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Original article

Diagnostic Value of Interleukin-33 in Neonatal Sepsis

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Abstract

Background: Most infant deaths are attributed to neonatal sepsis. Reduced morbidity and mortality can be achieved with prompt diagnosis. Due to the disease's rapid course, accurate utilization of disease-specific biomarkers is crucial for timely diagnosis and treatment. **Aim and objective:** This study aimed to find out serum IL33 levels in neonates with sepsis and to investigate its role in the identifying of neonatal sepsis. **Methods:** Case-control research was performed on 45 cases diagnosed with neonatal sepsis with positive blood cultures and 45 healthy neonates as controls. A peripheral blood sample was taken from the patients and controls then IL33 levels were measured by ELISA. **Results:** In newborn sepsis individuals, serum IL 33 levels were significantly greater than in healthy controls (203.9±63.1vs.127.4±30.5 ng/ml, respectively: p less than 0.001). Serum IL33 levels were significantly positively correlated with CRP levels in neonatal sepsis patients. IL33 is increased in survivor than in non-survivor cases**. Conclusion:** Elevated levels of interleukin 33 strongly correlate with neonatal sepsis so it might function as a reliable a diagnostic indicator for neonatal sepsis.

1. Introduction:

Neonatal sepsis is a clinical illness in infants within the first month of life characterized by hemodynamic abnormalities along with additional systemic clinical manifestations caused by the presence of pathogenic microorganisms (bacteria, viruses, or fungi) in otherwise sterile fluid, such as blood or cerebrospinal fluid (CSF) [1].

Neonatal Sepsis is a major determinant of neurocognitive sequelae [2] and remains one of the primary reasons for morbidity & mortality in newborns [3].

There are an assessed three million cases of neonatal sepsis every year, or 2202 cases for every 100.000 live births with a fatality rate of eleven percent to nineteen percent. [4].

Recognition of new biomarkers for the detection of neonatal sepsis is of great importance since early diagnosis of neonatal sepsis improves prognosis [5].

IL-33 relates to the IL-1cytokine family. T helper cells, eosinophils mast cells & basophils can all be stimulated to create Th2 cytokines by IL-33. Through interactions with its receptors and associated proteins, which are highly expressed on the surfaces of Th2 cells and also mast cells, IL-33 mediates its biological effects. [6]

Circulating IL-33 level was highly elevated in neonates with early onset sepsis compared with uninfected neonates [7] The purpose of this research was to evaluate serum IL33 levels in neonates with sepsis & to investigate its role in the diagnosis of neonatal sepsis.

2. Patients and Methods:

This was a prospective case-control trial was conducted which performed at the Neonatal intensive care unit at Beni-Suef University Hospital. The study was performed started from March 2021 till December 2021

Inclusion Criteria: Children who met the following diagnostic criteria for newborn sepsis were considered for inclusion in the study. A positive blood culture along with other clinical & laboratory evidence of sickness is diagnostic of neonatal sepsis [1]. Sepsis symptoms contain [8]: Respiratory distress or apnea, tachycardia or bradycardia, hypothermia or hyperthermia, arterial hypotension and/or poor perfusion, floppy infant, seizure, irritability, or lethargy & vomiting or feeding intolerance or ileus

Exclusion Criteria: Congenital infection, chromosomal abnormality, perinatal asphyxia, an inborn error of metabolism, congenital anomalies, liver diseases or renal failure, and malignant tumor or drug toxicity.

All cases were subjected to the following:

Full history taking including Maternal infection during gestation or parturition, Gestational age, multiple births, duration of rupture of membranes, complicated delivery, and medical intervention including endotracheal intubation, parenteral nutrition, or surgery.

Thorough clinical examination including Birth weight**,** vital signs (heart rate, respiratory rate, temperature & blood pressure), neonatal reflexes e.g. Moro and

suckling, and full examination of all systems (cardiac, respiratory, neurological, and abdominal).

Laboratory investigations including Complete blood count (CBC): was accomplished on an automated cell counter (colter), with the differential count done on Leishman-Geimsa stained peripheral blood film, C-reactive protein (CRP): employing a latex agglutination test and blood culture was obtained as routine in all cases. Measurement of Serum interleukin-33 level in the obtained sample: about (1-3ml) of blood was obtained in a plain tube to separate serum & stored immediately in plastic tube at -20°c for measurement of Serum interleukin-33 level by using an enzyme-linked immunosorbent assay (ELISA) kit catalog number SG-10295 according to the manufacturer instructions with Detection range: 28ng/L-900 ng/L. The unopened kit was stored at [2-8 °C], while the opened kit was held at [2-8 °C] for a maximum of one month.

Ethical Considerations: The study was explained to the parents. After obtaining approval from the Local Ethical Committee, written informed consent was obtained from the parents of the individuals participating in the investigation.

Approval No: FMBSUREC/06042021/Farag

Statistical Analysis: The data was analyzed using version 26 of the statistical package for social science (SPSS) for Windows. Regarding categorical variables, the two groups were compared using Chi-squared or Fisher exact. The T-test was utilized to compare cases and controls or Mann Whitney regarding scale variables according to their normality distribution. Spearman nonparametric correlation was used to correlate IL33 and other scale variables**.**

3. Results:

This case-control trial was done in order to assess interleukin33 (IL33) as a biomarker for neonatal sepsis. This study included 90 participants obtained from the NICU of Beni-Suef university hospital. They were divided into two groups, 45 cases diagnosed with neonatal sepsis & 45 healthy controls.

Table (1) Groups' demographic information:

Items		**Cases (n=45)**	**Controls(n=45)**	**P-value**
Gestational age in weeks		35.8±2.6(30-37)	37±3.2 (34-40)	0.054
Sex	**Females**	15(33.3%)	25(55.6%)	0.217
	Males	30(66.7%)	20(44.4%)	
Mode of delivery	**CS**	36(80.0%)	42(93.3%)	0.140
	NVD	9(20%)	3(6.7%)	

P-value is significant

The gestational age of the neonatal sepsis patients ranged from (30-37) weeks while the gestational age of the controls (34-40) weeks with no significant variance amongst the 2 groups. The neonatal sepsis group included 30 (66.7%) males and 15 (33.3%) females while the controls group consisted of 20 (44.4 %) males in addition 25 (55.6 %) females with no significant change among the 2 groups. The mode of delivery in 80% of neonatal sepsis patients was cesarean section while 93.3 % of controls were delivered by cesarean section with no significant difference amongst the 2 groups.

There was a significant alteration among the cases with sepsis and controls regarding their respiratory rate, as it was higher in cases with sepsis than in controls (51.1±8.9) per minute, and (45.5±10.1) per minute respectively with a p-value (0.007).

Table (2): Comparison among the researched groups regarding the laboratory parameters

Items	Cases (no=45)	Controls (no=45)	P-value
Hb(gm/dl)	12.3±2.6(7-19.5)	14±2.19-18.2)	0.001*
TLC X10^3(cell/mm^3)	13.1±9.7(4.1-62)	9.8±4.6(2-30)	0.043*
PLT X10^3(cell/mm^3)	285.4±201.6(36-885)	287.8±107.9(97-550)	0.942
CRP(mg/dl)	66.8±45.1 (median=60)	3.4±1.1	<0.001*

P-value is significant

There was a statistically significant change amongst sepsis cases & controls regarding their total leukocytic count which was higher in cases (13.1±9.7) than in control (9.8±4.6) with a p-value (of 0.043). There was also, a statistically significant difference between sepsis cases and controls regarding hemoglobin level which was lower in cases (12.3±2.) than in control (14±2.19) with a p-value (0.001) CRP levels were significantly greater in neonatal sepsis individuals than in controls with (66.8±45.1) in neonatal sepsis patients and (3.4±1.1) in controls with a p-value (<0.001). No significant difference amongst the 2 studied groups regarding platelet counts with a p-value (0.942).

Table (3) Comparison amongst the examined groups regarding the IL33:

Items	Cases (no=45)	Controls (no=45)	P-value
IL33(ng/ml)	203.9±63.1 (11.9-504.3)	127.4±30.5 (40.3-170)	<0.001*

P-value is significant

This table showed a Comparison amongst the investigated groups regarding the IL33 & show that the level of IL33 was significantly developed in cases with sepsis (203.9±63.1) than in controls (127.4±30.5) with p-value <0.001.

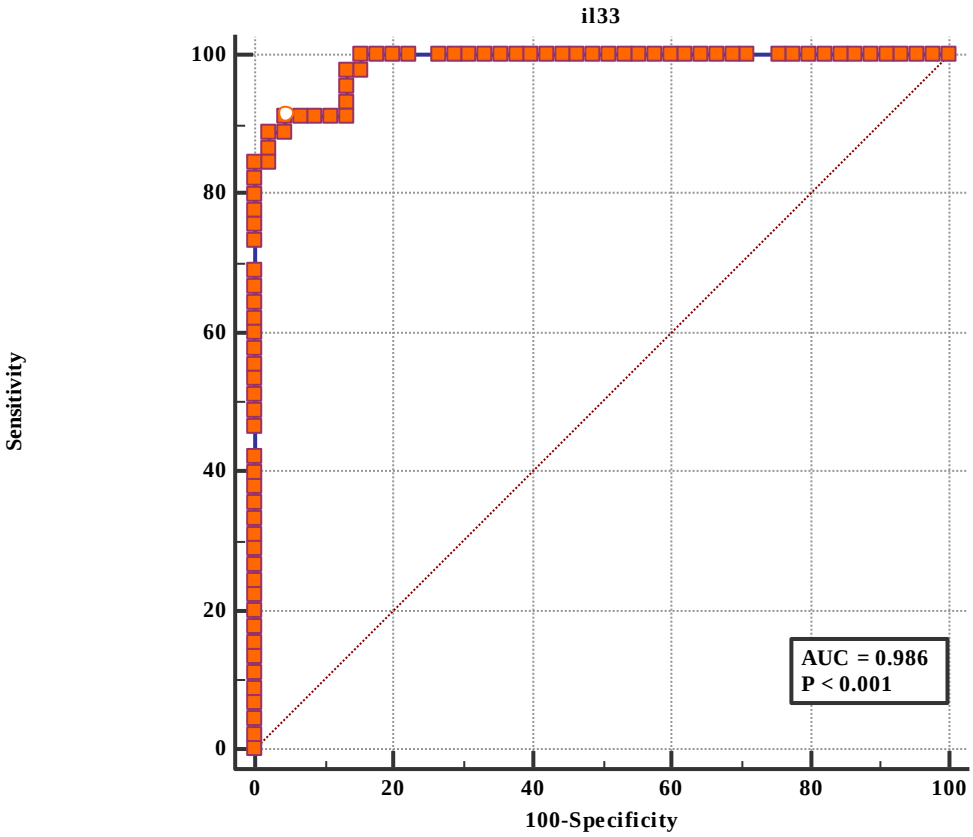

Figure (2) ROC curve analysis for prediction of Sepsis from IL33, showed that there was a significant role of IL33 in the estimation of sepsis at a cut-off 163.8 with Sensitivity 91.11%, Specificity 95.6%, PPV 95.3%, and NPV 91.5%.

Table (4) Correlation between the IL33 level and patients' parameters:

Spearman's rho	IL33	
Age	Correlation Coefficient (r)	.089
	P-value	.563
Weight	Correlation Coefficient (r)	.079
	P-value	.608
Hemoglobin	Correlation Coefficient (r)	.081
	P-value	.596
Total leucocytic count	Correlation Coefficient (r)	.169
	P-value	.268
Platelets	Correlation Coefficient (r)	-.058
	P-value	.703
CRP	Correlation Coefficient (r)	.373
	P-value	.012

IL33,interleukin 33, CRP,C-reactive protein.

The table showed that there was a significant linear positive moderate Connections among the IL33 level and CRP level in cases with sepsis (p-value 0.012).

Table (5) Association between the IL33 level and maturity, type of sepsis, and outcome in the sepsis group

Groups		N	Mean IL33	Std. Deviation	P-value
Prematurity	**FT**	29	195.197	38.6453	0.670
	PT	16	219.738	91.9886	
Type of sepsis	**EOS**	15	190.313	34.5420	0.311
	LOS	30	210.727	72.8920	
Mortality	**Survivors**	29	216.900	74.2929	0.010*
	Nonsurvivors	16	180.400	21.0936	

P-value is significant** ***MW: Mann Whitney U test

As shown in the previous table: Serum IL33 levels were not significantly different in different types of sepsis, between preterm and full-term while there was a significant association between discharge from NICU and a higher level of IL33 with a p-value (0.010).

4. Discussion:

Interleukin (IL)-33 is a cytokine that belongs to the IL-1 family. It has strong immunomodulatory effects, including the induction of eosinophil degranulation and maturation, the promotion of macrophage in addition neutrophil migration & the activation of Th2-related immune responses. **[9].**

The purpose of this research was to evaluate serum IL33's diagnostic utility in patients with newborn sepsis. The current study was carried out on 90 neonates, 45 of them have clinical pictures suggesting neonatal sepsis with positive acute phase reactant and positive blood cultures (confirmed cases). It was found that prematurity is the leading cause of neonatal sepsis 15 cases (33.3%) Oxygen delivery mechanism detected that most of the cases were on nasal oxygen (44.4%) followed by CPAP 26.7%and mechanical ventilation (13.3%).

Late-onset sepsis (LOS) was more common than early-onset sepsis (EOS) (66.7% and 24.4% respectively).

Shehab El-Din et al., [10] also reported that LOS was more common than EOS (55.8% and 44.2% respectively) in their study in Mansoura Egypt. In another study being performed in NICUs of the Children's Hospital of Ain Shams University & that of El-Hussein Hospital, Al-Azhar University, Egypt, early/late onset sepsis accounted for 35.4 & 64.6 percent, respectively **[11].** It was also in consensus with **Aydemir et al., [12]** who informed that (52.0%) of the cases were detected with LOS, whereas (48%) of the newborns were diagnosed with EOS. However, the opposite was documented by **Paul et al., [13]** EOS was observed more (65%) than LOS (35%).

Our results presented that neonatal sepsis was more common in males than females (66.7% and 33.3% respectively). These outcomes are in agreement with a research by **Ebrahem et al., [14]** who demonstrated that the percentage of neonate males was 68.8% while females were 31.1% and also agreed with a study by **Noha et al., [15]** who showed that The majority of those examined were male (65.5%). In addition, our findings concurred with **Jemal et al.'s [17]** finding that 53.5% of patients were males. Specific pathogenic variations have unknown causes as well as their underlying mechanisms are obscure & inadequately understood. It is influenced by genetic, immune in addition to hormonal factors, making it potentially complex.

There was a significant variance amongst documented sepsis group & the control group regarding weight. The mean weight of our cases was (2.3±0.6) kg and (2.6±0.6) for the control group. This result agreed with a trial by **Paul et al., [13]** who initiated that (77.5%) of neonates with sepsis had low birth weight (<2500 g) and out of these (30%) had very low birth weight (<1500g). And also agreed with a study by **Shehab El-Din et al., [10]** who found that (69.7%) of neonates with sepsis had low birth weights (<2500 g).

In the present research, TLC was significantly higher in cases than in controls with a p-value (0.043) while Hb was significantly decreased in cases than in controls with a p-value (0.001) and there was a mild difference regarding platelet levels, but it was non-significant with P value (0.942) between the two groups. This is in agreement with the trial by **Ebrahem et al., [14]** showed a significantly increasing mean of WBC count in the sepsis cases group as opposed to the control group (13.61 ± 0.68 versus 8.54 ± 0.39 x103/mm3, respectively). While the outcomes of the platelets count mean were not significantly reduced in the sepsis individuals group equated to the control group (257.98 ± 15.16 versus 269.51 ± 9.46 x109/L). This finding is also consistent with another investigation conducted in **Baghdad by Abood [18],** who found a variation in the mean WBCs count between sepsis patients and healthy controls. In addition, **Yang et al. [19]** found that the mean WBC count was higher in the sepsis group.

The outcomes of the present trial showed that serum IL33 levels were significantly higher in neonatal sepsis individuals than in controls (203.9±63.1 ng/ml&127.4±30.5 ng/dl respectively) with a significant P value <0.001. we also found that there was a significant role of IL33 in the prediction of sepsis at a cut off more than 163.8 ng/dl with Sensitivity 91.11%, Specificity 95.6%, PPV 95.3%, and NPV 91.5%. This agrees with the study by **Ebrahem et al., [14]** detected the mean of IL-33 in the sepsis neonates group compared to the control group (250.28 ± 15.75 versus 101.16 ± 3.38 pg/ml). Our findings were consistent with those of **Halil et al.'s [17]** study on septic neonates in Turkey, which

found a sharp rise in IL-33 on the first day after diagnosis, followed by a continuous decline over time (days three as well as seven). Our study concurred with **Yang et al.'s [19]** comparison of the EOS group to the non-EOS group, which revealed substantially higher median levels of IL-33 in the EOS group.

Our study showed statistically significant increased serum levels of IL33 between survivor neonates with sepsis relative to the non-survivor group.

This result agrees with the study by **Nascimento et al., [20]** in adults with sepsis IL33 is elevated among survivors with sepsis relative to the non-survivor group. And pointed out that targeting IL-33 could be an effective treatment for immunosuppression caused by sepsis. To determine the function of IL33 in the treatment of neonatal sepsis, more research is required.

The relatively small sample size in this single-center study and just a single measurement of IL 33 levels are the main study's limitations, but its key strength is that we only included neonates with sepsis that were confirmed by blood culture.

5. Conclusion:

Serum IL33 may be used as a promising biomarker for the diagnosis of neonatal sepsis.

6. References:

1. Shane AI, Sanchez PJ & Stoll BJ. Neonatal sepsis. Lancet, 2017; 390:1770-80.
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.
3. Mersha A, Worku T, Shibiru S, Bante A, Molla A. Neonatal sepsis and associated factors among newborns in hospitals of Wolaita Sodo Town, Southern Ethiopia. Research and Reports in Neonatology, 2019; 9:1.
4. Fleischmann-Struzek C, Goldfarb DM, Schlattmann P, Schlapbach LJ, Reinhart K, Kissoon N. The global burden of pediatric and neonatal sepsis: a systematic review. Lancet Respir Med., 2018; 6:223–30.
5. Rao L, Song Z, Yu X, Tu Q, He Y, Luo Y, Yin Y Chen D. Progranulin as a novel biomarker in diagnosis of early-onset neonatal sepsis. Cytokine. 2020 Apr 1; 128:155000.
6. Machado JR, Soave DF, da Silva MV. Neonatal sepsis and inflammatory mediators. Mediators Inflamm. 2014; 4:269681.
7. Halil H, Tayman C, Buyuktiryaki M, Okur N, Cakır U, Serkant U. Serum Interleukin-33 as a Biomarker in Predicting Neonatal

Sepsis in Premature Infants. Combinatorial Chemistry & High Throughput Screening, 2018; 21(7), 510-515.

8. Stocker M, Van Herk W, El Helou S, Dutta S, Fontana MS, et al., Procalcitonin-guided decision making for duration of antibiotic therapy in neonates with suspected early-onset sepsis: a multicentre, randomised controlled trial (NeoPIns). The Lancet. 2017 Aug 26; 390(10097):871-81.
9. Zhu JF, Xu Y, Zhao J, Li X, Meng X, Wang TQ. IL-33 protects mice against DSS-induced chronic colitis by increasing both regulatory B cell and regulatory T cell responses as well as decreasing Th17 cell response. J Immunol Res. 2018:1827901.
10. Shehab El-Din EM, El-Sokkary MM, Bassiouny MR, Hassan R. Epidemiology of Neonatal Sepsis and. Implicated Pathogens: A Study from Egypt. BioMed Research International, 2015; 1-11. 11.
11. Awad HA, Mohamed MH, Badran NF, Mohsen M, Abd-Elrhman AS. Multidrug-resistant organisms in neonatal sepsis in two tertiary neonatal ICUs, Egypt. Journal of the Egyptian Public Health Association, 2016; 91(1):31-8.
12. Aydemir C, Aydemir H, Kokturk F, Kulah C, Mungan AG. The cut-off levels of procalcitonin and C-reactive protein and the kinetics of mean platelet volume in preterm neonates with sepsis. BMC Pediatr. 2018; 18(1):253.
13. Paul D, IbneSattar A, Roy C. Role of neutrophil CD64 as an emerging biomarker in the evaluation of neonatal sepsis. Int Clin Pathol J. 2017; 4(5):114–118.
14. Ebrahem AM, Jameel SK, Abbas AH. Estimation Of Interleukin 27 And Interleukin 33 Serum Levels In Iraqi Neonatal Sepsis. Journal of Pharmaceutical Negative Results. 2022; 10: 136-41.
15. Noha FN, Doya LJ, Jouni O Perinatal Risk Factors and Early Onset of Neonatal Sepsis. Int J Pediatr Res, 2022; 8: 088.
16. Abdellatif M, Al-Khabori, Rahman AU, Khan AA, Al-Farsi A, Ali K. Outcome of Late-onset Neonatal Sepsis at a Tertiary Hospital in Oman. Oman medical journal, 2019; 34(4), 302–307.
17. Jemal M, Tinshku F, Nigussie Y, Kefyalew B, Alemu C. Trend Analysis of MultidrugResistant Bacterial Pathogens Causing Neonatal Sepsis at University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia: A Retrospective Study. International journal of microbiology, 2021; 9992994.
18. Abood RM. Laboratory assessment of C-reactive protein (CRP), procalcitonin (PCT) and serum amyloid A-2 protein(SAA2) as biomarkers of pediatric bacterial bloodstream infection, Master thesis, College of Medicine in Mustansiriya University, 2021.

19. Yang A, Liu J, Yue L, Wang H, Yang W, Yang G. Neutrophil CD64 combined with PCT, CRP and WBC improves the sensitivity for the early diagnosis of neonatal sepsis. Clinical chemistry and laboratory medicine, 2016; 54(2), 345–351.
20. Nascimento DC, Melo PH, Piñeros AR, Ferreira RG, Colón DF. IL-33 contributes to sepsis-induced long-term immunosuppression by expanding the regulatory T cell population. Nat Commun. 2017; 8:14919.