SERUM FERRITIN AS A DIAGNOSTIC MARKER OF BACTERIAL SEPSIS IN TERM NEONAT

By

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ABSTRACT

Background: *Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. Severe sepsis is defined as sepsis plus one of the following: Cardiovascular organ dysfunction OR acute respiratory distress syndrome OR two or more other organ dysfunctions (renal, neurologic, hematologic, or hepatic).*

Aim and objectives: *the main aim of this study was to evaluate the level of serum ferritin in neonatal sepsis as a diagnostic tool.*

Materials and Methods: *This prospective case control NICU of Al-Hussein and Bab El-Shaeryia Hospitals Al-Azhar University by simple random method after obtaining written informed consent from the parents or guardians. Detailed history regarding symptoms, past/medical history was collected from parents/ relatives. All neonates underwent anthropometric measurements, detailed general/systemic examination. Relevant laboratory investigations were done at admission, such as total white blood cells count, erythrocyte sedimentation rate, platelet count and serum ferritin levels. Bl.,sputum., c.s.f., &urine cultures were done when indicated.*

Results: *In the present study 90 neonate satisfying study criteria were studied, 60 as patient group &30 as healthy control group. The mean age in case group was (8.63±6.106 ms).and in control group (9.63±4.042 ms). With insignificant difference. According to S. Ferritin correlation with lab. parameters there was no significant correlation with WBCs, HB and Platelets, (p-value=0.230, 0.213, 0,480,) respectively. On the other hand, there was a significant correlation with ESR and CRP (p-value=0.001). Also, there was statistically significant CORRELATION between serum ferritin and blood, sputum cultures (p-value=0.001.*

Conclusion: *serum ferritin is a sensitive predictor of N. sepsis in neonates with sensitivity 93%, specificity 87%, negative predictive value 88.9% and positive predictive value 92.3%. Hence, it should be included in the septic screening of newborns.*

Key Words: *Sepsis, acute respiratory distress syndrome, mortality.*

INTRODUCTION

Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to an infection. The prevalence of neonatal sepsis is estimated to be 2824 cases per 1,00,000 live births worldwide, Sepsis contributes to 17%-20% of neonatal deaths **(Fleischmann et al., 2021)**. Various studies have shown that the most important measure in reducing mortality from sepsis is early identification of the condition and prompt initiation of therapy. Prognostic scores and biomarkers are commonly used in intensive care units to direct resources, to suggest a more vigorous monitoring or to predict a risk of early deterioration. **(Randolph et al., 2015)**, **(Brilli et al., 2016)**.

Various inflammatory markers like ferritin, C- Reactive protein and procalcitonin have attracted attention for their use as prognostic markers in neonatal sepsis patients **(Ng et al., 2016)**, **(Carcillo et al., 2016)**, **(Guven et al., 2012)**.

Ferritin is an iron storage protein, in inflammatory conditions there is a great production of ferritin which leads to a decrease in serum iron, believed to minimize the availability of iron to microorganisms **(Krol et al., 2013)**.

For this reason, ferritin in critically ill patients may be elevated, and it is correlated with severity in some diseases **(Carl et al., 2014)**.

Aim of the study: To evaluate the level of serum ferritin in neonatal sepsis as a diagnostic test and its prognostic value regarding severity &comorbidity.

Ethical considerations:

Approval by the ethical committee of the Pediatrics department at the Faculty of Medicine at Al-Azhar University under the registration number: 000488 was obtained before the study.

- Patients were enrolled in the study after taking informed oral and written consent from their parents.
- Patient data confidentiality was preserved during all study procedures.
- The patient and parents have the right to withdraw any time.
- There was no conflict of interest regarding the study or publication.

- There is no financial support or sponsorship.

We ensure that the participants are not physically or psychologically harmed during the study.

Sample size calculation:

Sample size calculation was done using G-power sotware version 3.1.9.4 for MS windows. we calculated that the minimum proper sample size was 60 patients' septic neonates & 30 healthy control. in each group to be able to reject the null hypothesis with 80% power at &=0.05 level using one way analysis of variance with test ratio and with accommodated 15% dropout rate.

Inclusion criteria:

- Age below 28 days.
- A neonate with any of the following symptoms:

- Temperature of more than 38.5° or less than 36°C.
- Tachycardia or bradycardia Tachypnea.
- Seizures, cyanosis, poor feeding or hypo activity.
- Diarrhea, vomiting, feeding intolerance.

Exclusion criteria:

- Age above 28 days.
- Preterm neonates.
- Hypoxic ischemic encephalopathy.
- Surgical cases.
- Hemorrhagic diseases of newborn.
- Conditions with iron overload whether primary or secondary.
- Ineffective erythropoiesis.
- Hematological malignancy.

PATIENTS AND METHODS

This prospective case- control study of the neonates that were collected from the NICU of Al-Hussein and Bab El-Shaeryia Hospitals Al-Azhar University after obtaining written informed consent from the parents or guardians during the period from May 2023. To September 2023 They, were selected by simple random method.

Methodology:

All included neonates were subjected to the following:

1. **Detailed history regarding** symptoms, past/medical history was collected from parents/ relatives.
2. **Complete general ex. including:** Vital signs,

anthropometric measurements, cyanosis &.jaundic.

3. **Systemic examination including:**

- Chest examination.
- Heart examination.
- Abdominal examination.
- CNS examination.

4. **Laboratory investigation including:**

- CBC (automated).
- CRP (latex).
- ESR (Westergren).
- Blood culture (Bactec).
- Urine culture (manual).
- CSF culture (manual).
- Sputum culture (manual).
- Serum ferritin level estimated by latex immunoturbidimetric method).

Statistical analysis:

All statistical calculations done using software MEDCAL. Shapiro-wilk test was applied to find whether data on ferritin levels will be parametric or non-parametric. Comparison of serum ferritin was done by Kruskal Wallis test. Receiver operating characteristics (ROC) analysis was used to identify cut-offs of serum ferritin level for determining the presence of sepsis and predicting poor outcome.

RESULTS

Our result will be demonstrated in the following tables:

Table (1): Descriptive analysis of studied cases

	Case Group (n = 60);	Control Group (n = 30)	Total patients	Test of Sig.	P Value
Age (days)					
Min.-Max.	3-27	3-26	3-27	A=6.8	0.65
Mean± S.D	8.63±6.106	9.63±4.042	7.63±5.301		
Sex					
Male	37	19	56	A=2.3	0.43
Female	23	11	34		

This table show that: There was no statistically significant difference regarding age and sex between cases and controls (p-value (0.65,0.43 respectively).

Table (2): Descriptive analysis of lab parameters

		N	Mean	SD deviation	Min.	Max.	p-value
WBCs (Thousand/mm)	case group	60	5.830	5.7723	1.6	35.0	0.001
	control group	30	12.267	2.3479	8.0	17.0	
	Total	90	7.976	5.7615	1.6	35.0	
Hb (gm/dl.)	case group	60	13.038	1.9246	6.2	17.0	0.184
	control group	30	13.567	1.3817	10.0	15.0	
	Total	90	13.214	1.7721	6.2	17.0	
Platelets (Thousand/mm)	case group	60	160.05	106.305	22	623	0.001
	control group	30	254.47	45.054	198	340	
	Total	90	191.52	100.778	22	623	
CRP (mg/dl)	case group	60	42.38	30.440	10	158	0.001
	control group	30	2.67	1.155	1	5	
	Total	90	29.14	31.131	1	158	
ESR	case group	60	24.00	11.527	10	60	0.001
	control group	30	3.90	1.062	2	5	
	Total	90	17.30	13.388	2	60	

This table shows that: there was a statistically significant difference regarding WBCs, Platelets, CRP and ESR between cases and controls (p-value=0.001), However there was no statistically significant difference between the two groups regarding of HB. level (p-value=0.184).

Table (3): frequency of cultures (blood, CSF, sputum and urine)

		Frequency	Percent
Blood	Klebsiella	15	25.0
	E. coli	9	15.0
	Pseudomonas	4	6.7
	Actinobacteria	3	5.0
	MRSA	3	5.0
	GBS	5;2	8.3
	No growth	21	35.0
CSF	Not done	59	98.3
	No growth	1	1.7
Sputum	Not done	51	85.0
	Klebsiella	6	10.0
	Pseudomonas	3	5.0
Urine	No growth	60	100

This table shows that: the most common organism was Klebsiella spp. Followed by E.coli spp. While No growth was present in35 % of cases in bl.culture.

Table (4): Correlation between serum ferritin and blood cultures

	N	Mean of serum ferritin	SD deviation	Minimum	Maximum	p-value
Klebsiella	15	614.27	90.613	492	778	0.001
E.Coli	9	431.33	130.437	158	635	
Psudomonas	4	675.25	92.222	550	772	
Actinobacter	3	582.33	124.645	440	672	
MRSA	3	542.67	35.907	502	570	
GBS	5	391.40	82.279	257	480	
No growth	21	138.43	131.920	19	592	
Total	60	400.60	235.921	19	778	

This table shows that There was statistically significant correlation between serum ferritin and blood cultures (p-value=0.001).

Table (5): Correlation between serum ferritin and sputum cultures

	N	Mean of serum ferritin	Std. Deviation	Minimum	Maximum	p-value
Not done	51	365.94	231.376	19	778	0.01
Klebsiella	6	665.17	74.759	542	758	
Pseudomonas	3	460.67	208.714	220	592	
Total	60	400.60	235.921	19	778	

According to serum ferritin and Sputum cultures There was significant correlation (p-value=0.001).

Table (6): Descriptive analysis of serum ferritin in patients &control group

	N	Mean of s.ferritin	SD. Deviation	Minimum	Maximum	p-value
case group	60	400.60	235.921	19	778	0.001
control group	30	61.10	20.337	33	110	
Total	90	287.43	250.865	19	778	

There was statistically significant difference in the term of serum ferritin between two groups p-value=0.001.

Table (7): Sensitivity& specificity of S.ferritin in diagnosis of n.sepsis& cut off point

Cut off value	115.00
Sensitivity	93 %
Specificity	87%
AUC	.871
CI 95%	(.791,.951)

DISCUSSION

Sepsis is a potentially fatal condition when the immune system's response to infection becomes uncontrolled, leading to failure of vital organs. Severe sepsis is characterized by the presence of sepsis together with at least one of the following: Cardiovascular organ dysfunction, acute respiratory distress syndrome, or two or more dysfunctions in other organs such as the kidneys, nervous system, blood, or liver **(Fleischmann et al., 2021)**.

Neonatal sepsis is a phrase used to describe a systemic

bacterial, viral, or fungal infection that causes hemodynamic abnormalities and other clinical symptoms as well as significant morbidity and death in the newborn **(Wynn, J L., 2016)**.

The primary factors contributing to sepsis-related mortality are infections associated with other comorbidities (such as encephalopathy, and hemolytic disease), lower respiratory infections, and diarrheal diseases **(Ng et al., 2016)**. The rise in plasma ferritin levels corresponded to the rise in plasma CRP levels seen in cases of acute pneumonia, TB, rheumatoid arthritis, and neutropenic sepsis, indicating that ferritin is functioning as an acute phase protein **(Carcillo et al., 2016)**.

Biomarkers for diagnosis of neonatal sepsis have been investigated that help in the early diagnosis of neonatal sepsis, before the onset of clinical manifestation so that early treatment of sepsis can be started and neonate can be properly managed.

Neonatal sepsis continues to be a significant contributor to illness and death, particularly in underdeveloped nations. In 2013, the World Health Organization (WHO) documented those 2.761 million newborns passed away during the neonatal period **(Guven et al., 2012)**.

Approximately one-fourth of these fatalities were attributed to infection. Early-onset infection refers to an infection that happens during the first three days of life. This kind of infection is often caused via vertical transmission, which occurs either before or during birth. Late-onset sepsis refers to the occurrence of infection after 72 hours after birth, and is caused by bacteria that are acquired either from the hospital or the community **(Krol et al., 2013)**.

Ferritin is a protein that stores iron. Inflammatory situations result in a significant increase in the synthesis of ferritin, which in turn causes a reduction in the amount of iron present in the bloodstream. This decrease in iron availability is thought to limit the access of microbes to iron. Consequently, ferritin levels may be increased in critically sick individuals, and this elevation is linked to the severity of certain disorders **(Carl et al., 2014)**.

The main results of this study were as follows:

According to age and sex of case and control groups There was no statistically significant difference **(Table 1)**.

In harmony with **(Varvoutis et al., 2022)** who found that there were no statistically significant differences between the case and control groups regarding age, and sex.

60 patient was enrolled in this study, percent of males was (62.2%) which is higher than percent of females (37.7%) but still statistically insignificant (P Value = 0.43) **(Table 1)** that was disagree with **(sarvatnida et al., 2022)** where male sex in their study was 38 (49%) and female group was 39(51%) out of 77 patients.

Regarding lab parameters between cases and controls, our results showed that: there was a statistically significant difference in the term of WBCs, Platelets, CRP and ESR (p-value=0.001), there was no statistically significant difference in the term of HB p-value=0.184 **(Table 2)**.

Regarding CBC in **(Table 2)** It was noticed that the levels of platelets were significantly lower in septic patients group compared to control group. however, the levels of CRP and ESR were significantly higher in septic patients group compared to control group (p<0.001). There was no statistically significant difference between the two groups regarding hemoglobin level (p= 0.184).

Our results were supported by **(Bank et al., 2022)** as they reported that platelet count was significantly lower in Cases group than control group (p value <0.001). Also **(Cao et al., 2022)** who found that no significat results with RBCs and HB and found a significant results in Platelets, CRP and ESR.

Also, WBCS. count was significantly lower in sepsis group than non-sepsis group (p-value=<0.001). Which is in disagreement with Hamam et al.,2019 who found that there was no significant difference between sepsis and control group (p- value >0.05) as regard Total Leucocytic Count.

C-reactive protein (CRP) is an acute phase reactant, produced in the liver which has a half –life of 24 to 48 hours. It is a commonly used marker to diagnose neonatal sepsis but as it takes 10 to 12 hours to respond to an infection, it is not reliable **(Ganesan, P et al., 2016)**.

The current study showed that, as regard comparison between the studied groups regarding inflammatory markers. It was noticed that the levels of CRP and ESR were significantly higher in septic patients group compared to control group (p<0.001). In accordance with our results,

Mondal et al., 2012 reported that CRP and ERS levels were significantly higher in septic group compared to control group. Also, **Chatterjee et al., 2017** revealed that the serum C-reactive protein (CRP) level was significantly raised in the clinically suspected neonatal sepsis groups than the control groups which is consistent with other studies. Our results declared that CRP and ESR has the most accurate blood lab parameter that associated with S. Ferritin which agreed to **(Shane et al., 2017)** as they reported that CRP and ESR levels were significantly higher in septic group compared to control group.

Moreover **(Cao (Pokhrel et al., 2018)** revealed that the serum C-reactive protein (CRP) level was significantly raised in the clinically suspected neonatal sepsis groups than the control groups which is consistent with other studies.

In harmony with our result. **(Pokhrel et al., 2018)**, found that there was statistically significant difference between serum ferritin and blood cultures and sputum culture which agree with our results.

According to frequency of cultures in our study we found that in blood the percentage of klebsiella 25%, E-coli 15%, Pseudomonas 6.7%, Actinobacteria 5%, MRSA 5% and GBS 8.3%. while in sputum culture klebsiella 10% and Pseudomonas 5% **(Tables 3, 4 and 5)**.

Our results were supported with study of **(Auriti et al., 2021)** as they reported that as regard the microorganisms identified in blood cultures; Klebsiella pneumoniae was the most common organism (63%) followed by coagulase-negative Staphylococci (12.5).

Also, in the study of **(Shane et al., 2017)** the most prevalent organism was Klebsiella (37.25%) followed by staphylococcus aureus (17.65%) in Disagreement with **(Parra-Llorca et al., 2023)** that found E-coli (37.25%) was more than klebsiella (17.65%) and Pseudomonas (11.2%).

According to S. Ferritin correlation with lab. parameter there was no statistically significant difference as regarding WBCs, HB and Platelets, p-value=0.230, 0.213, 0,480, on the other hand there was a statistically significant difference in the term of ESR and CRP p-value=0.001.

Our result stated that there was a significant difference in the serum ferritin between cases and controls (p value=0.001). Serum

ferritin was high in case group over control group in control group which ensure that serum ferritin has the upper hand in detecting the neonatal sepsis **(Table 6)**.

Various authors have reported association of high level of ferritin with detection and correlation with the severity of neonatal sepsis. **Arnab nandy et al., 2020** reported serum ferritin>1994 ng/ml and Garcia et al2021 reported serum ferritin >500 ng/ml and **Sarvatnida et al., 2022** reported the cut-off value of serum ferritin >1366ng/ml associated with neonatal sepsis and high mortality.

Our results also supported by **Peng et al., (2022)** who studied 36 Neonates with severe sepsis in Neonatal intensive care. Ferritin was 500 ng/mL in 12 Neonate, which agreed with our study that reported that was statistically significant difference and correlation in the term of serum ferritin between two groups p-value=0.001.

The difference between studies in cut-off may be due to difference in sample size, methods of selection and severity of cases.

Our study in agreement with previous studies that high ferritin levels is an effective tool for detection and prognosis of sepsis.

The ROC curve analysis between serum ferritin in both groups showed that cut-off value was 115 ng/ml.

Sensitivity and specificity 93%, 87% respectively, p-value 0.001 **(Table 7)**.

LIMITATIONS

- We had few limitations in our study as small number of included patients.
- Small period for follow up.

CONCLUSION

In neonatal septicemia serum ferritin has 87% accuracy for detection of neonatal sepsis with 93% sensitivity and 87% specificity, AUC 0.87. So, it is used as a marker in neonatal sepsis. Serum ferritin increased with increased severity of cases who had bad prognosis, so it can be used for early diagnosis and prognostic purposes in neonatal sepsis.

RECOMMENDATION

We recommend using S. ferritin for detecting neonatal sepsis because S.Ferritin has 87% accuracy with cut-off point value of 115 ng/ml. for detection of neonatal sepsis. And could be used as a marker in neonatal sepsis. The ration of Serum ferritin increased

with high severity of cases who had bad prognosis, so it can be used for early diagnosis and prognostic purposes in neonatal sepsis.

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