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Cytokines Profile in Patients with Bacterial Sepsis and Septic Shock in Intensive Care Unit (ICU) of a Hospital in Colombia

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Abstract

Introduction: The management of patients with sepsis in Intensive care unit (ICU) is a difficult task that may benefit from the use of non-invasive assessment by biomarkers. This study was conducted to evaluate early cytokine profile plasma (IL-1β, IL-6, IL-10 and TNF-α) in patients with sepsis and septic shock.

Methods: A total of 62 patients with a recent diagnosis of sepsis and septic shock severe were included. Plasma samples were collected for measurement of cytokine concentrations. Plasma levels of cytokines were determined with enzyme-linked immunosorbent assays. Mann–Whitney U-test for the comparison of quantitative variables, $\chi 2$ test for qualitative variables. ROC curves of the variables were found to be significant in the multivariate analysis.

Results: Patients who presented high levels of IL-1β (63%) and TNF-α (66.7%) during the first 48 hours, were more likely to develop septic shock (RR: 11,019, CI 2.782-43.643; RR: 8.444, CI 2.749-25.939, respectively).IL-1β and TNF-α plasma levels fairly a good predictor of septic shock during the first 48 hours of admission presented area under the curve0.916 and 0.890 ($p \leq 0.001$), respectively

Conclusion: The results of this study suggest that IL-1βand TNF-α, are better in predicting to develop septic shock in the first 48 hours.

Key words. Biomarkers, Cytokines, Interleukin-1β, IL-6, IL-10, FNT-α, Sepsis, Septic shock.

1. Introduction

Incidence and mortality due to sepsis are increasing worldwide in patients admitted to intensive care units (UCI), constituting a public health problem (Gaieski, Edwards, Kallan, &Carr, 2013; Shankar-Hari, Harrison, Rubenfeld, & Rowan, 2017). The incidence of sepsis is determined by the characteristics of the patient, the associated comorbidities and the pathogenic flora of the place (Knoop, Skrede, Langeland, & Flaatten, 2017; Vincent, Jones, David, Olariu, & Cadwell, 2019).

This uncertain diagnosis allows a delay in the initiation of antimicrobial therapy, which may influence the mortality of certain groups of patients with sepsis, and also increase the use of antimicrobial agents, with the ecological consequences that it causes (flora alteration, resistance development, etc.). For this reason an attempt has been made to find biomarkers that would facilitate a rapid and early diagnosis of patients with infections in ICUs and can contribute to a better management of the patient and to the reduction of mortality (Walley, 2013; Samraj, Zingarelli, B.; Wong, 2013).

Several biomarkers have been evaluated for prediction, diagnosis and prognostic of sepsis, among them, the cytokines:Interleukine-1β (IL-1β), Interleukine-6 (IL-6), Interleukine-10 (IL-10) and tumorNecrosis Factor-α(TNF-α) (Reinhart, Bauer, Riedemann, & Hartog, 2012; Cioara, Valeanu, Todor, Cristea, & Lupse, 2016). Assessing the alterations of these biomarkers in relation to factors related to the characteristics of the patient (especially their clinical situation), of the causal infectious process and of the clinical evolution have been considered in the context of the clinical routine and in the management of the septic patient.

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However, in Latin America, there are few studies in relation to determining common characteristics in patients with sepsis through the use of sensitive and specific biomarkers that allow a better diagnosis and prognosis of the disease (Rodríguez et al, 2011; Ruiz, & Castell, 2016). The objective of this study was to evaluate the cytokine plasma levels (IL-1β, IL-6, IL-10 and FNT-α) in patients with sepsis and septic shock in the ICU of a hospital in the city of Cali, Colombia.

2. Methods.

2.1 Study population

An open-label randomized controlled trial at tertiary hospital in the city of Cali, southwestern Colombia. A total of 62 patients (37 men and 25 women) with sepsis, admitted to ICU were included between July until September 2018.

Twenty healthy individuals served as controls for determination of cytokine plasma levels. The definition of sepsis was based on the presence of at least two criteria considered as organic failure with the qSOFA (Sequential Organ Failure Assessment) scale, at the time of admission and daily during their stay in ICU according to the current criteria defined in the third consensus of sepsis (Singer et al, 2016).

Dysfunction of the organ systems were defined as any of the following criteria: 1) Renal dysfunction: serum creatinine >2.0 mg/dl or urine output <0.5 ml/kg per hour despite adequate fluid resuscitation; 2) Liver dysfunction: serum bilirubin >2.0 gr/dl, or a threefold increase in serum aminotransferases; 3) Disseminated Intravascular Coagulation (DIC): international normalized ratio (INR)>1.2, elevated d-dimer, platelet <100.000/mm3); 4) Respiratory insufficiency: PaO2/FiO2 ratio ≤ 300; 5) Hypotension: systolic blood pressure≤90 mmHg or the mean arterial blood pressure ≤70 mmHg despite adequate fluid and vasopressor resuscitation; 6) Central Nervous System (CNS) dysfunction: acute alteration of the mental status.

Patients were then stratified according to the sepsis degree and the septic focus (pulmonary, abdominal, urinary, catheter, skin and soft parts, others unknown [including neurological, as well as endocarditis of infectious origin]).

The exclusion criteria considered were: age under 18 years old, pregnancy, therapeutic use of cytokines, heparin or thrombolytics, antibiotics treatment prior to admission, and patients who did not survive more than 24 hours.

2.2 Sociodemographic and Clinical variables.

Sociodemographic variables included age and gender. Clinical variables included: etiological agent reported, antibiotic treatment provided, date of admission, date of discharge, comorbidities, physical-chemical parameters (blood pressure, temperature, heart rate, etc.), cell count (hematocrit, leukocyte count), and all parameters included in the qSOFA scale were analyzed with frequency distribution and percentages.

2.3. Quantitation of levels of cytokines in plasma.

Venous blood samples for the determination of plasma levels of cytokines (IL1-β, IL-6, IL-10 and TNF-α) were obtained at admission and at 48 hours after admission in the ICU.

Blood was collected by venipuncture into 15-ml sterile vacutainers either containing EDTA (10 mmol/L) as anticoagulant. The tubes were centrifuged immediately for ten minutes at 3000 rpm, and the plasma was stored in aliquots at –70°C.

The analysis of cytokines (including IL-1β, IL-6, IL-10, and TNF-α) was performed using an enzyme immunoassay with Human ELISA kit (Elabscience Biotechnology Inc. USA) following manufactures' instructions. The detection range was 7.81-500 pg/mL, with intra and interassay coefficients of variation below 5%. Readings were carried out in an automated optical Spectramax reader at 450 nm. The sensitivity of the assay was <2pg/mL. The serum levels of this cytokine are below the detection limit in healthy subjects. Results were related to a dose-response curve obtained with recombinant human each cytokine and concentration expressed as picograms per milliliter (pg/mL).

2.4 Statistical analysis

Days of hospital stay were analyzed as quantitative variables, along with plasma levels of each cytokine at admission and at 48 hours after admission in the ICU, with a view to assessing their evolution during patient stay in the ICU were analyzed with measures of central tendency and dispersion as the average and standard deviation (SD), respectively.

The Kolmogorov-Smirnov goodness of fit test was used to check the data for parametric distribution. For normally distributed variables, mean and standard deviation were used, for non-normally distributed variables median and 25th-75th percentiles were used. Comparison of the quantitative variables was carried out using the Mann-Whitney U-test, while categorical variables were compared between groups with the deχ^2 test, with a P-value <0.05 that was considered statistically significant. Correlations between variables were determined by using the Pearson correlation analysis for normally distributed variables, and by the Wilcoxon correlation analysis for each cytokine at time admission and at 48hours in patients with sepsis and septic shock. All statistical analyses were performed by SPSS Vs 25.00 for Windows (SPSS Inc, Chicago, Ill).

Analysis of classifiers was conducted using receiver operating characteristic (ROC) curves, and the area under the curve (AUC) was calculated for IL-1β, IL-6, IL-10 and TNF-α regarding sepsis or shock septic. Logistic regression analysis was also used to estimate Rate Risk (RR), expressed with their 95% confidence intervals (95% CI) for sepsis outcome in relation to levels of IL-1β, IL-6, IL-10 and TNF-α.

2.5 Ethical issues.

This study was conducted in accordance with the Helsinki Declaration and approved by the Medical Ethics Committee of the Clinica Versalles (Act No. 05-013). All patients or legal representatives were sufficiently informed about goals and procedures. Samples and data were obtained after written informed consent was signed.

3. Results.

The average age of the population was 52 years (± 19.47 SD) but was lower in the deceased patients and median duration of stay in the ICU was 7 days. The patients' characteristics are shown in table 1. Twenty-seven patients (43.5%) had pneumonia,18 (29%) had intra-abdominal infections, 12 (19.4%) had pyelonephritis, 4 (6.5%) had skin and joint infections, and 7 (11.3%) had bacteremia of unknown origin. The most frequent comorbidities were arterial hypertension, diabetes mellitus and Chronic obstructive pulmonary disease. qSOFA score median values at inclusion were higher in deceased patients when compared with survivors during hospital stay.

Microbiological investigations were positive in 72.6% of cases, Gram negative organisms were most frequent among survivors than non-survivors (65.4% vs. 30%, p=0.037). Nineteen patients (30.6%) developed septic shock within 48 h after admission, whereas the remaining 43(69.4%) patients had no hemodynamic deterioration during the first 48 h. The ICU mortality rate was 16.1% (10/62). Septic shock was more frequent among non-survivors than survivors (60% vs. 25%, p=0.028).

Table 1. Patient characteristics at admission as a function of in-hospital mortality

	All (n=62)	Non-survivors (n=10)	Survivors (n=52)	*P*-value
Demografics				
Gender (%) Female	25 (40.3)	4 (40)	21 (40.4)	0,982
Male	37 (59.7)	6 (60)	31 (59,6)	
Age (mean ± SD)	52 (19,477)	51.4 (16.972)	52.13 (20.07)	0.338
Primarysite of infection (%)				
Lung	25 (40.3)	6 (60)	19 (36.5)	0.166
Urinarytract	11 (17.7)	0	11 (21.2)	0.109
Abdomen	16 (25.8)	2 (20)	14 (26.9)	0.647
Softtissueinfections	3 (4.8)	0	3 (5.8)	0.436
Multiplesites of infection	3 (4.8)	1 (10)	2 (3.8)	0.406
Unknownfoci	4 (6.5)	1 (10)	3 (5.8)	0.618
Comorbidities (%)				
Hypertension	26 (41.9)	5 (50)	21 (40.4)	0.573
Diabetes mellitus	16 (25.8)	3 (30)	13 (25)	0.741
Coronaryarterydisease	10 (16.1)	2 (20)	8 (15.4)	0.716
Malignancy	4 (6.5)	0	4 (7.7)	0.365
COPD	18 (29)	4 (40)	14 (26,9)	0.404
Liverdisease	9 (14.5)	1 (10)	8 (15.4)	0.658
Neurologicaldisease	5 (8.1)	1 (10)	4 (7.7)	0.806
Renal disease	5 (8.1)	0	5 (9.6)	0.306
Evolvingorgandysfunction (%)				
Acute renal failure	9 (14.5)	2 (20)	7 (13.5)	0.591
Respiratoryfailure	10 (16.1)	2 (20)	8 (15.4)	0.716
Haematologicalprocess	3 (4.8)	1 (10)	2 (3.8)	0.406
Microbes (%)				
Gram-positive bacteria	8 (12.9)	3 (30)	5 (9.6)	0.078
Gram-negative bacteria	37 (59.7)	3 (30)	34 (65,4)	0.037*
Unidentified bacteria	17 (27.4)	4 (40)	13 (25)	0.33
Outcomes				
qSOFA>2 (%)	23 (37,1)	5 (50)	18 (34,6)	0.356
ICU stay≥ 7 days (%)	37 (59.7)	6 (60)	31 (59.6)	0.982

COPD- Chronic Obstructive Pulmonary Disease; SOFA – Sequential Organ Failure Assessment;
*significant difference: $p \leq 0.05$

3.1 Biomarkers at admission.

Plasma cytokine levels for healthy individuals were undetectable or <4.36 pg/mL for IL-1-β, <8.12 pg/mL for IL-6, <11.2 pg/mL for IL-10 and <9.1 pg/mL for FNT-α.

On ICU admission, patients with septic shock showed significantly high plasma levels of IL-1-β (RR:5.743,CI 41.453–22.703), $p \leq 0.001$.The rest of biomarkers did not shown statistical differences among patients with sepsis and septic shock (table 2). The plasma levels of all cytokines measured not differ significantly in survivors and non-survivors.

Table 2. Plasma concentrations of biomarkers in patients with sepsis/septic shockat time admission

	Sepsis n= 43	**RR [95% IC]**	**Septic shock** n=19	**RR [95% CI]**	***P*-value**
IL-1-β (pg/mL), Median [25th-75th]	300 [130-500]		450 [360-500]		0,339
IL-1-β High (%)	20 (54.1)	0.588 [0.427-0.808]	17 (45.9)	5.743 [1.453-22.703]	0.001*
IL-6 (pg/mL), Median [25th-75th]	60 [7-300]		70 [30-300]		0.314
IL-6 High (%)	22 (66.7)	0.921 [0.662-1.280]	11 (33.3)	1.208 [0.564-2.589]	0.624
IL-10 (pg/mL), Median [25th-75th]	43 [27-74]		43 [16-68]		0.328
IL-10 High (%)	20 (76.9)	1.204 [0.871-1.664]	6 (23.1)	0.639 [0.280-1.459]	0.272
FNT-α (pg/mL), Median [25th-75th]	110 [60-200]		200 [110-210]		0.289
FNT-α High (%)	19 (59.4)	0.742 [0.529-1.041]	13 (40.6)	2.031 [0.886-4.655]	0.078

Me = Median, CI = Confidence Interval, TNF = Tumor Necrosis Factor.
*Significant difference: p≤0.05

3.2 Biomarkers at 48 hours.

Median plasma IL-1βlevels were significantly higher in patients with septic shock than subjects with sepsis (500 pg/mL [450–500 pg/mL] vs 200 pg/mL, [120–300 pg/mL]; p=0.004. The same trend was observed in the plasma FNT-α levels, in patients who developed septic shock, the median was230 pg/mL [210–250 pg/mL], while in patients with sepsis, the median was determined in60pg/mL [61–110 pg/mL]; p=0.002(table 3).

We chose to use the cut-off used in their study (> 350 pg/mL, 150pg/mL, 66 pg/mL and 130 pg/mL) to categorize our sample of patients in groups with Low and High plasmaIL-1β,IL-6, IL-10 and FNT-α levels, respectively. Patients who presented high levels of IL-1β (63%) and TNF-α (66.7%) during the first 48 hours of admission to the ICU, were more likely to develop septic shock(RR: 11,019, CI 2.782-43.643;RR: 11,019, CI 2.749-25.939, respectively)with p.≤0.001.

Table 3. Plasma concentrations of biomarkers in patients with sepsis/septic shock at 48 hours in ICU.

	Sepsis (n= 43)	**RR [95% CI]**	**Septic shock (n=19)**	**RR [95% CI]**	***P*-value**
IL-1β (pg/mL), Median [25th-75th]	200 [120-300]	-	500 [450-500]	-	0.004*
IL-1β High (%)	10 (37.0)	0.393 [0.239-0.647]	17 (63.0)	11,019 [2.782-43.643]	≤0.001*
IL-6 (pg/mL), Median [25th-75th]	7 [7-230]	-	50 [7-200]	-	0,337
IL-6 High (%)	11 (57.9)	0.778 [0.510-1.186]	8 (42.1)	1,646 [0.790-3.427]	0.193
IL-10 (pg/mL), Median [25th-75th]	45 [30-69]	-	66 [46-90]	-	0,296
IL-10 High (%)	18 (60.0)	0.768 [0.544-1.084]	12 (40.0)	1,829 [0.832-4.021]	0.122
FNT-α (pg/mL), Median [25th-75th]	61 [60-110]	-	230 [210-250]	-	0.002*
FNT-α High (%)	8 (33.3)	0.362 [0.204-0.642]	16 (66.7)	8,444 [2.749-25.939]	≤0.001*

*significant difference: p≤0.05

Patients who did not survive had a higher plasma cytokine level than those who survived, although this difference was not significant (data not shown).

3.4 ROC analyses.

Receiver operator curves were generated to determine the cut-off values for optimal sensitivity and specificity for the IL-1β, IL-6, IL-10 and TNF-α plasma levels for patients with sepsis or shock septic.

On ICU admission, only TNF-α levels fairly a good predictor of septic shock in our patient population (Fig. 1A; Table 4). The area under the curve (AUC) for TNF-α was 0.659 (p = 0.047). However, patients who developed septic shock during the first 48 hours of admission to the ICU presented AUC for IL-1βof 0.916 (p ≤0.001) with sensitivity of 84% and specificity of de 93%; Cut-off 415pg/mL. In this case TNF-α, AUC was 0.890 with p<0.001, sensitivity of 84%,specificityof 91%,Cut-off 157.5 pg/mL(Fig. 1B; Table 4).The patients with higher values than these cut-off levels had a higher probability of developing septic shock compared with the patients with lower values.

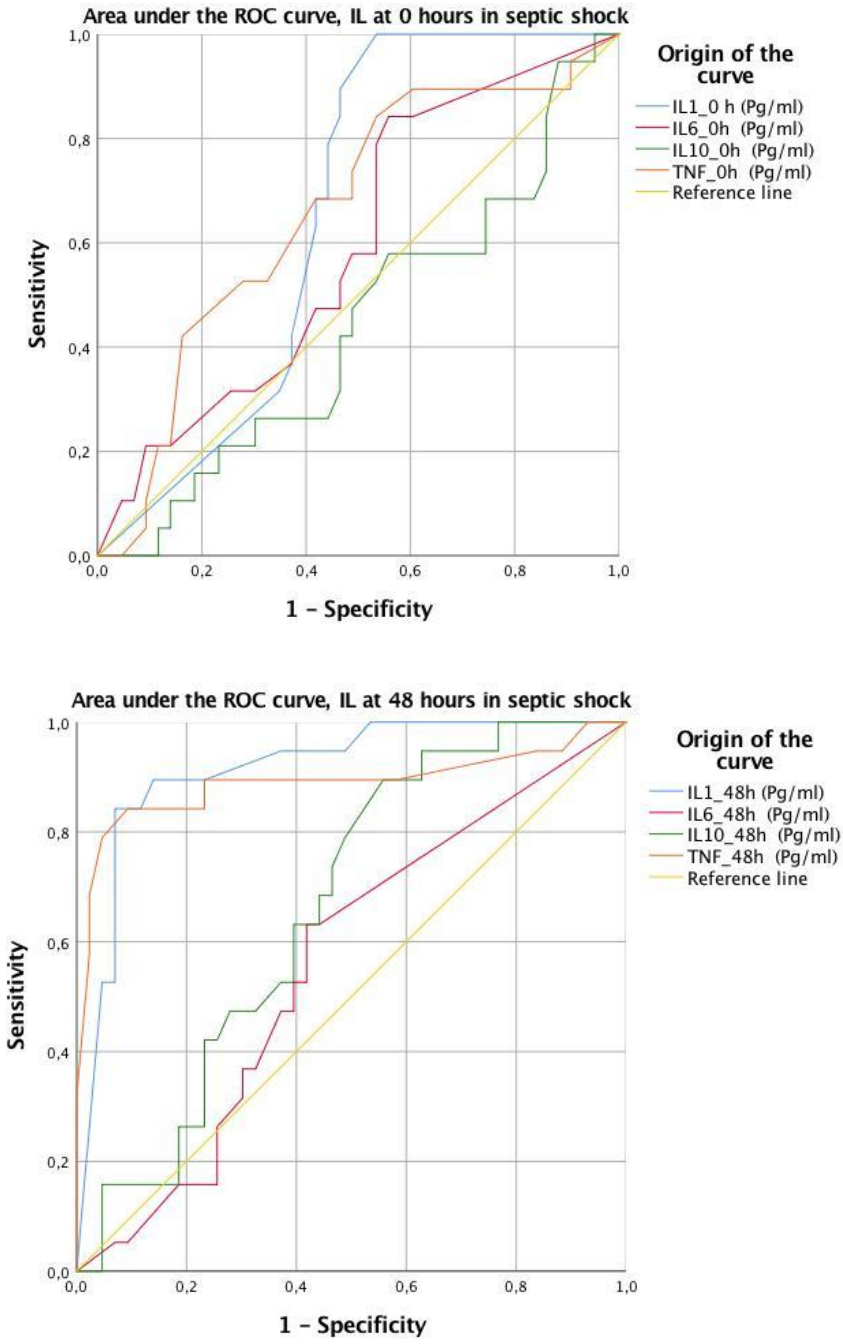

Fig. 1 Survival ROC curves for quotient of IL-1β, IL-6, IL-10 and TNF-α levels at admission (A) and at 48 hours after admission in the ICU (B).Area under the ROC curve for IL-1β, IL-6, IL-10 and TNF-α plasma levels in patients with septic shock. ROC = receiver operating characteristic;

Table 4. Area under the ROC curve for individual cytokine measurements and the presence of sepsis and septic shock.

	SEPSIS				SEPTICO SHOCK				
Mediator	**Cut-off (Pg/ml)**	**Sensitivity (%)**	**Specificity (%)**	**AUC (%)**	**Cut-off (Pg/ml)**	**Sensitivity (%)**	**Specificity (%)**	**AUC (%)**	**p value**
At admission									
IL1β	480	35	68	0.346	290	54	47	0.654	0.055
IL6	162.5	37	63	0.409	14	84	44	0.591	0.255
IL10	59.5	44	74	0.561	9	95	12	0.439	0.445
FNT-α	305	5	100	0.341	86	84	47	0.659	0.047*
48 Hours									
Mediator	**Cut-off (Pg/ml)**	**Sensitivity (%)**	**Specificity (%)**	**AUC (%)**	**Cut-off (Pg/ml)**	**Sensitivity (%)**	**Specificity (%)**	**AUC (%)**	**p value**
IL1β	-	-	-	0.084	415	84	93	0.916	≤0.001*
IL6	215	26	84	0.441	14	63	58	0.559	0.464
IL10	235	2	100	0.339	44	90	44	0.661	0.045*
FNT-α	-	-	-	0.110	157.5	84	91	0.890	≤0.001*

*significant difference: p≤0.05

4. Discussion.

As established at the International Sepsis Forum, a molecular biomarker of sepsis should be able to reflect the biochemical changes of the cellular and subcellular response at the plasma level (Singer et al, 2016). Several studies have established associations between the plasma levels of certain cytokines with the development of sepsis(Xiao et al, 2015; Matsumoto et al, 2018).

It has been reported that IL-1β, IL-16, IL-10 and TNF-α are critical cytokines in the pathophysiology of sepsis and play a pivotal role in the progression of the sepsis response(Wu et al, 2009; Schulte, Bernhagen, &Bucala, 2013). In our study was aimed at assessing the levels of this cytokines in the blood plasma of patients in the early onset of sepsis. Cytokine levels were not related to septic focus, comorbidities or the characteristic of the etiologic agent and none of the inflammatory markers in our study were found to be predictive of multi-organ failure.

Patients with septic shock showed at admission significantly High plasma IL-1β levels. Although the role of IL-1β in sepsis has not been extensively studied, some researchers suggest that IL-1β may play a role in sepsis. It has been reported that there is an significant increase in IL-1β level as early as 12 hours in mice with sepsis (Roderburg et al, 2016). Mera et al,2011 reported that during the first seven days after admission ofpatients with sepsis, IL-1β exhibited persistent increases in those who died.

Interestingly, patients with sepsis showed a rapid IL-1β reduction, while patients with septic shock persistently high IL-1β concentrations during the first 48 hours of admission to the ICU. The results of this study suggest that IL-1β is an important marker of septic shock in our study population. Plasma IL-1β level at a threshold of 415pg/mL and higher is 84% sensitive and 93% specific for diagnosis of septic shock.

Another significant finding is that plasmaTNF-α, particularly on 48 hours of admission to the ICU, also shows septic shock predicting capacity, patients with septic shock persistently High plasma TNF-α levels. In this trial, plasma TNF-α level at a threshold of 157.5 pg/mL and higher is reported to be 84% sensitive and 91% specific for diagnosis of septic shock. These findings are in accordance with previous studies that have established the role of TNF-α with the severity of the disease. Bosa et al, TNF-α concentrations were significantly higher in patients with septic shock than in those with severe sepsis (Comim et al, 2011). The concentration of TNF-α is correlated with severity of sepsis and TNF-α elevated is poor prognosis in sepsis patients.

Numerous studies have reported that the concentration of IL-1β and TNF-α are correlates with the severity of sepsis (Reinhart, Bauer, Riedemann, & Hartog, 2012; Cioara, Valeanu, Todor, Cristea, &Lupse, 2016; Comim et al, 2011; Almawash, 2018).In this sense, the use of anti-TNF and anti-IL-1β to prevent death in septic patients are usefulness (Almawash, 2018). Although some studies indicate the role of Il-6 and Il-10 as predictors of severity of sepsis and mortality (Ramnath et al, 2009; Jekar et al, 2015). In this study not found significant differences between the high levels of these cytokines in the type of sepsis or patient survival.

Mortality in our study was a 16.1% and patients with septic shock results in an attributable mortality of about 60%. However,the results of this study show that there were no difference in IL-1β, IL-6, IL-10 and TNF-α plasma level in the first hoursof admission to the ICU between survivors and non-survivors of sepsis or septic shock. A limitation of this study was that a relatively small number of patients and the use of data from a single institution were included. It is necessary to expand the sample, including patients of various institutions that help clarify the role of different cytokines in the pathogenesis of sepsis of great importance for the treatment of patients with sepsis.

5. Conclusion.

Measurement of plasma IL-1β and TNF-α in patients with septic shock indicated that the plasma levels of these cytokines level were significantly enhanced, suggesting that IL-1β and TNF-α were better to predict the sepsis severity within the first 48 hours in our study population

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Conflicts of interest. The authors have no conflict to interests to declare.

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