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Original Article

Serum Cortisol Level and Monocyte HLA-DR Expression in Late Onset Neonatal Sepsis: A Case-Control Study

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Abstract

Background: An important source of illness and death in the nursery is neonatal sepsis, which is why it is important to understand the background of the condition. The symptoms of neonatal sepsis are often vague and could be caused by other illnesses, making diagnosis difficult. These symptoms include respiratory distress, apnea, poor feeding and hypotension.

Objectives: Investigate the role of serum cortisol levels and expression of Human Leukocyte Antigen-DR on monocytes (mHLADR) in early diagnosis of late onset neonatal sepsis.

Methods: Sixty neonates with a diagnosis of late-onset neonatal sepsis were split into two groups for this case-control study: One group consisted of 30 neonates with definite sepsis, while the other included 30 neonates with probable sepsis and 30 healthy neonates of the same age and sex as a comparison. Flow cytometric evaluation of HLA-DR on monocytes, serum cortisol, C-reactive protein, blood cultures, and complete blood count were all investigated to all groups.

Results: Compared to probable sepsis, the group with definite sepsis had significantly lower mHLA-DR levels and significantly higher cortisol and CRP levels (p-value = 0.0001 for all). Not only that, compared to controls, those with definite or probable sepsis had much higher levels of cortisol and CRP and much lower values of mHLA-DR (p-value = 0.0001 for all).

Conclusion: While cortisol levels and HLA-DR expression on monocytes are useful indicators for the early detection of late-onset neonatal sepsis, they are not sufficient alone for a definitive diagnosis.

Keywords: Neonatal sepsis; Cortisol; Monocyte Expression, HLA-DR; Flow cytometry

Introduction

Pathogenic bacteria can penetrate the blood circulation and assault the reproductive system of neonates during the first month of life, causing systemic signs of infection and hemodynamic abnormalities, which is known as neonatal sepsis [1].

Damage to tissues and organ failure can occur in neonatal sepsis, leading to neurocognitive consequences and death [2].

There are two subtypes of neonatal sepsis, defined by the time of onset: early-onset and late-onset.

Prenatal and perinatal conditions, such as prolonged amniotic membrane rupture, maternal chorioamnionitis, and group B streptococcus (GBS) infection in the mother, are the main causes of early-onset sepsis (EOS), which is identified within the first 72 hours of life.

Hospital-acquired late-onset sepsis (LOS) is most common between days 10 and 22 of life , and it is typically identified after 72 hours of life [3].

Indwelling vascular catheters, prolonged mechanical ventilation, necrotizing enterocolitis, and other medical conditions increase the risk of LOS. A prolonged hospital stay and a poor prognosis are common outcomes for neonates with late-onset sepsis [4]. Because late-onset sepsis typically has non-typical early signs, its delayed diagnosis can lead to septic shock [5].

Reduced organ failure and death can be achieved with early detection of sepsis and subsequent rapid treatment. Problems arise with early identification, though, because the gold standard test—blood cultures—take two to four days to confirm the diagnosis, and neonates who were treated by antibiotics while still in utero may become negative [4, 5].

There is an increasing demand for rapid diagnostic indicators for sepsis because to the fact that antibiotics are often given to neonates who do not actually have septic infections, putting the babies at risk of needless treatment and adding to financial burdens [6].

The expression of cell adhesion molecules on interacting leukocytes increases during bacterial infections; these cell surface antigens are new markers in sepsis diagnosis and may be easily recognized using the advanced technology of flow cytometry [7].

The surface of monocytes, macrophages, dendritic cells, and B cells express HLA-DR. An adaptive immune response relies on it. Immunoparalysis, an anti-inflammatory immune response commonly seen in sepsis, has been linked to a reduction in mHLA-DR molecules [8, 9].

A key component of the anti-inflammatory response elicited by the central nervous system during septic shock is endogenous cortisol [10]. Its ability to reduce mHLA-DR expression has been documented in various in vitro studies and clinical contexts [11].

A greater Basal plasma cortisol level is associated with a higher risk of mortality in patients. Surprisingly, septic shock that is dependent on catecholamines can benefit from low doses of corticosteroids [12].

Subjects and Methods

Subjects

Sixty neonates admitted to the Neonatal Intensive Care Unit (NICU) at Minia University Children's Hospital between October 2023 and April 2024 were studied, with a control group of thirty neonates that seemed to be in good health also included.

Included neonates with late onset neonatal sepsis, defined as the condition detected based on clinical signs of sepsis and laboratory studies, and ranging in age from 4 days to 28 days. Here is how the neonates were categorized: Thirty neonates exhibiting clinical signs of sepsis and a positive blood culture were included in the definite sepsis group (group 1). Group 2: Neonates with clinical signs of sepsis, positive results from two screening parameters, and sterile blood culture. Thirty neonates that appeared to be in good health, with normal laboratory parameters and ages

and sexes that were comparable to the first two groups made up the control group (group 3). We did not include neonates with hypoxic-ischemic encephalopathy, congenital abnormalities, inborn metabolic problems, or those who were aged ≤ 72 hours in this study. We took a thorough history of each neonate, including their prenatal, natal, and postnatal details, as well as the date and circumstances of sepsis start, any risk factors for sepsis, and any symptoms of neonatal sepsis that were detected during the clinical examination. Symptoms include as: sleepiness, elevated body temperature, rapid heart rate, distended abdomen, grunting, increased prefeed aspirate, difficulty suckling, tachypnea, hypothermia, apnea, and bradycardia [13].

Collecting samples

Under complete aseptic precautions, collection of five milliliters of venous blood in the early morning: Pediatric Bactec bottles were inoculated with one milliliter of blood for aerobic blood cultures (only for sepsis groups). two milliliters of blood were drawn into an Ethylene diamine tetra acetic acid (EDTA) tube for complete blood count (CBC) and flow cytometry evaluation of HLA-DR expression on monocytes, and two milliliters of blood were drawn into a plain tube, were allowed to clot before centrifugation and subsequent analysis for serum C-reactive protein (CRP) and serum cortisol.

Neonates thought to have sepsis had their blood collected before antibiotic treatment began.

Laboratory methods

To determine the organisms responsible for the infection, blood cultures are taken using (BD™ BACTEC™ FX40 Automated Blood Culture System, Becton Dickinson, USA). In cases where the cultures were positive, additional subcultures are performed for identification and antibiotic sensitivity test done using (VITEK-2, bioMérieux, France).

Automated cell counter (CELLTAC G, NIHON KOHDEN CORPORATION, Japan) was used to assess complete blood count. Microscopical analysis of Lieshman-stained blood films verified the differential leucocytic count. CRP was measured with a GENRUI, biotech Inc. kinetic assay, China. serum cortisol measured by enzyme-linked immunosorbent assay (ELISA) (Diagnostics Biochem Canada Inc., Canada (Catalog No. CAN-C-270). Within 24 hours of sample collection, the expression of HLA-DR on monocytes was assessed by flow cytometry. Using staining agents. To summarize: We made sure to label both the test tube and the isotypic control tube with the appropriate numbers for each sample. To the tubes, one hundred ul of blood sample was added. Additionally, the test tube was supplemented with 10 μL of an anti HLA-DR FITC conjugated antibody. After incubating both tubes for 15 minutes in the dark at room temperature, they were washed with Phosphate buffered saline (PBS) to remove any unbound antibodies. Next, 2 ml of lysing solution was added to lyse the red cells then incubated for 10 minutes in the dark at room temperature. After centrifugation for 5 minutes, the supernatant was discarded and 2 ml of PBS were added as wash repeated twice. Final flow cytometric analysis required cells were re-suspended in 300 μL of PBS. The BD FACS canto II, U.SA., was used for the analysis. The data processing was done using the Diva software.

Ethical consent

The parents of the neonates were given a thorough explanation of the study and their written agreement was obtained. The research was carried out in accordance with the 1975 Declaration of Helsinki, as amended in 2008, and it was authorized by Minia University's Institutional Review Board and Medical Ethics Committee (Approval number: 864:8:2023, Date of approval: 14 August 2023).

Data analysis using statistical methods

We used Windows version 22 of the Statistical Package for the Social Sciences (SPSS) application. We provided the quantitative results as mean± SD. The qualitative data was displayed using the percentage for frequency distribution. We compared percentages using the Z test and compared means using the Student's sample t test, analysis of variance (ANOVA), and chi-square test. Using receiver operating characteristic (ROC) curve analysis, we found the best cutoff values, how well various markers and scores performed as detectives, and how sensitive and specific they were for detecting late onset neonatal sepsis. For all relevant tests, a cutoff threshold of less than 0.05 was utilized.

Results

In this study, the Definite Sepsis group were 14 males and 16 females, they had a mean gestational age of 37±2.5 weeks and a mean postnatal age of 12.1±4.3 days, and the Probable Sepsis group were 17 males and 13 females, with a mean gestational age of 37.6±2.4 weeks and a mean postnatal age of 10.4±4.08 days. While the Control group were 16 males and 14 females, their mean gestational age was 38.4 ±1.3 weeks and mean postnatal age was 8.5±4.5 days. There were no statistically significant differences between the three groups regarding demographic data (Table 1).

In comparison between neonates with probable and definite sepsis regarding the clinical signs, there was no statistically significant difference in clinical signs, but only significant difference regarding blood culture results.

The isolated organisms were Klebsiella Pneumoniae from 14 neonates (46.6%) and Escherichia coli from 6 neonates (20%), staphylococcus pneumoniae and staphylococcus aureus each from 3 neonates, (10%) for each organism, Methicillin-resistant Staphylococcus aureus (MRSA) from 2 neonates (6.6%) and Pseudomonas aeruginosa was

isolated from only one neonate (3.3%). Neonates with negative blood cultures (n = 30) were considered the probable sepsis group (clinical sepsis) (Table 2).

Regarding hemoglobin level and platelets count, there was statistically significant decrease in hemoglobin level and platelets count in the definite sepsis group when compared to the probable sepsis group and control group and statistically significant decrease in hemoglobin level and platelets count in the probable sepsis group when compared to the control group (P value= 0.0001* for all) and (P value = 0.0001* for all) respectively. While total leucocyte count (TLC), there was statistically significant increase in total leucocytic count in the definite sepsis group when compared to the probable sepsis group and the control group and statistical significant increase in total leucocytic count in the probable sepsis group when compared to the control group (P value = 0.0001* for all) (Table 3).

Concerning blood cells surface markers evaluated by flow cytometry, mHLA-DR percent was statistically significant decreased in definite sepsis group when compared to probable sepsis group and control group and statistical significant decrease in mHLA-DR percent in probable sepsis group when compared to control group (P-value = 0.0001* for all) , while statistically significant increase in cortisol level and CRP level in definite sepsis group when compared to probable sepsis group and control group and statistical significant increase in cortisol level and CRP level in probable sepsis group when compared to control group (P value = 0.0001* for all) and (P-value = 0.0001* for all) respectively (Table 4).

ROC curve analysis for detection of sepsis for comparison of definite to controls revealed that mHLADR at a cut-off value of ≤ 23.5 had AUC (0.99±0.009) with highest sensitivity (96.7%) and specificity (90%), (P value = 0.0001*), while cortisol at a cut-off

value of ≥25 had AUC (1±0.0) with highest both sensitivity and specificity (100%) for both, (P value = 0.0001*) (Table 5 and Figures 1 & 2).

ROC curve analysis for detection of sepsis for comparison of probable to controls revealed that mHLA-DR at a cut-off value of ≤ 42 had AUC (0.83±0.05) with highest sensitivity (80%) and specificity (73.3%%), P 0.0001*), while cortisol at a cut-off value of ≥ 22 had AUC (1±0.0) with highest sensitivity (100%) and specificity (100%), (P-0.0001*) (Table (6) and Figure (3 and 4).

Table (1): Demographic data of included neonates

Variables	Definite sepsis group (n = 30)	Probable sepsis group (n = 30)	Control group (n = 30)	p-value
Gestational age (weeks)				
Range	34 - 39	35 – 40	36 - 39	0.326
Mean±SD	37±2.5	37.6±2.4	38.4 ±1.3	
Postnatal age (days)				
Range	8 – 17	6 – 15	5– 14	0.326
Mean±SD	12.1±4.3	10.4±4.08	8.5±4.5	
Sex:				
Male N (%)	14 (46.6%)	17(56.6%)	16 (53.3%)	0.372
Female N (%)	16 (53.3%)	13 (43.3%)	14 (46.6%)	

*: Significant difference at P value < 0.05

Table (2): Clinical signs of the two sepsis groups and blood culture results of the definite sepsis group

Variables	Definite sepsis group (n = 30) n (%)	Probable sepsis group (n = 30) n (%)	p-value
Tachycardia	8 (26.6 %)	6 (20%)	0.2
Fever	8 (26.6 %)	11 (36.6%)	0.4
Increased prefeed aspirate	13 (43.3 %)	11 (36.6%)	0.3
Abdominal distension	10 (33.3%)	10 (33.3 %)	0.5
Grunting	10 (33.3%)	6(20%)	0.15
Chest retraction	19 (63.3%)	20 (66.6%)	0.4
Hypothermia	11 (36.6%)	5 (16.6%)	0.13
Poor suckling	25 (83.3%)	18 (60%)	0.07
Bradycardia	3 (10%)	2 (6.6%)	0.24
Tachypnea	14 (46.6%)	11(36.6%)	0.22
Apnea	15 (50%)	10 (33.3%)	0.11
Blood culture:			
- Klebsiella	14 (46.6%)	0 (0%)	< 0.0001*
- Escherichia coli	6 (20%)	0 (0%)	0.009*
- Streptococcus pneumoniae	3 (10%)	0 (0%)	0.07
- Staphylococcus aureus	3 (10%)	0 (0%)	0.07
- MRSA	2 (6.6%)	0 (0%)	0.1
- Pseudomonas aeruginosa	1 (3.3%)	0 (0%)	0.3

*: Significant difference at P value < 0.05

Table (3): Comparison of the different studied groups as regard hematological parameters

Data		Definite No=30	Probable No=30	Controls No=30	P	p1	p2	p3
Hemoglobin level(g/dl)	**Range Mean±SD**	7.3-15.5 10.2±2.2	9.7-17 11.9±1.9	12.3-16.6 14.5±1.1	0.0001*	0.0001*	0.0001*	0.0001*
Total leucocytic count ($x10^3$ /µl)	**Range Mean±SD**	6-33.6 18.6±7.05	5-15 10.6±2.4	5-11.1 8.7±1.3	0.0001*	0.0001*	0.0001*	0.0001*
Platelet count ($x10^3$ /µl)	**Range Mean±SD**	50-220 133.7±39.1	90-298 196.1±49.8	210-399 304.8±52.	0.0001*	0.0001*	0.0001*	0.0001*

P = Between all groups, P1=Definite vs controls, p2= probable vs controls, p3= definite vs probable.

*: Significant difference at P value < 0.05

Table (4): Comparison of the different studied groups as regard mHLA-DR, Serum cortisol and CRP

Data		Definite No=30	Probable No=30	Controls No=30	P	p1	p2	p3
mHLADR (%)	**Range**	5-25	10-58	20-76	0.0001*	0.0001*	0.0001*	0.0001*
	Mean±SD	14.2±6.3	33.5±12.9	53.9±15.8				
Cortisol (ug/ dl)	**Range**	30-51	24-43	9-20	0.0001*	0.0001*	0.0001*	0.0001*
	Mean±SD	44.2±5.1	32.6±5.3	14.1±3.6				
CRP (mg/ l)	**Range**	25-75	9-35	1.8-5.9	0.0001*	0.0001*	0.0001*	0.0001*
	Mean±SD	54.3±15.4	19.8±7.02	3.6± 1.2				

P = Between all groups, P1, Definite vs controls, p2= probable vs controls, p3= definite vs probable.
mHLADR: Monocytic human leukocyte antigen–DR ., CRP: C reactive protein

Table (5): AUC, Sensitivity and Specificity of mHLA-DR and Cortisol Level for comparison of definite to controls

Data	AUC	Cutoff	Sensitivity	Specificity	p-value
mHLA-DR	0.99±0.009	≤ 23.5	96.7%	90%	0.0001*
Cortisol	1±0.0	≥ 25	100%	100%	0.0001*

Table (6): AUC, Sensitivity and Specificity of mHLA-DR and Cortisol Level for comparison of probable to controls

Data	AUC	Cutoff	Sensitivity	Specificity	p-value
mHLA-DR	0.83±0.05	≤ 42	80%	73.3%	0.0001*
Cortisol	1±0.0	≥ 22	100%	100%	0.0001*

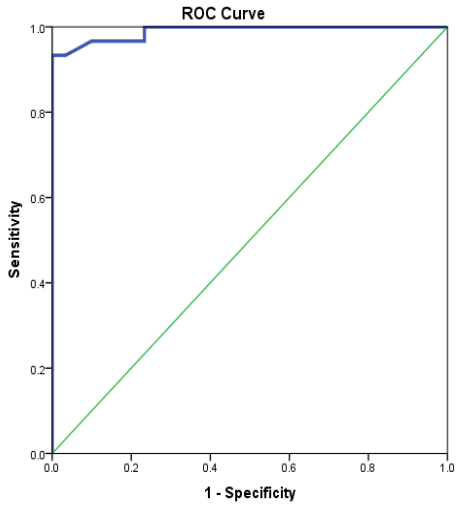

Figure (1): ROC Curve analysis of mHLA-DR for comparison of definite to controls

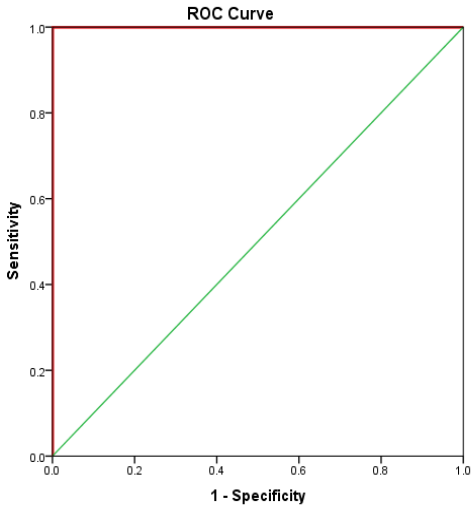

Figure (2): ROC Curve analysis of Cortisol Level for comparison of definite to controls

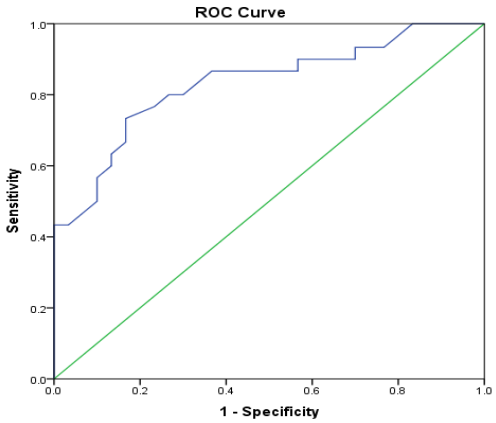

Figure (3): ROC Curve analysis of mHLA-DR for comparison of probable to controls

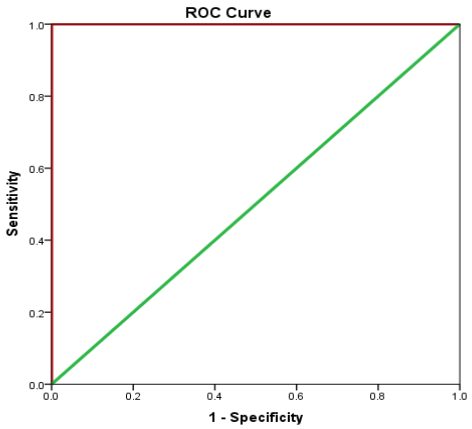

Figure (4): ROC Curve analysis of Cortisol Level for comparison of probable to controls

Discussion

Newborn sepsis diagnosis is still difficult. The signs of sepsis, the frequent sampling. Knowing the right biomarker to help diagnose sepsis is crucial due to the time it takes for blood culture results and the ideal timing of antibiotic treatment. Three groups of neonates were used in this study: one group had definite sepsis based on a positive blood culture, another group had probable sepsis due to the presence of signs of sepsis but negative blood cultures, and the third group was a control group of

healthy neonates. The invading microorganisms released Cytokines that promote inflammation cause the liver to produce acute-phase proteins, such as C-reactive protein (CRP), which is an essential component of the humoral immune response to bacterial invasion [14].Our findings corroborate those of previous studies that found significantly increased CRP levels in neonates with clinically definite sepsis; these studies served as a diagnostic tool for differentiating between healthy neonates and those with definite or probable sepsis [15, 16]. Both the existence and severity of sepsis can be indicated by CRP levels, which were found to be considerably greater in the definite sepsis group compared to the probable sepsis group. The size of the CRP response to sepsis depends on the underlying pathogen [14].

Sensitivity is 60% during sepsis due to the slow initial concentration increase and the need for serial measurements; furthermore, it is not specific because elevated concentrations can be observed in other conditions like tissue necrosis, surgery, recent vaccination, and meconium aspiration [7, 14].

The platelet count was substantially lower in the definite septic group compared to the probable septic group, according to the hematological parameters examined in our study. Consistent with our findings, Omran et al. (2021) discovered a significantly reduced platelet count in both the septic and non-septic groups [17]. Tosson et al., (2021) found no statistically significant change in platelet count between the septic and non-septic groups (P=0.47) [18], which contradicts our findings. Additionally, TLC was found to be considerably greater in the definite septic group compared to the probable -septic group, which is in agreement with the findings of Mubaraki et al., (2023) , who found that TLC was significantly higher in septic group [19].

Omran et al. (2021) and Tosson et al. (2021) disagree with our findings, since

they failed to detect a statistically significant difference between the septic and non-septic groups [17, 18]. The most reliable way to diagnose bacterial sepsis is with a blood culture, but the results can take a long time due to factors including the low bacteremia that is typically expected and the fact that antibiotic therapy is commonly started before the blood culture is taken. And to get the most out of the blood culture, it's best to take it out as the temperature is rising, according to hospital regulations and guidelines [20]. Thirty neonates, who did not show signs of blood culture growth, were included in this investigation. Although thirty neonates showed positive results in the blood culture, Klebsiella (46.6%), Escherichia coli (20%), streptococcus pneumoniae (10%), staphylococcus aureus (10%), methicillin-resistant Staphylococcus aureus (6.6%), and Pseudomonas aeruginosa (3.3%) were the most frequently found microbes in blood cultures. Among the gram-negative bacteria isolated, klebsiella pneumoniae accounted for nearly half (46.6%). These findings corroborated those of with Elmashad et al. (2019) [21] and Ramavath et al., (2023) [22]. Our findings are at odds with those of Tosson et al. (2021) who reported that MRSA was the most frequently isolated bacterium in their cases [18]. The majority of LOS pathogens were Coagulase-negative staphylococci (CONS), according to other researchers like Hammoud et al., 2017 [23]. There is a wide range of organism findings from one NICU to another and from one region to another, thus it is necessary for each hospital to modify its antibiotic policy accordingly. It is still challenging to diagnose sepsis at an early stage; so, new indicators for neonatal sepsis are needed to improve diagnostic sensitivity and specificity and, frequently, to track treatment progress. However, at this time, no biomarker exists that can distinguish between systemic inflammation and sepsis.

When diagnosing sepsis, flow cytometry can be useful. You only need a small amount of blood, and you'll see effects quickly. We can start treating patients based on their immune systems, which are evaluated by flow cytometric expression of cell surface markers [24]. There was a strong correlation between mHLA-DR and the likelihood of sepsis in this investigation. In the peripheral blood of neonates with septic shock, Winkler et al. (2017) found a greater number of monocytes but decreased expression of HLA-DR [25]. In neonates with sepsis, Genel et al. (2010) found a decreased HLA-DR level that had predictive value [26]. In contrast, HLA-DR expression on monocytes did not differ significantly across infected, non-infected, and control groups in a study by Ng et al. (2006) [27].

Das et al,. (2002) also discovered that neonates with sepsis had a higher mean serum cortisol level. This could be because sepsis causes a marked increase in cortisol production by the adrenal cortex, which is driven centrally [28]. The cortisol level was also significantly higher in the definite sepsis group compared to the probable sepsis group. An increase in cortisol levels was also found in neonatal septic shock by Bhat et al., (2022) [29].

The ROC curve for mHLA-DR demonstrated a high level of sensitivity and specificity with an area under curve of 0.99±0.009 and 0.83±0.05, respectively. In contrast, the ROC curve for cortisol showed an area under curve of (1±0.0) and a high level of sensitivity and specificity for both confirmed and suspected cases compared to controls (P = 0.0001*). Therefore, it would be more effective to combine the two tests when diagnosing late-onset neonatal sepsis.

Limitation: This study does have a few caveats. Some examples include a bigger sample size, continuous monitoring of cortisol levels, and HLA-DR expression on monocytes as the disease progress.

Conclusion

Serum cortisol levels rise and monocyte HLA-DR expression in blood decreases in cases of definite neonatal sepsis compared to those of probable neonatal sepsis, these markers are more sensitive and specific than C-reactive protein (CRP) when used to diagnose late-onset neonatal sepsis. Early detection of late-onset neonatal sepsis can improve outcomes and shorten neonatal hospital stays by allowing for the prompt initiation of appropriate treatment.

Data Availability

The datasets used and/or analyzed during this study available from the corresponding author on reasonable request.

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Author's contributions

Everyone who wrote the paper had a hand in coming up with the idea, carrying it out, and revising the final product. Data gathering and manuscript draught preparation were shared responsibilities among EM, NI, and MM. NI and MM interpreted the results of the laboratory work. EE analyzed the results statistically. After reviewing the final manuscript, all authors gave their approval.

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Conflict of interest

We declared no conflict of interest concerning the study.

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References

1. Balayan S, Chauhan N, Chandra R, Kuchhal NK and Jain U. Recent advances in developing biosensing based platforms for neonatal sepsis. *Biosens Bioelectron.* 2020; 169:112552. doi: 10.1016/j.bios.2020.112552
2. Liang LD, Kotadia N, English L, Kissoon N, Ansermino JM, Kabakyenga J, et al. Predictors of mortality in neonates and infants hospitalized with sepsis or serious infections in developing countries: a

systematic review. *Front Pediatr*. 2018; 6:277. doi: 10.3389/fped.2018.00277

3. Dong Y and Speer CP. Late-onset neonatal sepsis: recent developments. *Arch Dis Child Fetal Neonatal Ed*. 2015; 100: F257–263. doi: 10.1136/archdischild-2014-306213
4. Greenberg RG, Kandefer S, Do BT, Smith PB, Stoll BJ, Bell EF, et al. Late-onset sepsis in extremely premature infants: 2000–2011. *Pediatr Infect Dis J*. 2017; 36:774–779.
5. Liu Z, Feng J and Shi L. Clinical analysis and examination of neonatal sepsis. *J Pak Med Assoc*. 2020; 70:120–124.
6. De Rose D U , Ronchetti M P, Santisi A, Bernaschi P, Martini L, Porzio O, Dotta A and Auriti C . Stop in Time: How to Reduce Unnecessary Antibiotics in Newborns with Late-Onset Sepsis in Neonatal Intensive Care. Trop Med Infect Dis.; 2024:19;9(3): 63. doi: 10.3390/tropicalmed9030063.
7. Aydin M, Barut S, Akbulut HH, Ucar S, Orman A et al. Application of fow cytometry in the early diagnosis of neonatal sepsis. Ann Clin Lab Sci. 2017; 47(2):184–190.
8. Pradhan R, Jain P, Paria A , Saha A, Sahoo J, Sen A et al . Ratio of neutrophilic CD64 and monocytic HLA-DR: a novel parameter in diagnosis and prognostication of neonatal sepsis. Cytom Part B Clin Cy 2016; 90(3):295–302.
9. I, Walter P Carney and Edwin P Rock .Utility of monocyte HLA-DR and rationale for therapeutic GM-CSF in sepsis immunoparalysis. Front Immunol. 2023 Feb 7; 14:1130214. doi: 10.3389/ fimmu.2023. 1130214
10. GladnessD. Nethathe , Jeffrey Lipman , Ron ald Anderson , Peter J. Fuller et al.. Glucocorticoids with or without fludrocortisone in septic shock: a narrative review from a biochemical and molecular perspective] British Journal of Anaesthesia . 2024; 132 (1): 53-65.
11. Yves Le Tulzo 1, Celine Pangault, Laurence Amiot, Valérie Guilloux, Olivier Tribut, et al. Monocyte Human Leukocyte Antigen–DR Transcriptional Downregulation by Cortisol during Septic Shock .American Journal of Respiratory and Critical Care Medicine.2004; 169(10):1144-1151.
12. Ioannis Ilias, Alice G. Vassiliou, Chrysi Keskinidou, Charikleia S. Vrettou, Stylianos Orfanos, Anastasia Kotanidou et al. Changes in Cortisol Secretion and Corticosteroid Receptors in COVID-19 and Non COVID-19 Critically Ill Patients with Sepsis/Septic Shock and Scope for Treatment. Biomedicines 2023, 11(7): 1801

13. Kar SS, Dube R, Mahapatro S and Kar SS. The Role of Clinical Signs in the Diagnosis of Late-onset Neonatal Sepsis and Formulation of Clinical Score. Indian Journal of Clinical Practice. 2013; 23: 654-660.
14. Hofer N, Zacharias E, Müller W and Resch B. An update on the use of C reactive protein in early-onset neonatal sepsis: current new tasks. insights Neonatology. 2012; 102(1):25–36.
15. Hotoura E, Giapros V, Kostoula A, Spyrou P and Andronikou S. Pre inflammatory mediators and lymphocyte subpopulations in preterm neonates with sepsis. Inflammation. 2012: 35(3):1094-1101.
16. Kurt A, Aygun A, Godekmerdan A, Kurt A, Dogan Y, Yilmaz E . Serum IL-1β, IL-6, IL-8, and TNF-α levels in early diagnosis and management of neonatal sepsis. Mediators of Inflammation. 2007: 7:31397. doi: 10.1155/2007/31397.
17. Omran A, Sobh H, Abdalla MO, El-Sharkawy S, Rezk AR and Khashana A. Salivary and Serum Interleukin-10, C-Reactive Protein, Mean Platelet Volume, and CRP/MPV Ratio in the Diagnosis of Late-Onset Neonatal Sepsis in Term Neonates. Journal of Immunology Research. 2021; 2021:4884537.
18. Tosson A, Koptan D, Aal RA and Elhady MA. Evaluation of serum and salivary C-reactive protein for diagnosis of late-onset neonatal sepsis: a single center cross-sectional study. Jornal de Pediatria. 2021; 97:623-628.
19. Mubaraki MA, Faqihi A, AlQhtani F, Hafiz TA, Alalhareth A, Thagfan FA, et al. Blood Biomarkers of Neonatal Sepsis with Special Emphasis on the Monocyte Distribution Width Value as an Early Sepsis Index. Medicina. 2023;59(8):1425.
20. Mancini N, Carletti S, Ghidoli N, Cichero P, Burioni R and Clementi M. The Era of Molecular and Other Non-Culture-Based Methods in Diagnosis of Sepsis. Clin Microbiol Rev2010 Jan;23(1):235–251. doi: 10.1128/CMR.00043-09
21. Elmashad GM, Elsayed HM, Omar ZA, Badr EA and Omran OM. Evaluation of serum amyloid A protein as a marker in neonatal sepsis. Menoufia Medical Journal. 2019;32(3):1094-1098.
22. Ramavath C, Katam SK, Vardhelli V, Deshabhotla S and Oleti TP. Examining the Utility of Rapid Salivary C-Reactive Protein as a Predictor for Neonatal Sepsis: An Analytical Cross-Sectional Pilot Study. Diagnostics. 2023;13(5):867.

23. Hammoud MS, Al-Taiar A, Al-Abdi SY, Bozaid H, Khan A, AlMuhairi LM, et al. Late-onset neonatal sepsis in Arab states in the Gulf region: two-year prospective study. International Journal of Infectious Diseases. 2017; 55:125-130.
24. Mahmoodpoor A, Paknezhad S, Shadvar K, Hamishehkar H, Movassaghpour A, Sanaie S et al . Flow cytometry of CD64, HLA-DR, CD25, and TLRs for diagnosis and prognosis of sepsis in critically ill patients admitted toS the intensive care unit: a review article. Anesth Pain Med.2018; 8(6): e83128.
25. Winkler MS, Rissiek A, Priefler M, Schwedhelm E, Robbe L, Bauer A, Zahrte C et al., Human leucocyte antigen (HLA-DR) gene expression is reduced in sepsis and correlates with impaired TNFα response: a diagnostic tool for immunosuppression? PLoS One. 2017;12(8): e0182427 27.
26. Genel F, Atlihan F, Ozsu E and , Ozbek E. Monocyte HLA-DR expression as predictor of poor outcome in neonates with late onset neonatal sepsis. J Infection. 2010; 60(3):224–228.
27. Ng PC and Lam HS. Diagnostic markers for neonatal sepsis. Curr Opin Pediatr .2006;18(2):125–131.
28. Das BK, Agarwal PK, Agarwal J and Mishra OP: Serum cortisol and thyroid hormone levels in Neonates with sepsis . Indian J Pediatr. 2002; 69 (8): 663 – 665.
29. Bhat V, Saini SS, Sachdeva N, Walia R, Sundaram V and Dutta S. Adrenocortical Dysfunctions in Neonatal Septic Shock. Indian J Pediatr; 2022 ;89(7):714-716. DOI: 10.1007/s12098-021-03955-7.

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