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ORIGINAL ARTICLE

Study of Serum Apolipoprotein A-V Level as a Prognostic Biomarker for Sepsis in the Pediatric Intensive Care Unit

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ABSTRACT

Background: Sepsis is a major cause of illness and mortality. Children in pediatric intensive care units (PICU) in developed nations experience a sepsis mortality rate that exceeds 50%. For efficient patient care, evaluating disease severity at admission is critical for prognosis, management, and optimal use of resources. Aim of the work: To assess the prognostic value of serum apolipoprotein A-V levels in children with sepsis admitted to the PICU.

Methods: This study was conducted in the PICU in collaboration with the clinical pathology department of Zagazig University Hospital, on patients aged between 28 days and 16 years who were admitted with sepsis. We evaluated patients with complete sepsis screening and serum apolipoprotein A-V levels.

Results: The mean age of the studied group was 46±55.6 months. More than half of the study group had associated comorbidities and congenital heart disease was most common, followed by renal disease, and neurologic disorders. Acinetobacter baumannii complex, coagulase-negative Staphylococcus, and Klebsiella pneumoniae were the most prevalent pathogens affecting previously healthy children. In contrast, pseudomonas aeruginosa and Staphylococcus aureus were the most prevalent pathogens in children with comorbidities. Half of patients were survivors. An apolipoprotein A-V level of 89.5 ng/ml predicted mortality due to sepsis with 73.4% sensitivity, 52.7% accuracy, and 32.1% specificity.

Conclusions: Serum apolipoprotein A-V levels are sensitive in predicting sepsis-related mortality in the PICU for early therapy within the first few hours after sepsis presentation, which can prevent predictable progression and poor outcomes such as organ failure and death.

Keywords: Apolipoprotein A-V; Sepsis; PICU; Prognosis; Mortality.

INTRODUCTION

Pediatric sepsis is primarily responsible for infant illness and mortality [1]. Unfortunately, sepsis-related morbidity and mortality may develop because of the body's hyperimmune response to systemic bacterial infections. Organ malfunction and tissue destruction are the possible effects of sepsis. Sepsis can also develop into septic shock, characterized by prolonged hypotension, significant tissue destruction, and high mortality [2].

According to estimates, the fatality rate of sepsis is 20% worldwide [3]. Therefore, it is essential to identify and treat sepsis within the first few hours after pediatric intensive care unit (PICU) admission to enable pediatric doctors to provide appropriate acute care and prevent worst-case situations [4].

Sepsis causes severe metabolic alterations, such as the release of stress and inflammatory mediators [5, 6]. Early detection of patients at risk of sepsis-related morbidity and mortality is challenging and, at the same time, valuable for improving survival rates and clinical outcomes. In addition, determining disease severity at admission is crucial for efficient patient management, accurate prognosis, and optimal resource utilization [7, 8].

Acute sepsis causes mobilization of lipid mediators, which control inflammation and lipid metabolism. Lipid homeostasis is regulated by apolipoprotein A-V (APOA5). Sepsis causes dyslipidemia, including low levels of high-density lipoprotein, and high triglyceride levels [9]. The apolipoprotein A-V function in pediatric patients with sepsis needs to be better understood. Novel studies have aimed to assess whether serum apolipoprotein A-V levels upon admission to the PICU correlate with prognosis in such patients [3]. Therefore, this study aimed to evaluate the diagnostic and predictive values of serum apolipoprotein A-V levels in children hospitalized in the PICU with sepsis.

METHODS

This prospective observational study was conducted at the PICU in collaboration with the Clinical Pathology Department of Zagazig University Hospital. The duration of the study was six months. All patients aged between 28 days and 16 years with a sepsis diagnosis were admitted to the PICU of Zagazig University Children's Hospital and were included in the study. The Surviving Sepsis Campaign international guidelines for managing septic shock and sepsis-associated organ dysfunction in children in 2020 served as the foundation for the definition of sepsis in the study. Sepsis-associated complications were evaluated [3, 10]. Patients who died within 24 h, neonates and patients above 16 years of age, children with a history of genetic disease, and inborn errors of metabolism (IEM) affecting lipid profile were excluded from the study.

Each patient underwent a thorough clinical examination, including serial Glasgow Coma Scale, vital signs, oxygen saturation, fluid input and output assessment, nutritional state assessment, and assessment of sepsis according to the worldwide guidelines for the care of septic shock and sepsis-associated organ failure in children by the Surviving Sepsis Campaign in 2020 [3]. Within twenty- four hours of admission, pediatric sequential organ failure assessment (PSOFA) score was assessed. Routine laboratory investigations were performed, including the following: complete blood count, served with a Sysmex, Japan, automated cell counter, model XN 330; coagulation profile, performed with the CS 2100 automated blood coagulation analyzer (Sysmex, Japan); liver and kidney function tests, calcium, magnesium, phosphorus level assay, triglycerides, cholesterol, high-density lipoprotein, and , low-density lipoprotein which were all performed on a Cobas 8000 autoanalyzer, c702 module by spectrophotometry; C-reaction protein (CRP), which was performed on a Cobas 8000 autoanalyzer, c702 module by immunoturbidimetric methodology; procalcitonin, which was carried out using a Roche Cobas 8000 autoanalyzer, e 602 module by electrochemiluminescence; all biochemical tests were performed using dedicated reagents supplied by the manufacturer and according to the manufacturer recommendations (Roche diagnostics, Switzerland); and a blood gas analyzer, ABL80 FLEX BASIC instrument (Radiometer, Denmark), which was used to measure electrolytes and arterial blood gases. Bacteriological examination: According to each case, blood cultures, as well as cultures from various sites, such as sputum, urine, and central venous line, are performed. Positive blood samples and other cultures were subjected to identification and antibiotic susceptibility testing. Blood culture bottles were incubated in BACT/ALERT3D equipment (Biomerieux, France), whereas different cultures were incubated in plates of suitable media according to each site on a VITEK2 Compact instrument (Biomerieux, France).

The serum apolipoprotein A-V assay was collected from each participant under sterile conditions and placed in a plain vacutainer tube for serum separation. The sample was discarded for twenty minutes at ambient temperature, then centrifugated at 2000 rpm for 20 min, and the resulting serum was stored at -20°C. The test kit was supplied by the Shanghai Sunred Biological Technology Co. Ltd. Ltd., China (Catalog no. 201-12-5981). The kit uses an enzyme-linked immunosorbent assay (ELISA) with a double-antibody sandwich to determine the level of serum apolipoprotein A-V in each specimen. The manufacturer's recommendations were followed, and a standard curve was used to calculate the serum apolipoprotein A-V levels.

Administrative design:

Approval was obtained from the Zagazig University Institutional Review Board (IRB), and the parents of the patients who took part in this study gave their permission after informing them about the research, the steps that were done, and their capability to withdraw at any time. Written informed consent was obtained from all participants' patients. The study was done according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis:

To process the data, SPSS version 23 was used for data checking, entering, and analysis. The

student's t-test, chi-square test (X2), Fischer's exact test, receiver operating characteristic (ROC) curve, correlation coefficient test, and Kaplan–Meier survival curve were used. Statistical significance was set at $p \leq 0.05$.

RESULTS

The study group's mean age was 46±55.6 months, ranging from 1.6 to 168 months. Approximately half of the study participants were female (53.3%) and 46.7% were male. More than half of the study group had associated comorbidities (56.7%), and congenital heart disease was the most common (16.7%), followed by renal disease (15%) and neurologic disorders (13.4%). Bacterial infections were the most common (65.0%). Half of our patients were survivors and the others were non-survivors **(Table 1**). The mean, median, and range of the laboratory test results are presented in **Table 2**. Acinetobacter baumannii complex (15%), coagulase-negative Staphylococcus (11.7%), and Klebsiella pneumoniae (11.7%) were the most prevalent pathogens affecting previously healthy children, whereas Pseudomonas aeruginosa (10%), Staphylococcus aureus (8.4%), and COVID-19 (8.4%) were the most prevalent pathogens in children with comorbidities **(Table 3).** Serum apolipoprotein A-V level of 89.5 ng/ml had 73.4% sensitivity, 52.7% accuracy, and 32.1% specificity for predicting mortality due to sepsis **(Table 4). Figure 1** shows that the average survival duration after sepsis was 18.6± 1.4 days with 95% CI (15.8-21.4 days).

Table 1: Descriptive data of the study group

Variables	**The studied group (No= 60 (%)** Mean ± SD Median (Range)
Age (months)	46±55.6 12 (1.6-168)
Sex Male Female	 28 (46.7%) 32 (53.3%)
Heart rate (beats per minute)	128.9±21.9 130 (81-172)
Respiratory rate (breaths per minute)	28.9±15.8 24 (16-91)
Diastolic blood pressure (mmHg)	66.7±14.9 66 (35-97)
Systolic blood pressure (mmHg)	107.2±20.1 104.5 (68-151)
Comorbidities No Yes	 26 (43.3%) 34 (56.7 %)
Comorbidities Congenital heart disease Renal disorders Neurologic disorders Metabolic disorders	 10 (16.7%) 9 (15%) 8 (13.4%) 7 (11.66%)
Type of infections Bacterial Viral Fungal Mixed infections (Viral & bacterial)	 39 (65.0%) 15 (25.0%) 1 (1.7%) 5 (8.3%)
PSOFA score	8.1±3.07 8 (2-16)
Outcome Died Survived	 30 (50%) 30 (50%)

Table 2: Laboratory investigations of the studied group

Variables	**The studied group No= 60 (%)** Mean ± SD Median (Range)
Culture results Positive Sterile	 44 (73.3%) 16 (26.7%)
WBCs (10^3/mm3)	14.5±7.8 12.7 (3.4-38.5)
Hb (g/dl)	10.4±1.6 10.1 (6.8-15.2)
Platelets (10^3/mm3)	286.4±145.4 268 (44-605)
PT (seconds)	14.7±4.5 13.2 (10.1-29.3)
PTT (seconds)	36.9±10.1 34.9 (12.5-64.7)
INR	1.2±0.42 1.13 (0.1-2.38)
Blood urea nitrogen (mg/dl)	27.2±26.2 17.9 (1.6-117)
Creatinine (mg/dl)	1.7±2.5 0.46 (0.03-10.7)
Na (mEq/l)	135.7±6.6 136 (120-156)
K (mEq/l)	4.1±1.1 4.1 (2.1-7.9)
Total calcium (mg/dl)	9.07±1.3 8.9 (5.6-13.1)
Mg (mg/dl)	2.3±0.56 2.2 (1.19-3.74)
C-reactive protein (mg/l)	63.7±80.2 24.7 (0.06-336.1)
Total Protein (mg/dl)	5.9±0.94 5.8 (3.75-7.9)
Albumin (gm/dl)	3.4±0.62 3.49 (2.1-5.32)
Phosphorus (mg/dl)	5.3±2.6 4.9 (0.46-13.7)
Apolipoprotein (ng/ml)	601.5±657.1 364.6 (42.1-2890.6)
Procalcitonin (ng/ml)	17.8±30.7 3.2 (0.2-100)

Table (3): List of pathogens among the studied group

Organism	The studied group	
	No (60)	%
Acinetobacter baumannii complex	9	15
Coagulase negative staphylococcus	7	11.7
Klebsiella pneumoniae	7	11.7
Pseudomonas aeruginosa	6	10
Escherichia coli	6	10
Staphylococcus aureus	5	8.4
Staphylococcus haemolyticus	4	6.7
Covid-19	5	8.4
Cytomegalovirus	1	1.6
Hepatitis A virus	1	1.6
Candida	1	1.6
Mixed microorganisms	8	13.3

Table 4: The predictive performance of serum apo-lipoprotein level for PICU mortality due to sepsis

Variables	Serum Apo-lipoprotein level
Cut off point	89.5 (ng/ml)
The area under the curve	0.56
Significance	0.4
95% Confidence interval	(0.41-0.7)
Sensitivity	73.4%
Specificity	32.1%
Predictive value positive	51.9%
Predictive value negative	54.7%
Accuracy	52.7%

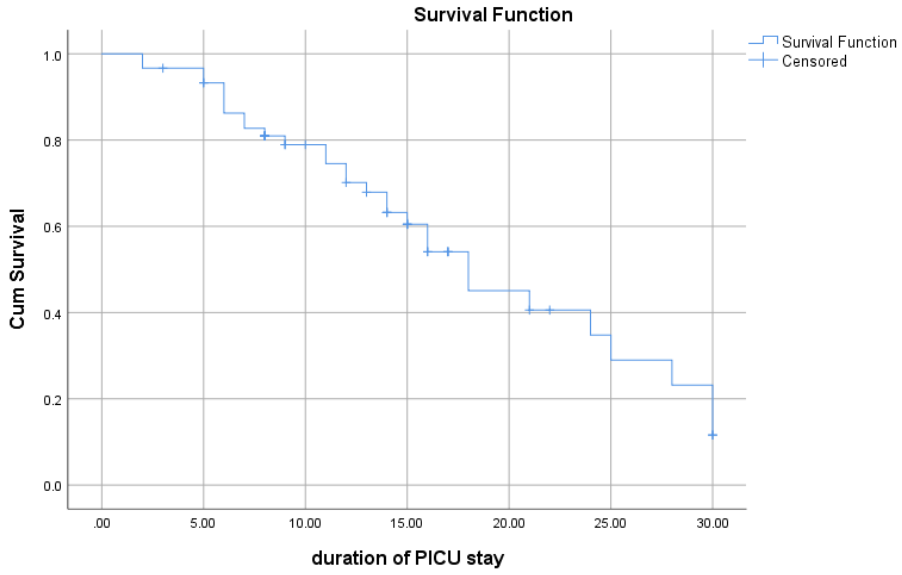

Figure 1: Kaplan Meier survival curve for the survival rate of children with sepsis in PICU

DISCUSSION

Sepsis is a major cause of illness and mortality. Pediatric sepsis, which has a 2.8% incidence rate, is the cause of 0.7% of all hospital admissions among inpatients in the United States. Epidemiological studies using clinical data have

linked pediatric sepsis to up to 8% of all PICU hospitalizations [11, 12]. The mortality rate associated with sepsis in PICU in developing countries is > 50%. Statistics from the World Health Organization (WHO) have shown that 80% of deaths below four years of age fall within the category of sepsis-related mortality. Early detection of patients at risk of sepsis-related morbidity and mortality is challenging and, at the same time, valuable for improving survival rates and clinical outcomes. In addition, determining disease severity at admission is crucial for efficient patient management, accurate prognosis, and optimum resource utilization [7, 8].

Sepsis causes severe metabolic alterations, and many researchers have attempted to identify the prognostic indicators of these alterations. Although several biomarkers have been used to recognize sepsis severity, their predictive abilities must be improved. C-reactive Protein, interleukins, tumor necrosis factor, procalcitonin [13], plasma lipids, lipoproteins, and bilirubin [14] are examples of inflammatory cytokines. In addition to other laboratory data, sepsis predictive indicators have been identified, including the sequential organ failure assessment (SOFA) [15, 16], quick SOFA [17, 18], and other scores [19]. Therefore, there is a need to develop new techniques and to identify reliable biomarkers to enhance sepsis detection and prognosis.

Lipid homeostasis is regulated by apolipoproteins A-V (APOA5). The acute-phase processes that cause sepsis cause low levels of high-density lipoprotein (HDL) cholesterol, and high triglyceride levels define dyslipidemia [9]. The apolipoprotein A-V function in pediatric patients with sepsis needs to be better understood. Therefore, this study aimed to evaluate the diagnostic and predictive value of serum apolipoprotein A-V levels in children hospitalized in the PICU with sepsis. Novel studies have aimed to assess whether serum apolipoprotein A-V levels upon admission to the PICU correlate with prognosis in such patients [3].

The median age of the studied group was 12 months, ranging from 1.6 to 168 months. Approximately half of the participants in the study (53.3%) were female, while 46.7% were male. According to Wang et al. [5]**,** the study group's median age was 19 (5-60) months, and 58% were male. Based on the study by Humoodi et al. [20], 47.8% of sepsis patients were female, with the majority (66.3%) being children under six.

According to our findings, 56.7% of the study group had associated comorbidities, with congenital heart disease being the most frequent (15.0%). This is in accordance with a study by Weiss et al. [3], which showed that children with specific preexisting comorbid conditions, such as congenital and acquired heart disease, are more likely to develop sepsis and have a worse prognosis following sepsis. However, Humoodi et al. [20] found that malignancy was the most common comorbidity (74.2%) in their research group, followed by neuromuscular diseases (14.2%).

In the current study, half of our patients were survivors and the others were non-survivors. This high mortality rate is due to patients' characteristics, consistent with previous studies conducted in the same PICU [21, 22].

In our study, Acinetobacter baumannii complex (15%), coagulase-negative Staphylococcus (11.7%), and Klebsiella pneumoniae (11.7%) were the most prevalent pathogens affecting previously healthy children, whereas Pseudomonas aeruginosa (10%), Staphylococcus aureus (8.4%), and COVID-19 (8.4%) were the most prevalent pathogens in children with comorbidities. Saleh et al. [9] reported that among patients with sepsis, 68% had gram-negative infections, 17% had gram-positive infections, and 15% had mixed microflora infections.

In the current study, a serum apolipoprotein A-V level of 89.5 ng/ml had 73.4% sensitivity, 52.7% accuracy, and 32.1% specificity for predicting mortality due to sepsis. According to Wang et al. [5]**,** apolipoprotein A-V level attained an area under the curve of 0.753. The serum ApoA5 was 1096 ng/mL, with 86.7% specificity and 55.6% sensitivity. At a threshold value of 822 ng/mL, serum ApoA5 levels 83.6% specificity and 75% sensitivity.

In accordance with the study by Saleh et al. [9]**,** APOA5 can correctly predict PICU mortality. According to ROC curve analyses, APOA5, CRP, pancreatic stone protein (PSP), and copeptin accurately predicted pediatric sepsis. APOA5 level >250 ng/ml was the most important predictor, with 96% sensitivity, and 90% specificity. Copeptin, CRP, and PSP levels had AUCs of 0.960, 0.917, and 0.868, respectively.

Those with sepsis have lower high-density lipoprotein and decreased apolipoprotein A-V levels, which may be biomarkers for sepsis [23]. Proteomic analysis of plasma apolipoproteins and cholesterol revealed that patients with sepsis have a lipid metabolic imbalance, suggesting a potential target for future treatment [5].

CONCLUSION: Serum apolipoprotein A-V levels showed fair sensitivity for predicting PICU mortality due to sepsis. Early detection of sepsis severity based on apolipoprotein A-V levels and precise therapy within the first few hours after

presentation can prevent predictable progression and poor outcomes, leading to clinically manifested organ failure, death, and multiple organ system failure. Therefore, it is a promising prognostic biomarker for sepsis in critically ill children.

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