\L) than the control group (50.61 ± 12.55ng\L), the P-value was <0.001.
Conclusion: Early-onset sepsis risk calculator and vitamin D have high sensitivity and specificity in predicting sepsis among infants exposed to PROM, these may decrease the unnecessary use of prophylactic antibiotics among infants > 34 weeks of gestation. Estimation of vitamin D in neonates with equivocal EOSR calculator score may assist in the early management.

Keywords: Premature rupture of membrane -Early Onset Sepsis Risk Calculator - Vitamin D –Full-term infants –Near term infants - Early-onset neonatal sepsis.

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INTRODUCTION

Early-onset sepsis (EOS) still a worrying and possibly serious disease. Identified tests still lack sensitivity and specificity to manage EOS 1. Maternal risk factors that are known to cause EOS include premature rupture of membranes (PROM), chorioamnionitis, abnormal vaginal discharge, and repeated per-vaginal examinations 2. There is a need for rapid, safe, and accurate methods to estimate the probability of infection among the neonates exposed to PROM. Maternal and infant clinical characteristics can help to identify newborn infants who are at risk of infection and guide the administration of empirical antibiotic therapy. Early-onset sepsis risk score calculator is still not widely used in NICUs, there is inconsistency regarding some items in the score as the lower limit of gestational age and the choice of incidence of sepsis, which may decrease sensitivity to detect cases with positive blood culture. Also, there is a delay in the management of equivocal cases which may aggravate their condition. Vitamin D has been implicated in early immunity and there is evidence that it plays role in PROM and labor, there are epigenetic alterations through DNA methylation in genes that regulate the extracellular matrix reorganization and affect membrane integrity3. PROM increases the risk of ascending infection. Therefore, we adopt the assumptions that a combination of measurement of EOSR score values and vitamin D may assist in the evaluation of newborn infants that might develop sepsis due to exposure to PROM.

Aim of The Work

Study the diagnostic value of early-onset sepsis risk calculator (EOSR) and vitamin D as markers for early diagnosis of sepsis among the newborn infants > 34 weeks who were exposed to premature rupture of membrane for more than 18 hours.

Patients and Methods

Patients

This observational case-control study was performed in the neonatal intensive care unit (NICU) of Itay Al-Baroud shareyah society Hospital from March to September 2020. The study included 100 newborns > 34 weeks of gestation and exposed to PROM >18 hours' infants. They were allocated into two groups: septic group and control group according to the development of EOS.

Septic group: Included 50 newborn infants who fulfilled the inclusion criteria; the history of PROM > 18 hours and more than 34 weeks of gestation and developed early-onset sepsis.

Control group: Included 50 newborn infant with no proven or suspected infection and did not expose to PROM.

METHODS

All newborn infants were subjected to full history taking and clinical examination. Estimation of the EOSR calculator was done at birth and after clinical examination according to Kaiser Method 4.

This interactive calculator generates the probability of early-onset sepsis per 1000 babies. It depends upon specified maternal risk factors along with the infant's clinical presentation. We used the calculated incidence of EOS in our NICU, which was 4/1000 live birth. This score was performed twice, after birth and then after clinical examination. The infants were graded well-appearing, equivocal, or clinical illness. Hospital records were used to determine the incidence of EOS during the previous 6 months to improve the accuracy of the score. The use of antibiotics before and during the study was evaluated.

Laboratory investigations were done after birth and included a complete blood picture (CBC), blood culture, CRP. Vitamin D level was estimated using ELISA, Kit catalog number ER001-1from assay pro company.

Ethical approval

Ethical approval was obtained from the ethical committee of the Faculty of Medicine for girls, ALAzhar University before the study. The number of approvals is 202002160. Informed consent was taken from the parents after explaining the purpose of the study.

Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0**.** The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation, median, and interquartile range (IQR). The significance of the obtained results was judged at the 5% level. Qualitative data were described using numbers and percentages. Chi-square test was used to compare categorical variables, Fisher's Exact test for the countless than 5 cells. The student t-test was used for normally distributed quantitative variables while the Mann Whitney test. Spearman coefficient was used for abnormally quantitative variables. The receiver operating characteristic curve (ROC) is used to detect cutoff values, sensitivity, specificity as well as positive and negative predictive values.

RESULTS

The results are shown in table 1 to 5

There was a significant decrease in gestational age, birth weight, head circumference, and abdominal circumference in the septic group than the control group.

The EOSR calculator mean values increased significantly at birth and after clinical examination in the septic group than the control. EOSR calculator values showed a significant positive correlation with GA and birth weight.

Blood culture was positive among 72% of cases with sepsis**.** Vitamin D decreased significantly in the septic group than the control group. The mean values of vitamin D were not significantly increased among the survival cases**.** Vitamin D showed a significant positive correlation with gestational age and birth weight and a negative correlation with I.T ratio and CRP. The best cut-off points of vitamin D to detect EOS was ≤27 with a sensitivity of 92.0%, a specificity of 98.0%, PPV of 97.9%, NPV of 92.5%, and total accuracy of 99.0%. The best cut off point of EOSR calculator values at birth was >1.23 with a sensitivity of 98%, a specificity of 96%, PPV of 96.1%, NPV of 98%, and total accuracy of 99%, while the best cut off point of EOSR calculator values after clinical examination to detect sepsis was >0.69 with a sensitivity of 100%, a specificity of 98%, PPV of 98%, NPV of 100% and total accuracy of 100%.

The cases with positive blood culture showed a significant increase in total white blood cells (WBC), immature to total neutrophil ratio, CRP as well as EOSR calculator values, but there was a decrease in vitamin D mean values than the negative blood culture cases. There was a significant positive correlation between duration of PROM, values of EOSR calculator at birth, and after clinical examination. Vitamin D showed a significant negative correlation to duration of PROM.

DISCUSSION

The researchers at Kaiser developed a multivariate predictive model to estimate the risk of EOS in infants 34 weeks and older's gestation to reduce the abuse of antibiotics to well-appearing infants who were exposed to PROM 4,5. As we adopt the assumptions that maternal vitamin D deficiency associated with PROM and preterm labor, the combination of measurement of EOSR score and vitamin D status may assist in the evaluation of newborn infants >34 weeks of gestation that might develop sepsis due to exposure to PROM > 18 hours especially among equivocal cases. Hundred newborn infants were included in the study fifty cases with proven sepsis as sepsis group and fifty normal neonates as a control group. Every neonate in both groups was subjected to assessment by EOSR calculator at birth and after clinical examination to segregate the newborn infants and determine their need for the antibiotics; 70% were classified as equivocal,24% as ill as 7 % were normal. The median value of the EOSR calculator was significantly higher in a septic group than in the control group p was < 0.001. The increase may be due to the maternal and infants' conditions as the duration of PROM, heart and respiratory rates as well as a fever that elevate the score among the sepsis cases. These findings were similar to those of Kuzniewicz et al. 6. Moreover, the EOSR calculator median value was increased among the positive blood culture cases. Comparable findings were reported by Sharma et al.**7.**

The sensitivity of the EOSR calculator to correctly identify EOS among newborn infants exposed to PROM was 98% at birth and 100% after clinical examination, while specificity was 96% and 98%. The current study demonstrates that the use of the EOSR score can predict the cases of sepsis and subsequently decrease the number of asymptomatic infants who receive the empiric antibiotic treatment due to their exposure to PROM, however, still the equivocal cases need further assessment to prove if they will develop sepsis or not.

Antibiotic overuse may cause short and long-term morbidity including adverse drug events and antimicrobial resistance especially in developing countries with limited resources 8. Quality improvement intervention by safe use of antibiotics increased patient safety and reduced errors from antibiotics and decrease adverse events9.

The study showed the benefit of the use of Vitamin D to increase the accuracy of early diagnosis of sepsis among infants exposed to PROM. There was a highly significant decrease in vitamin D mean values in a septic group (8.57 ± 9.14ng\L) than the control group (50.61 ± 12.55 ng\L). This finding was supported by Behera et al.10**.** Neonates with vitamin D deficiency are at higher risk for developing sepsis than those with normal vitamin D levels. Vitamin D plays important role in immunity, vitamin D receptors are found nearly in all types of immune cells, including activated CD4+ and CD8+ T cells, B cells, neutrophils, macrophages, and dendritic cells.9**,** 11. These cells affect the innate and adaptive immune reactions to pathogens. It also can modulate immunoglobulin production 12,13.

The values of vitamin D were evaluated with findings of blood culture; blood culture still the gold standard for the diagnosis of neonatal septicemia 14. In the current study, 72% of the septic group had positive blood culture. These results more or less as reported by ELMeneza *et al.*15 Vitamin D was significantly decreased in cases with positive blood culture group P value was < 0.001. Vitamin D is proposed to impede both grams positive and negative bacterial growth and induces the release of antimicrobial proteins in cells of the innate immune system. Vitamin D also prevents direct invasion of pathogenic bacteria by enhancing the clearance of these invading organisms at

sites such as the respiratory tract.16 In contrast to our finding, Say et al. found no significant difference between vitamin D level and blood culture results17.

There was no significant difference between survival and non-survival septic cases regarding the values of the EOSR score or vitamin D. Similarly, a meta-analysis by Deshmukh et al. showed that implementation of sepsis calculator was not associated with a difference in mortality 18. PROM was reported in 100% of septic cases with a mean duration of 85.32 ± 61.2 hours. There was a significant positive correlation between duration of PROM and values of EOSR calculator and negative correlation with mean values of vitamin D. Maternal vitamin D deficiency is associated with PROM that increases the risk of ascending infection, vitamin D has been implicated in epigenetic alterations through DNA methylation in genes that regulate the extracellular matrix reorganization and affect membrane integrity3. Vitamin D-binding protein in cervicovaginal fluid independently predicts intra-amniotic infection and preterm delivery 19. So, neonates exposed to PROM are at greater risk of sepsis due to ascending infection as well as subsequent deficiency of vitamin D, which lower their immunity. Adding evaluation of vitamin D status to values of the EOSR score may improve the prediction of sepsis in neonates exposed to PROM, especially those with equivocal scores.

The current study showed that the use of combined EOSR score (that include objective maternal data with developing valuable neonatal clinical findings) as well as the measurement of vitamin D status in newborn infants may aid in early diagnosis and management of EOS in newborn infants > 34 weeks of gestation and decrease unnecessary use of antibiotics. Safe use of antibiotics will decrease adverse events20.

CONCLUSION

Early-onset sepsis risk calculator and vitamin D have high sensitivity and specificity in predicting sepsis among infants exposed to premature rupture of membranes, these may decrease the unnecessary use of prophylactic antibiotics among infants > 34 weeks of gestation. Estimation of vitamin D during the first day of life in neonates with equivocal EOSR calculator score may assist in the early diagnosis of sepsis and management.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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Table 1: Comparison between the two groups according to demographic data

	Septic group		Control group		Test of Sig.	P-value
	No.	%	No.	%		
Gender						
Male	31	62.0	27	54.0	χ^2=0.657	0.418
Female	19	38.0	23	46.0		
Gestational age (weeks)						
Min. – Max.	35.0 – 39.0		35.0 – 39.0		t=3.366*	0.001*
Mean ± SD.	36.56 ± 1.18		37.34 ± 1.14			
Birth weight (kg)						
Min. – Max.	1.85 – 3.72		2.28 – 3.79		t=3.814*	<0.001*
Mean ± SD.	2.73 ± 0.49		3.05 ± 0.33			
Length (cm)						
Min. – Max.	2.36 – 51.0		42.0 – 52.0		t=1.517	0.134
Mean ± SD.	45.59 ± 7.20		47.22 ± 2.47			
Head circumference(cm)						
Min. – Max.	30.0 – 36.0		32.0 – 36.0		t=3.322*	0.001*
Mean ± SD.	33.93 ± 1.53		34.81 ± 1.08			
Abdominal circumference(cm)						
Min. – Max.	27.0 – 33.0		26.0 – 35.0		t=2.902*	0.005*
Mean ± SD.	29.97 ± 1.43		30.91 ± 1.79			

χ^2: Chi square test t: Student t-test *: Statistically significant at $p \leq 0.05$

Table 2: Comparison between the two groups according to clinical examination and EOSR calculator

	Septic group		Control group		χ^2	P
	No.	%	No.	%		
Clinical examination						
Equivocal	35	70.0	0	0.0	χ^2= 88.679*	<0.001*
Clinical illness	12	24.0	0	0.0		
Well appearing	3	6.0	50	100.0		
EOSR calculator at birth/ risk per 1000 births						
Min. – Max.	1.20 – 85.01		0.21 – 2.30		U=9.0*	<0.001*
Median (IQR)	10.26(5.3 – 24.0)		0.47(0.39 – 0.67)			
EOSR calculator (after clinical examination)/ risk per 1000 births						
Min. – Max.	0.82 – 663.27		0.08 – 0.95		U=1.0*	<0.001*
Median (IQR)	63.21(25.5 – 142.6)		0.19(0.16 – 0.27)			

χ^2: Chi square test U: Mann Whitney test

Table 3: Comparison between the two studied groups according to vitamin D

Vitamin D (ng\L)	Septic group (n = 50)	Control group (n = 50)	U	P-value
Min. – Max.	1.0 – 32.0	27.0 – 78.0	17.0*	<0.001*
Median (IQR)	4.0(3.2 – 9.2)	52.0(38.5 – 57.0)		
	Survival	Non-Survival	P<0.001*	p=0.052
Min-Max	1.0 – 32.0	2.43 – 3.53		
Median (IQR)	4.0(3.26 – 9.60)	2.88(2.65 – 3.20)		
	Male	Female	263.50	0.535
Min-Max	1.0 – 32.0	2.0 – 25.20		
Median (IQR)	3.70	4.50		

U: Mann Whitney test

Table 4: Sensitivity, specificity of vitamin D and EOSR calculator in septic and control group

	AUC	P	95% C.I		Cut off	Sensitivity	Specificity	PPV	NPV
			LL	UL					
Vitamin D (ng\L)	0.993	<0.001*	0.984	1.003	≤27	92.0	98.0	97.9	92.5
EOSR calculator at birth/ risk per 1000 births	0.996	<0.001*	0.990	1.002	>1.23	98.0	96.0	96.1	98.0
EOSR calculator after clinical examination / risk per 1000 births	1.000	<0.001*	1.0	1.0	>0.69	100.0	98.0	98.0	100.0

AUC: Area Under a Curve CI: Confidence Intervals
NPV: Negative predictive value PPV: Positive predictive value

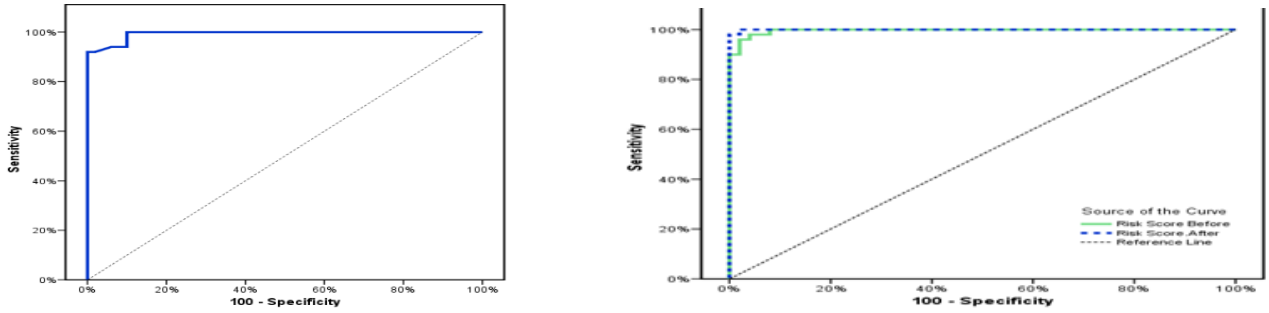

Figure 1:ROC curve for Vitamin D ROC curve for EOSR calculator at birth and after clinical examination

Table 5: Comparison between negative and positive blood culture in sepsis group regarding complete blood count, C-reactive protein, vitamin D and EOSR calculator

P-value > 0.05: Non-significant (NS); P-value <0.05: Significant(S); P-value< 0.01:

		Negative blood culture	Positive blood culture	Test value	P-value	Sig.
		N= 14	**N= 36**			
Decrease platelet count(x10³cmm)	No	9 (64.3%)	15 (41.7%)	2,066*	0.151	NS
	Yes	5 (31.25%)	21 (58.3%)			
Total white blood cells (x10³ cmm)	Mean ± SD	11.53 ± 3.44	16.61 ± 9.64	-3.816•	0.000	HS
	Range	7.5 – 27	3.7 – 35.5			
Immature/total neutrophil ratio	Mean ± SD	0.08 ± 0.07	0.32 ± 0.14	-11.301•	0.000	HS
	Range	0.01 – 0.3	0.03 – 0.72			
C-reactive protein (mg/dl)	Negative	3(21.4%)	0(0.0%)	8.207	0.004	HS
	Positive	11(78.6%)	36(100.0%)			
Vitamin D (ng/l)	Median (IQR)	19.08 (5 –28)	3.65 (3.20 – 5.50)	-3.513ᔕ	0.000	**HS**
	Range	3.22 – 78	1 – 26.4			
EOSR calculator at birth/ risk per 1000 births	Median (IQR)	0.53 (0.4 – 1.23)	15.83 (7.5 – 30.86)	**-4.692 ᔕ**	**0.000**	**HS**
	Range	44 – 0.21	240 – 1.99			
EOSR calculator after clinical examination/ risk per 1000 births	Median (IQR)	0.22 (0.16 – 0.6)	86.25 (40.2 – 192.72)	**-6.725 ᔕ**	**0.000**	**HS**

highly significant(HS)
*: Chi-square test, •: Independent t-test, ᔕ: Mann- Whitney test