Evaluation of Left ventricle ejection fraction, Serum Ferritin and C - reactive protein as Early Prognostic Markers in Children with Sepsis.

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Abstract

Sepsis, still cause morbidity and mortality in children admitted to the pediatric intensive care unit (PICU). Several diagnostic and biological markers have y been studied to monitor unfavorable evolution of sepsis in critically ill patients but didn't established. Objective: This study aimed to evaluate the Left ventricle ejection fraction, serum Ferritin and C - reactive protein and find out their value as early prognostic markers for outcomes in pediatric sepsis in PICU. Patients and Methods: 40 patients admitted to a PICU diagnosed with sepsis. Echocardiography study: to figure out the ejection fraction (EF) of the left ventricle, serum levels of C-reactive protein and ferritin on D1 and D3 after admission. Patients followed to detect their outcomes and mortality. Results: We found with cardiac dysfunction by echocardiogram and elevated ferritin levels on D1 had longer hospital stay in, prolonged duration of mechanical ventilation use, higher maximum inotropic score, and fewer ventilator-free hours. Both low Left ventricle ejection fraction and high serum Ferritin are associated with unfavorable outcomes. Conclusion: Cardiac dysfunction by echocardiogram (EF $<$ 55%) and serum ferritin values ([?]300 ng/mL) on D1 of admission in pediatric patients with sepsis admitted to the PICU, were significantly associated with unfavorable outcomes.

Introduction:

Sepsis, still cause morbidity and mortality in children admitted to the pediatric intensive care unit (PICU). Systemic inflammatory response syndrome (SIRS) within the presence of suspected or proven infection constitutes sepsis. Clinical presentations progress in severity from sepsis to severe sepsis, septic shock, and multiple organs failure.[1, 2] Several diagnostic and biological markers have y been studied to monitor unfavorable evolution of sepsis in critically ill patients but didn't established in developing countries. [3, 4, 5] In pediatric sepsis, myocardial dysfunction is one of the causes of clinical deterioration causing systolic or diastolic ventricular dysfunction and contributing to shock and mortality. [6'7] sepsis induced myocardial dysfunction (SIMD) has been defined as a reversible decrease in ejection fraction (EF) of both ventricles, with dilatation of ventricles and less response to fluid resuscitation and catechol amines. [8] However, the left ventricular EF is a load dependent index that represents the after load and contractility between the left ventricular, rather than the intrinsic contractile myocardial feature. In septic shock, while left ventricular intrinsic contractility is seriously reduced, left ventricular EF can be normal if the afterload is seriously depressed.[9] The true clinical significance of cardiac dysfunction caused by sepsis is still elusive but using Echocardiography as an important non-invasive tool for evaluation of cardiac function in critically ill children is very important.

Ferritin is a commonly distributed protein for the storage of iron and has a role in as part of the acute phase response of infection and causes an iron-deficient state by reducing the iron available as a serum and this was thought as a defensive mechanism for reducing the iron available to invading species. [10] SIR is triggered in critical disease due to sepsis, and elevated levels of pro-inflammatory cytokines are present in

early disease phases. The pro-inflammatory cytokines: interleukin 6, interleukin 8, and tumor necrosis factor promote synthesis of ferritin and increase the serum ferritin levels. [11] Inflammatory biomarkers, such as C-reactive protein is useful in identifying the presence of significant bacterial infection in infants and young children. CRP is one of the groups of IL-6-regulated acute-phase reactants proteins and shows potential for determining seriousness and prognosis of sepsis. A rapid decline in CRP rates in septic patients was reported to correlate with good response to initial antimicrobial therapy. [12]

Aim of the Work

This study aimed to evaluate the Left ventricle ejection fraction as a measurement for cardiac dysfunction, serum Ferritin and C - reactive protein and find out if we can use them as early prognostic markers for outcomes in pediatric sepsis.

Patients and Methods

This a prospective observational cohort study included 40 patients; were recruited during the period from September 2019 to April 2020 at Minia University Children and Maternal hospital, department of pediatrics, PICU. This study was approved by the ethical committee and a written consent was obtained from each parent to agree to participate in the study. We declared no conflict of interest concerning the study. All enrolled children underwent detailed history taking and clinical examination. The patients data including age, gender, anthropometry, admission disease category (respiratory, cardiovascular, gastrointestinal, neurological and others). Routine investigations like complete blood count, serum ferritin, CRP, ESR, renal and liver function tests, serum glucose and electrolytes including sodium (Na), potassium (K), calcium (Ca), albumin, and arterial blood gases were performed. Other relevant investigations were required for selected cases such as blood culture, CSF examination, brain imaging as CT or MRI, and ECG. Calculation of pediatric risk of mortality score (PRISM)[13] was done at the time of admission in PICU. All patients received treatment according to the PICU protocol. Enrolment in the study did not change the normal treatment procedures. All patients were followed until discharge from hospital or death. The patient prognosis was measured by length of PICU stay, need and duration of mechanical ventilator support and outcome at the end of hospital stay. Our patients had clinical diagnosis or suspicion of sepsis, diagnosis or suspicion of sepsis made by the presence of two or more of subsequent four criteria: tachycardia, tachypnea, temperature change, leukocytosis, or leukopenia for age within the presence of confirmed or suspected infection based on 2001 SCCM/ ACCP/ ATS/ eSCiM/ SiS Consensus Conference. [14] We exclude: congenital heart disease, presence of confirmed or suspected endocrine disease, diagnosis of congenital or acquired immunosuppression, severe liver impairment.

Assessment of cardiac dysfunction was done by transthoracic echocardiography to work out left ventricular ejection fraction on day of admission D1 &D 3. The device used is logic v2 GE echo with 3 and 6 MHz transducer. All assessments were performed using an equivalent device and by an equivalent pediatric cardiologist with experience within the Pediatric Cardiology Service of the Hospital. The left ventricular end-diastolic dimension (LVEDD) is estimated at the top of the T-wave, the R-wave of the cardiac cycle and therefore the left ventricular end-systolic dimension (LVESD) is calculated using the subsequent equation: FS (%) =LVEDD – LVESD /LVEDD ×100. The EF may be a measurement, expressed as a percentage, of what proportion blood the ventricle pumps out with each contraction, calculated using the subsequent equation: EF (%) =LVEDV – LVESV /LVEDV ×100. The Pediatric Index of Mortality 2 (PIM2) was calculated on the first day of the PICU, according to the routine of this service. A PIM2 value of 6% was chosen because the cutoff point for severity, because it's that the upper limit of historical mortality during this service.

[15]

Blood sampling:

Four milliliters of blood were drawn from the patients under complete aseptic conditions, 2 ml of blood were added to the plain tube and incubated for a period of 20 min at 37 c and then placed in centrifugation 10 minutes at 3000 rpm to separate the serum and stored at - 20 till the day of analysis of *both ferritin and*

CRP levels at admission (D1) and after 72 h (D3) of admission .

(A) Serum ferritin: The normal range for ferritin levels in children at our clinical laboratory is 10–250 ng/mL, with some variability by age and gender. This number is given for orientation only; each laboratory should have its own reference range. The quantitative test kit for ferritin is based on a solid-phase enzyme combined with an immune sorbent assay. The assay method uses one anti-ferritin antibody in the antibody enzyme conjugate solution for solid phase (microtiter wells) immobilization, and another mouse monoclonal anti-ferritin antibody.

(B) C-reactive protein: CRP is estimated by latex agglutination assay using the AVITEX CRP kit. The CRP latex test is a rapid agglutination procedure for direct detection and semi quantization of CRP on a slide. The principle of test involves an immunological reaction between CRP (as an antigen) and the corresponding antibody fixed to the surface of latex particles. CRP in the sample reacts with IgG-coated polystyrene latex particles in the reagent forming visible agglutination.The normal range for CRP levels in children at our clinical laboratory is up to 6 mg/L. [16]

Statistical analysis:

The analysis of the data was administered using the IBM SPSS 20.0 statistical package software and MedCalc version 12.2.1.0 (MedCalc Software, Ostend, Belgium). Data were expressed as median, interquartile range (IQR), mean ± standard deviation (SD) for quantitative measures additionally to both number and percentage for categorized data. The normality of data was assessed using the Kolmogorov-Smirnov test. Mann Whitney test was used for comparison between independent groups and Wilcoxon test for dependent groups for non-parametric data. The Chi-square test or Fisher's exact test were used to compare categorical variables. The receiver operating characteristic (ROC) curve was computed and therefore the area under the ROC curve was used to evaluate the ability of serum ferritin and ejection fraction to predict the occurrence of mortality. The optimum cutoff was defined because the value that maximized the area under the ROC curves. Spearman correlation was used to describe the association between two variables. Multiple binary logistic regressions were used to create a predictive model. A P-value of 0.05 or less was considered significant.

Results

Our study included 40 critically ill children, with diagnosis or suspicious of diagnosis of sepsis. They were 16 (40.0%) males and 24 (60.0%) females, their age ranged from 1 month to 6 years, their PRISM score at admission ranged from 5- 25 with Mean ± SD 15.35±7.9. Patients' demographic, clinical and laboratory data are shown in **tables 1and 2** . As shown in **Table (3),** most cases had increased serum ferritin level and CRP above normal range and both significantly decreased between day 3 and day of admission D1 (p <0.001) and also there is statistically significant increase between day 3 and day of admission D1 as regard to Ejection fraction (p=0.001). Our study detected that there was a statistically significant difference between patients with cardiac dysfunction (EF<55%) and those without cardiac dysfunction (EF [?]55%) as regard to serum Ferritin level (ferritin level increased in pediatric patients with cardiac dysfunction) **(table 4).** The Outcome of studied cases: non-survivors were 24 (60%) patients, while the survivors were 16 (40%). The Median value of serum Ferritin level is significantly higher among non-survivors than survivors pediatric patients with sepsis (p=0.021) and the Median value of Ejection fraction is significantly lower among non-survivors than survivors septic pediatric patients (p=<0.001**) (Table 5).**

Table (6) shows that serum ferritin level has a sensitivity of 62.5% and specificity of 81.2% in prediction of mortality with (p value=0.002) with cut-off point>550, while ejection fraction has sensitivity of 83.3% and specificity of 87.5% in prediction of mortality with (p value=<0.001) with cut-off point[?]56%Also as shown in the same, serum ferritin level had a sensitivity of 68.4% and specificity of 66.7% in prediction of cardiac dysfunction with (p value=0.016) with cut-off point>510. Univariate and multivariable binary logistic regression analysis for predictors of mortality, are shown in **table 7** which clarifies that factors found to be significantly associated with mortality by univariate analysis were entered the multivariable model to detect the significant predictors of mortality. Cardiac dysfunction and serum ferritin were found to be predictors of mortality. Patients with cardiac dysfunction were more likely to die than patients without

cardiac dysfunction (p= 0.035). The increase in serum ferritin by 10 units was associated with a 10% increase in the odds of the child dying (AOR= 1.01, 95% CI = 1.0 - 1.02). **Figure** shows positive correlation between ferritin and CRP at day of admission (rho= -0.339, p=0.032).

(Table 1) Demographic and clinical laboratory data for studied 40 patients at presentation

Demographic data	**Demographic data**	**No & %**
Age (months)	**1 month – 2 years 2 – 6 years**	**34(85%) 6 (15%)**
Sex	**Male/ Female**	**16 (40.0%)/ 24 (60.0%)**
Residence	**Rural/ Urban**	**26 (65%)/14 (35%)**
Weight (kg)	**Range Mean± SD**	**3.5-23 9.1±4.4**
Length/Height (cm)	**Range Mean± SD**	**55-120 75.9±17.7**
Consanguinity	**+ve/ -ve**	**12 (30%)/ 28 (70%)**
Family history of similar condition	**+ve/-ve**	**9 (22.5%)/ 31 (77.5%)**
Diagnosis prior to PICU admission	**Diagnosis prior to PICU admission**	**No & %**
Pneumonia CNS infection	**Pneumonia CNS infection**	**20 (50.0%) 12 (30.0%) 11**
Viral encephalitis	**Viral encephalitis**	**(27.5%) 8 (20.0%) 1 (2.5%)**
Gastro-enteritis	**Gastro-enteritis**	
Meningococcemia	**Meningococcemia**	

(Table 2) Laboratory data for studied 40 patients at presentation

Laboratory tests	Range	Median	Mean± SD
Hemoglobin (g/dl)	**8-10**	**9**	**9±1.5**
Total leucocyte count (x10^9/L)	**9.5-23.5**	**15.5**	**16.8±9.3**
Platelets (x10^9/L)	**82.5-310**	**194.5**	**222.6±174.9**
Alanine transaminase (ALT)	**10-485**	**46**	**86.8±96.8**
Aspartate transaminase (AST)	**11-354**	**44.5**	**60.2±64.3**
Urea (mg/dl)	**11-120**	**36**	**42.4±26.8**
Creatinine (mg/dl)	**0.3-2**	**0.7**	**0.7±0.4**
Prothrombin time (seconds)	**11-25**	**16.5**	**16.8±3.7**
Albumin (g/dl)	**2.5-5.5**	**3.6**	**3.6±0.6**
sodium (mmol/L)	**125-168**	**141**	**142.7±10.9**
potassium (mmol/L)	**2.5-6.5**	**3.5**	**4.1±0.9**
Calcium (mg/dl)	**2-10**	**8**	**8±1.8**
C-reactive Protein (mg/dl)	**24-104**	**48**	**61.6±40.4**
Ferritin(ng/ml)	**240- 1150**	**510**	**535± 183.3**

Table (3): Serum Ferritin level, CRP & Ejection fraction (EF) among studied cases at day of admission D1 and day 3

	D1	**D3**	**Wilcoxon test**	**p value**
Ferritin (ng/ml) Median (IQR) Mean±SD	**522 (395-652) 554.4±223.3**	**400 (335.5-562) 447.8±183.8**	**-3.677**	**<0.001***

	D1	D3	Wilcoxon test	p value
C-reactive Protein (mg/dl) Median (IQR) Mean±SD	**48 (24-104) 61.6±40.4**	**24 (12-48) 37.6±34**	**-3.648**	**<0.001***
EF% Median (IQR) Median ±SD	**55 (41.5-60) 52.28±11.2**	**60 (55-68.5) 59.65±9.64**	**-3.417**	**0.001***

Table (4) Relation between Serum Ferritin level and cardiac dysfunction (EF %) at admission among studied cases

	With cardiac dysfunction EF< 55%	Without cardiac dysfunction EF [?]55%	ZMWU test	p value
	(n=19)	(n=21)		
Ferritin Median (IQR) Mean±SD	**568 (457-754) 625.6±214.3**	**463 (365-568) 490±216.2**	**-2.181**	**0.029***

Table (5) Outcome regarding serum ferritin level and Ejection fraction% among studied cases

	Outcome	Outcome	ZMWU	p value
	Non survivors (n=24) (60.0%)	Survivors (n=16) (40.0%)		
Ferritin Median (IQR) Mean±SD Range	**565 (453.5-769.5) 630.7±244.2 (254-1150)**	**456.5 (347.5-530) 440±122.4 (240-650)**	**-2.306**	**0.021***
EF% Median (IQR) Mean±SD Range	**44 (36.5-55.5) 46.17±10.82 (33-70)**	**60 (58.5-65.5) 61.44±5.3 (50-72)**	**-4.106**	**<0.001***

Table (6) serum ferritin level EF% and as predictive value of mortality among the studied cases

	Cut-off	AUC	P value	Sensitivity	Specificity	PPV	NPV
Ferritin	**>550**	**0.741**	**0.002***	**62.5%**	**81.2%**	**83.3%**	**59.1%**
EF%	**[?]56**	**0.887**	**<0.001***	**83.3%**	**87.5%**	**90.9%**	**77.8%**

Table (7): Univariate and multivariable binary logistic regression analysis for predictors of mortality

Predictors	Mortality	Mortality	Mortality	Mortality
	Crude OR (95% CI)	p value	Adjusted OR (95% CI)	p value
EF				
No cardiac dysfunction	**1.00 (Reference)**		**1.00 (Reference)**	

Predictors	Mortality	Mortality	Mortality	Mortality
Cardiac dysfunction	**45. (4.86-416.5)**	**0.001**	**63.22 (1.35-2963)**	**0.035***
Serum Ferritin	**1.01 (1.00-1.02)**	**0.005**	**1.01 (1.00-1.02)**	**0.038***
CRP	**1.01 (0.99-1.02)**	**0.344**	**-**	**-**
PRISM score	**2.14 (0.20-22.7)**	**0.526**	**-**	**-**
Duration of MV (hr)	**1.03 (1.01-1.04)**	**0.002**	**1.00 (0.96-1.04)**	**0.874**
Duration of inotropes (hr)	**1.03 (1.01-1.05)**	**0.008**	**0.99 (0.95-1.03)**	**0.538**
Max. inotropic score	**1.03 (1.01-1.06)**	**0.004**	**1.00 (0.95-1.05)**	**0.924**

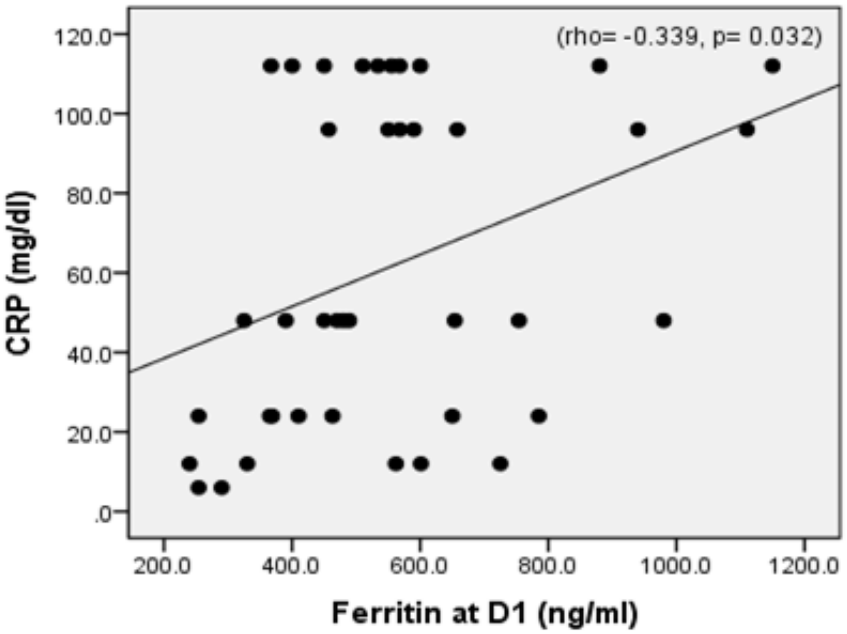

Figure (1): Scatter graph showing the correlation between ferritin and CRP at day of admission

Discussion

Sepsis is an important cause of mortality and morbidity in PICU, early identification and therapeutic interventions are the cornerstone in the outcome of sepsis, early recognition of sepsis is not always straightforward and clinical signs at presentation can be misleading.[17] Cardiac dysfunction is one of the main causes of clinical deterioration in pediatric patients.[7] Echocardiogram already used in the management of patients with septic shock during volumetric resuscitation and to choose the best vasoactive drug assessment and also can be used as marker of sepsis. [18, 19]

Our study aimed to verify the association between serum ferritin levels, CRP and cardiac dysfunction with unfavorable outcomes in pediatric sepsis. This present study was conducted on 40 infants and children suffering from sepsis of different causes admitted in PICU at Minia University Children and Maternal hospital, 85% of cases were between 1 month and 2 years, they were 24 females (60%) and 16 males (40%). The diagnoses of the studied cases were three main categories: pneumonia (50%), central nervous system (CNS) infection (30%), and gastroenteritis (20%). The etiology of increase the risk of pneumonia may include environmental factors, genetic susceptibility, and ethnic causes. [20] This come in line with the results of **Weiss et al., 2014** , who carried a study on the global epidemiology of pediatric sepsis and found that the most frequent site of infection was the respiratory system with pneumonia (40%).[21] Our current study

showed that cardiac dysfunction (EF<55%) was present among 47.5% of the studied cases. This finding of cardiac dysfunction in sepsis (low Ejection fraction) is higher than studies published by **Raj et al., 2014**[6] 37% of cases; **Pulido et al., 2012**[22] 27% of cases; and **Furian et al., 2012**[23] 33% of cases. Ferritin was the most prominent inflammatory marker in our study. It is an iron-storing protein, responsible for releasing it in a controlled way. In inflammatory processes, a great production of ferritin occurs, inducing a decrease in serum iron, believed to decrease the availability of iron to microorganisms. For this reason, ferritin in critically ill pediatric patients may be increased, and it is associated with severity in some illnesses. [24] C-reactive protein is an acute-phase reactant; it rises within six hours of the onset of inflammation and may rise to 10,000 folds with a peak at 48 hours, and disappears equally rapidly after the destructive process has stopped. CRP is not only useful in detecting the presence of an inflammatory disease but is also useful in following its progress and effectiveness of treatment. In particular serial measurements of CRP are very useful, for instance when one has to decide about the duration of antibiotic therapy in infection. [25] the present study showed that there was a highly statistically significant difference between a day of admission D1 and third day as regard to C-reactive protein with median value of CRP was higher on the day of admission 48 mg/L (24-104) compared to third day that was 24 mg/L (12-48) with P-value <0.001. Also, there was a highly statistically significant difference between a day of admission D1 and the third day as regard to serum ferritin level with median value of Ferritin level was higher on the day of admission 522 ng/ml (395-652) compared to third day that was 400 ng/ml (335.5-562) with P-value <0.001.

In the current study, we found that the median value of Ferritin level was significantly higher among nonsurvivors septic cases 565 ng/ml (453.5-769.5) than survivors septic cases 456.5 ng/ml (347.5-539) with p-value = 0.021. This result agrees with **Bennett et al., 2011**[26] who found that very high serum ferritin levels are associated with high mortality in pediatric intensive care patients. Our results showed that the median value of Ejection fraction% was significantly lower among non-survivors 44 % (36.5-55.5) than survivors with sepsis 60 % (58.5-65.5) with P-value <0.001. This comes in concordance with **Elgazzar et al., 2018** [27] whose study was carried out on 50 pediatric patients admitted to PICU with septic shock and found decreased EF% was higher among died than improved septic cases. We found that the median value of serum Ferritin level was significantly higher among cases with cardiac dysfunction than cases without cardiac dysfunction, this come in accordance with **Tonial et al., 2017**[28] who found that the mean value of serum Ferritin level was significantly higher among cases with cardiac dysfunction than those without cardiac dysfunction.

In our study, we found a statistically significant difference between cases with cardiac dysfunction and those without cardiac dysfunction as regard to the duration of mechanical ventilation (hr) with p-value <0.001, duration of inotropes (hr) with p-value 0.002, and maximum inotropic score with P-value <0.001 while there was no statistically significant difference between cases with cardiac dysfunction and those without cardiac dysfunction as regard to duration of PICU admission (days) with P-value 0.713. **Garcia et al., 2007**[29] found a statistically significant difference between cases with cardiac dysfunction and those without cardiac dysfunction as regards to duration of mechanical ventilation (hr) with P-value = 0.011, duration of PICU admission (days) with P-value = 0.02, and maximum inotropic score with P-value = 0.001. while there was no statistically significant difference between cases with cardiac dysfunction and those without cardiac dysfunction as regards to duration of inotropes (hr) with P-value = 0.231. The receiver operating characteristics area under the curve (ROC-AUC) value of ferritin level > 550 ng/ml as cut point was 0.741 has a sensitivity of 62.5% and specificity of 81.2%. In prediction of mortality among studied patients, factors found to be significantly associated with mortality by univariable analysis were entered the multivariable model to detect the significant predictors of mortality, cardiac dysfunction, and serum ferritin were found to be predictors of mortality. Patients with cardiac dysfunction were more likely to die than patients without cardiac dysfunction (p=0.035), the increase in serum ferritin by 10 units was associated with a 10% increase in the odds of child to die (AOR= 1.01, 95% CI = 1.0 - 1.02). These also come in line with**Garcia et al., 2007**[29] who found ferritin level > 500 ng/ml is associated with a higher mortality rate with P-value 0.02.

Conclusion

Cardiac dysfunction by echocardiogram (EF < 55%) and serum ferritin values ([?]300 ng/mL) on D1 of ad-

mission in pediatric patients with sepsis admitted to the PICU, were significantly associated with unfavorable outcomes.

Study limitations

Some limitations of this study should be indicated. The first is related to EF measurement by echocardiogram, which is a professional-dependent assessment. This method was chosen, despite its limitations, because it is available in most PICU services in Minia university hospital. The second is the limited number of patients.

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