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ORIGINAL ARTICLE

# Prognostic value of vascular endothelial growth factor in sepsis syndrome

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**KEYWORDS**

Sepsis;
VEGF;
Microalbuminuria

**Abstract** *Background:* Serum vascular endothelial growth factor (VEGF) levels are increased in sepsis.

*Purpose:* To investigate the prognostic value of the serum VEGF level in critically ill septic patients regarding the clinical course and final outcome.

*Methods:* A total of 40 critically ill septic patients were included in a prospective, randomized, single center study. All patients were subjected to the measurement of VEGF levels on admission day (VEGF1) and 48 hours later (VEGF2). CRP levels and Microalbuminuria levels were also measured on admission. APACHE IV and SOFA scores were calculated. Clinical outcome (duration of stay in the ICU, need for MV, need for inotropic/vasopressor support, need for hemodialysis, and survival) was recorded.

*Results:* In relation to healthy subjects, the mean VEGF 1&2 levels were significantly higher in the septic patients (142 + 28.98 vs 750.5 + 380.34 and 802.07 + 292.65 ng/l; $p = 0.001$ and $< 0.001$ respectively). Septic patients who required MV, inotropic/vasopressor support and hemodialysis, and also those who died had significantly higher VEGF1 levels compared to those who didn't require them ($p = 0.002$, 0.006, 0.008 and 0.001 respectively). VEGF2 level was significantly higher only in those who required inotropic/vasopressor support ($p = 0.024$). VEGF1 and 2 levels were significantly positively correlated with CRP level, Albumin/Creatinine ratio and APACHE IV score. ROC analysis of the data indicated a sensitivity of 85.15% and a specificity of 92.3% when a VEGF 1 level of 410 ng/l was taken as a predictor of ICU mortality.

*Conclusion:* The admission VEGF is a useful marker for the evaluation of septic patients.



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## 1. Introduction

The prognosis of patients is important in risk stratification and for the efficient use of hospital resources. Predicting the outcome of patients in the intensive care environment is of particular significance to ensure that resources are used appropriately. Incidence of sepsis is increasing. Severe sepsis, which occurs when sepsis progresses to involve acute organ dysfunction, results in more than 200,000 annual fatalities, and the number of cases is projected to increase [1].

Vascular permeability increases in response to systemic inflammation mediated by endotoxin and various cytokines. Macrophages and lymphocytes can produce vascular endothelial growth factor (VEGF) [2,3]. The vascular permeability factor was isolated in 1983 [4]. VEGF was identified in 1989, and in the same year, these two substances were found to be identical [5,6].

There are seven different VEGFs (VEGF-A,-B,-C,-D,-E,-F and placental growth factor PlGF), which have different physiological and biological properties. There are at least 6 VEGF-A isoforms of different sizes (with 121,145, 165, 183, 189 and 206 amino acid residues) [7,8]. VEGFs are involved, for instance, in wound healing, cardiovascular diseases, tumour growth and progression, ocular neovascularization and inflammatory diseases such as rheumatoid arthritis. In particular, VEGF-A acts on endothelial cells, causing vasodilatation by induction of endothelial nitric oxide synthase [9]. VEGF-A also has antiapoptotic effects on endothelium [10]. More importantly, VEGF-A was found to be an important mediator of vascular permeability [5]. Also, VEGF is a potent hypoxia-induced mediator in the formation of new capillaries (angiogenesis). VEGF-induced angiogenesis was also found to play an important role in the etiology of several additional diseases associated with abnormal angiogenesis as tumor angiogenesis [11–13] and in wound repair [14].

A number of prognostication tools have been developed for prediction of outcome in the critically ill septic patients, such as scoring systems (including Acute Physiology and Chronic Health Evaluation IV "APACHE IV" [15] and The Sequential Organ Failure Assessment score "SOFA" [16]) and chemical biomarkers (including CRP [17–19], procalcitonin [20], microalbuminuria [21,22] and inflammatory cytokines as IL6 and IL8 [23]).

The aim of this work is to investigate the prognostic value of VEGF concentrations in critically ill septic patients, and also to compare this prognostic value of VEGF with other biochemical markers for the prognosis of sepsis (CRP and microalbuminuria) and with the APACHE IV and SOFA scoring systems.

## 2. Patients and methods

### 2.1. Patients

Forty patients who had been diagnosed with sepsis and were admitted to the Critical care department at Cairo university hospital from September 2013 to August 2014 were enrolled in this prospective observational single centre study. The study protocol was approved by the ethics committee. This study did not interfere with normal routine patient management.

**Inclusion criteria:** (1) Age $\geqslant$ 18 years old (2) Informed consent given by the patient or immediate relative (first degree) (3) Sepsis (ACCP/SCCM criteria) [24]: (a) Clinically suspected infection as per the treating physician or confirmed infection and (b) 2 or more of the following: Temperature $>38$ °C (100.4°F) or $<36$ °C (96.8°F), heart rate (HR) $>$ 90/min, respiratory rate (RR) $>$ 20/min or $PaCO_2 < 32$ mmHg, White blood cell count $> 12{,}000/mm^3$ or $<4000/mm3$ or $>10\%$ immature neutrophils. ***Severe sepsis*** is defined as sepsis plus sepsis-induced organ dysfunction or tissue hypoperfusion. ***Septic shock*** is defined as sepsis-induced hypotension persisting despite adequate fluid resuscitation. ***Sepsis-induced hypotension*** is defined as a systolic blood pressure (**SBP**) $<90$ mmHg or mean arterial pressure (**MAP**) $<70$ mmHg or a **SBP** decrease $>40$ mmHg in the absence of other causes of hypotension.

**Exclusion criteria** included trauma, burns, acute myocardial infarction and patients with a history of autoimmune disease or malignancy.

Patients who were diagnosed as having sepsis at ICU admission and did not meet any of the exclusion criteria were included into the study on the day of ICU admission, and subsequently followed up until the day of discharge or demise.

The blood samples were also collected from 10 healthy age matched subjects as a control.

### 2.2. Evaluation of patients

All included patients were subjected to the following

#### 2.2.1. Full clinical evaluation

Including a history and physical examination with a special emphasis on vital signs (BP, HR, Temperature and RR) and Glasgow coma scale (GCS); these were evaluated on the day of admission and then followed up daily (every 2 h for vital signs and once daily for GCS).

#### 2.2.2. Laboratory investigations

- **Routine Labs:** ***CBC (complete blood count):*** Hemoglobin, Hematocrit, White blood cells and platelet count, ***Coagulation profile:*** PT, PC, INR and PTT, ***Kidney Function Tests:*** Na, K, Creatinine and Urea, ***Liver Function Tests***: ALT (Alanine aminotransferase), AST (Aspartate aminotransferase), BIL (bilirubin) and albumin and ***ABGs*** (arterial blood gases).
  These routine Labs were withdrawn on study day 1 and subsequently thereafter every day until ICU discharge or demise.
- **Labs specific for this study:** ***Total VEGF (Vascular Endothelial Growth Factor)*** [25,26]: VEGF was measured using a double-antibody sandwich enzyme-linked immune sorbent assay (ELISA) on the day of admission (VEGF1) and after 48 h i.e. on the morning of the third day (VEGF2). VEGF was added to the monoclonal antibody enzyme well, which was pre-coated with an incubated human VEGF monoclonal antibody. Then, VEGF antibodies labeled with biotin were added and combined with Streptavidin-HRP to form an immune complex, which was then incubated and washed again to remove the uncombined enzyme. After this, a chromogen solution was added, causing the color of the liquid to

turn blue, and with the effect of acid, the color finally becomes yellow. Both the chroma of color and the concentration of VEGF of sample were positively correlated. ***CRP (C-reactive protein)***[18]: Measured by ELISA on the day of admission, which is based on the principle of a solid phase enzyme-linked immunosorbent assay utilizing a mouse monoclonal antibody against distinct determinants for immobilization on the microtiter wells, and a goat anti-CRP antibody conjugated to horseradish peroxidase (HRP) for detection. ***Microalbuminuria*** [22]: It is generally expressed as the urinary albumin to creatinine ratio to correct the variations in urinary flow rate. Urinary micro albumin was measured using the immunoturbidimetric method and urinary creatinine through a modified kinetic Jaffe reaction (Dimension RxL Max, Dade Behring Inc., U.S.A) on the day of admission. Microalbuminuria was defined by Albumin/Creatinine Ratio values between 30 and 299 mg/g creatinine. Albumin/Creatinine Ratio > 300 mg/g creatinine is considered as clinical proteinuria. Albumin/Creatinine Ratio < 30 mg/g creatinine is normal.

#### 2.2.3. Clinical and outcome data

Length of ICU stay, final outcome of survival rates and need for organ supportive measures (Vasopressors, MV and/or Hemodialysis) were reported for all patients until ICU discharge or demise.

#### 2.2.4. Application scoring systems

**APACHE IV** score [15], which is a severity of disease classification system, was evaluated on the day of admission. After admission, an integer score is computed based on several measurements; higher scores imply a more severe disease and a higher risk of death. **SOFA** score [16] was evaluated on study day 1 and serially every other day until ICU discharge or demise. This score determines the extent of a person's organ function or rate of failure.

#### 2.2.5. The statistics

Continuous variables were summarized using range, mean ± SD. Categorical variables were summarized using frequencies and relative frequencies. A comparison of quantitative variables between the study groups was conducted using a Mann Whitney *U* test for independent samples. Accuracy was represented using the terms *sensitivity* and *specificity* of VEGF levels. A receiver operator characteristic (ROC) analysis was used to determine the optimum cut off value for the studied diagnostic markers. Correlation between various variables was done using a Spearman rank correlation equation for non-normal variables. A probability value ($p$ value) of less than 0.05 was considered statistically significant. All statistical calculations were done using SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 22 for Microsoft Windows.

## 3. Results

### 3.1. Demographic and baseline clinical data at ICU admission

Table 1.

### 3.2. VEGF and baseline clinical characteristics

On admission, VEGF was measured in the serum of all included patients, whereas VEGF 2 was measured only in the serum of 29 patients (as 11 patients died during the first 48 h of ICU admission). The mean level of VEGF1 was 750.50 ± 380.34 ng/l, approximately 6 folds higher than control subjects, (142 ± 28.98 ng/l, $P = 0.001$). The mean VEGF 2 level was 802.07 ± 292.65 ng/l, approximately 7 folds higher than control subjects, ($P < 0.001$), but there was no significant correlation between admission VEGF and VEGF 2, ($P = 0.272$). Moreover, there was no significant correlation between plasma VEGF1 and VEGF 2 levels and age ($p = 0.258$ and 0.068 respectively). Also, there was no significant correlation between the plasma VEGF1 and VEGF 2 levels and sex ($p = 0.171$ and 0.433). Also there was no significant correlation between postoperative and medical patients ($p = 0.570$ and 0.229).

The elevated levels of VEGF in the studied septic patients increased with the increasing severity of the septic condition at ICU admission, at which point it could be seen that the patients with a diagnosis of septic shock at ICU admission had significantly higher plasma VEGF1 levels (911.18 ± 449.86 ng/l) than those with sepsis (575 ± 212.94 ng/l, $p = 0.009$). However, no significant difference was found between plasma VEGF1 levels in patients with severe sepsis (1043.33 ± 353.46 ng/l) and those with sepsis and septic shock ($P = 0.142$ and 0.604 respectively) Table 2.

### 3.3. VEGF and severity of illness during ICU stay (need for organ supportive measures)

The mean level of the VEGF1 was significantly higher in patients who required MV (29 patients) (836.21 ± 399.85 ng/l) than those who did not require it (11 patients) (524.56 ± 197.55 ng/l, $P = 0.002$), and also in those who required inotropic/ vasopressor support (26 patients) (853.84 ± 406.55 ng/l) versus the group who did not require it (14 patients) (565.71 ± 208.45 ng/l, $P = 0.006$). In addition, higher VEGF1 level was found in those who required hemodialysis (11 patients) (824.83 ± 407.16 ng/l) versus those who did not require it (29 patients) (554.55 ± 204.27 ng/l, $P = 0.008$). Also, the mean VEGF 2 level was significantly higher in patients who required inotropic/ vasopressor support (20 patients) (927.37 ± 279.64 ng/l) versus those who did not require it (9 patients) (720 ± 169.12 ng/l, $P = 0.024$). On the other hand, there were no significant differences seen in the mean VEGF 2 level between patients who did not require MV (6 patients) (813.33 ± 132.77 ng/l) and those who required it (23 patients) (799.13 ± 323.97 ng/l), ($P = 0.871$) and also in those who required hemodialysis (8 patients) (737.50 ± 197.76 ng/l) and those who did not require it (21 patients) (826.67 ± 322.36 ng/l, $P = 0.378$).

### 3.4. VEGF and length of ICU stay, CRP, microalbuminuria, APACHE-IV and SOFA

An insignificant correlation was found between VEGF1 level and the length of ICU stay, ($r = 0.015$, $p = 0.927$); however, a significant positive correlation was found between VEGF 2 level and the length of ICU stay ($r = 0.448$, $p = 0.015$).

**Table 1** Demographic and clinical data of patients entered into the study.

| Variables | | Values |
|---|---|---|
| Age (years) | Mean ± SD (Range) | 69.25 ± 8.79 (53–82) |
| Male: Female sex | (Ratio) | 19: 21 (0.904) |
| *Clinical history* | | |
| Hypertension | N(%) | 12 (30%) |
| Diabetes mellitus | N(%) | 18 (45%) |
| Medical: Post-operative cases | (Ratio) | 11: 29 (0.379) |
| *Etiology of sepsis* | | |
| Chest Infection | N(%) | 14 (35%) |
| Urinary tract infection | N(%) | 10 (25%) |
| Soft tissue infection | N(%) | 10 (25%) |
| Intra abdominal sepsis | N(%) | 3 (7.5%) |
| CNS infection | N(%) | 3 (7.5%) |
| *Severity of sepsis at ICU admission* | | |
| Sepsis | N(%) | 20 (50%) |
| Severe sepsis | N(%) | 3 (7.5%) |
| Septic shock | N(%) | 17 (42.5%) |
| *Baseline hemodynamic* | | |
| Heart rate (beat/min) | Mean ± SD | 107.3 ± 28.43 |
| Respiratory rate (breath/min) | Mean ± SD | 23.7 ± 6.61 |
| Temperature (°C) | Mean ± SD | 37.69 ± 0.80 |
| Systolic blood pressure (mmHg) | Mean ± SD | 97.5 ± 17.20 |
| Diastolic blood pressure (mmHg) | Mean ± SD | 61.57 ± 10.25 |
| Mean blood pressure (mmHg) | Mean ± SD | 73.45 ± 12.32 |
| Central venous pressure ($CmH_2O$) | Mean ± SD | 8 ± 6.65 |
| GCS | Mean ± SD | 13.9 ± 1.13 |
| *Baseline Laboratory investigations* | | |
| Hemoglobin (gm/dl) | Mean ± SD | 10.25 ± 1.76 |
| Hematocrite (%) | Mean ± SD | 30.60 ± 4.42 |
| Leucocytes (cell/$mm^3$) | Mean ± SD | 13.46 ± 7.75 |
| aPTT (s) | Mean ± SD | 36.6 ± 8.29 |
| INR | Mean ± SD | 1.54 ± 0.57 |
| ALT (U/L) | Mean ± SD | 63.15 ± 37.40 |
| AST (U/L) | Mean ± SD | 57.37 ± 39.52 |
| Total bilirubin (mg/dl) | Mean ± SD | 1.72 ± 1.44 |
| Albumin (g/dl) | Mean ± SD | 2.29 ± 0.39 |
| Blood urea (mg/dl) | Mean ± SD | 121.05 ± 71.88 |
| Serum creatinine (mg/dl) | Mean ± SD | 2.34 ± 1.36 |
| Sodium (mEq/L) | Mean ± SD | 134.15 ± 6.96 |
| Potassium (mEq/L) | Mean ± SD | 3.88 ± 0.79 |
| *Clinical coarse and outcome* | | |
| Need for mechanical ventilation | N(%) | 29 (72.5%) |
| Duration of mechanical ventilation (day) | Mean ± SD | 4.47 ± 3.28 |
| Need for inotropic/vasopressor support | N(%) | 26 (65%) |
| Need for hemodialysis | N(%) | 11 (27.5%) |
| Duration of ICU stay (day) | Mean ± SD | 3.53 ± 1.81 |
| Mortality | N(%) | 24 (60%) |

**N:** number; **aPTT:** activated partial thromboplastin time; **AST:** alanine aminotransferase, **AST:** aspartate aminotransferase; **GLS:** Glasgow coma scale

VEGF1 level was significantly positively correlated with the CRP level at ICU admission ($r = 0.475$, $p = 0.002$), with microalbuminuria level at ICU admission ($r = 0.623$, $p < 0.001$) and also with APACHE IV score ($r = 0.397$, $p < 0.001$), whereas this was not the case with the SOFA score ($r = 0.153$, $p = 0.345$). Table 3 and 4.

### 3.5. VEGF and final outcome

The ICU mortality rate was 60% (24 of 40 patients). The mean level of VEGF1 in non-survivors was 893.33 ± 408.26 ng/l, which was significantly higher than that in survivors (542.50 ± 207.54 ng/l, $P = 0.001$). However, no significant difference was found between the VEGF 2 level in non survivors (792.22 ± 356.71 ng/l) and survivors (889.09 ± 37 ng/l), ($P = 0.268$).

### 3.6. Prognostic ability of VEGF

The receiver operator characteristic (ROC) curve was calculated for the use of VEGF1 level as a predictor of ICU mortality. The area under the ROC (AUROC) curve for VEGF1 to predict ICU mortality was 0.746 (95% confidence interval, 0.594–0.898, $p = 0.009$). The optimal cutoff value for VEGF1 to predict ICU mortality was 410 ng/l. This cutoff value gave a sensitivity of 85.15% and a specificity of 92.3% for ICU mortality.

AUROC curves were also calculated for CRP, Microalbuminuria, APACHE IV and SOFA scores in the prediction of ICU mortality. The AUROC curve for CRP was 0.462 (95% CI, 0.281–0.643); the best cut-off value to predict ICU mortality was 25 mg/L with a sensitivity of 56.5% and a specificity of 35.3%. The AUROC curve for Microalbuminuria was 0.382 (95%CI, 0.207–0.757); the optimal cut-off value was 357 mg/g with a sensitivity of 50% and a specificity of 21.4%. The AUROC curve for SOFA score was 0798. (95%CI, 0.633–0.963); the best cutoff score was 9 with a sensitivity of 81.8% and a specificity of 66.7%. The AUROC curve for APACHE IV score was 0.404 (95%CI, 0.218–0.589); the optimal cut-off score was 98 with a sensitivity of 78.6% and a specificity of 100%.

## 4. Discussion

The risk stratification of severely ill patients remains problematic, resulting in an increased interest in potential circulating markers such as VEGF. Some studies have shown that serum VEGF level is increased in polytrauma and severe sepsis [2,3].

In the current study there was no significant correlation between VEGF level and age ($P = 0.258$) or sex ($P = 0.171$). This finding is concurrent with the result of Karlsson et al. [27] who demonstrated that neither age nor sex correlated with VEGF level.

The VEGF1 and 2 levels were significantly higher in all included septic patients when compared with the levels detected in healthy controls ($P = 0.001$ and $< 0.001$ respectively). However, no significant differences were observed between the VEGF1 and VEGF2 levels ($P = 0.272$).

**Table 2** VEGF and severity of sepsis at ICU admission of patients entered into the study.

| Severity of sepsis | No. | Mean (ng/l) ± SD | Min. | Max. | Med. | P Value |
|---|---|---|---|---|---|---|
| *VEGF 1* | | | | | | |
| Sepsis | 20 | 575 ± 212.94 | 280 | 870 | 480 | *0.142 |
| Severe sepsis | 3 | 1043.33 ± 353.46 | 810 | 1450 | 870 | **0.009 |
| Septic shock | 17 | 911.18 ± 449.86 | 400 | 1600 | 820 | ***0.604 |
| *VEGF 2* | | | | | | |
| Sepsis | 15 | 787.12 ± 163.99 | 440 | 940 | 860 | *0.917 |
| Severe sepsis | 3 | 700 ± 183.85 | 640 | 900 | 770 | **0.588 |
| Septic shock | 11 | 863.65 ± 436.26 | 440 | 1660 | 830 | ***0.641 |

* Significance between sepsis vs severe sepsis.
** Significance between sepsis vs septic shock.
*** Significance between severe sepsis vs septic shock.

**Table 3** Correlation of VEGF(ng/l) and CRP(mg/l) and Microalbuminuria (mg/g) of patients entered into the study.

| Biomarker | No. | Mean ± SD | Minimum | Maximum | Median | R | P Value |
|---|---|---|---|---|---|---|---|
| VEGF 1 | 40 | 750.50 ± 380.34 | 280 | 1600 | 775 | +0.475 | *0.002 |
| CRP 1 | | 42.20 ± 22.96 | 12 | 96 | 38 | ++0.623 | ** < 0.001 |
| Microalbuminuria | | 498.02 ± 285.32 | 44 | 872 | 495 | | |
| VEGF 2 | 29 | 802.06 ± 292.65 | 440 | 1660 | 850 | +++0.631 | *** < 0.001 |
| CRP | | 45.06 ± 22.66 | 22 | 96 | 48 | ++++0.607 | **** < 0.001 |
| Microalbuminuria | | 53.56 ± 233.51 | 163 | 872 | 581 | | |

\+ Correlation between VEGF 1 vs CRP 1.
\++ Correlation between VEGF 1 vs microalbuminuria 1.
\+++ Correlation between VEGF 2 vs CRP 2.
\++++ Correlation between VEGF 2 vs microalbuminuria 2.
* Significance between VEGF 1 vs CRP 1.
** Significance between VEGF 1 vs microalbuminuria 1.
*** Significance between VEGF 2 vs CRP 2.
**** Significance between VEGF 2 vs microalbuminuria 2.

**Table 4** Correlation of VEGF(ng/l) and APACHE IV and SOFA of patients entered into the study.

| Biomarker | No. | Mean ± SD | Minimum | Maximum | Median | R | P Value |
|---|---|---|---|---|---|---|---|
| VEGF 1 | 40 | 750.50 ± 380.34 | 280 | 1600 | 775 | +0.397 | *0.011 |
| APACHE 1 | | 87.53 ± 15.19 | 65 | 112 | 86 | ++0.153 | ** < 0.345 |
| SOFA 1 | | 9.65 ± 4.19 | 3 | 17 | 9.5 | | |
| VEGF 2 | 29 | 802.06 ± 292.65 | 440 | 1660 | 850 | +++0.410 | *** < 0.034 |
| SOFA 3 | | 12.58 ± 4.54 | 2 | 17 | 14 | ++++0.291 | **** < 0.133 |

\+ Correlation between VEGF 1 vs APACHE 1.
\++ Correlation between VEGF 1 vs SOFA 1.
\+++ Correlation between VEGF 2 vs APACHE 1.
\++++ Correlation between VEGF 2 vs SOFA 3.
* Significance between VEGF 1 vs APACHE 1.
** Significance between VEGF 1 vs SOFA 1.
*** Significance between VEGF 2 vs APACHE 1.
**** Significance between VEGF 2 vs SOFA 3.

These results were similar to the results found by Karlsson et al. [27], who reported that the median VEGF level in septic patients on day 0 was 423 ng/l, which is higher than the median VEGF level of healthy control (260 ng/l), $P = 0.029$. After 72 h, VEGF levels were still higher in septic patients, with median VEGF level of 521 ng/l versus healthy control ($P = 0.003$). However, no significant correlation was found between VEGF levels on day 0 and after 72 h.

In similarity with the above findings, Nathan et al. [28] demonstrated that the patients who had been diagnosed with sepsis or septic shock at admission to the ICU had significantly higher levels of plasma VEGF than non-infected healthy control [132 ± 112 ng/l,166 ± 171 ng/l respectively versus 58 ± 38 ng/l], ($P < 0.01$).

Regarding the severity of sepsis at ICU admission, the current study demonstrated that the elevated levels of admission VEGF in the studied septic patients increased with the increasing severity of the septic condition at ICU admission to such an extent that the patients with a diagnosis of septic shock had significantly higher plasma admission VEGF levels than those with sepsis, ($P = 0.009$); however, no significant relation was found between the plasma admission VEGF levels in patients with severe sepsis and those with sepsis or septic shock ($P = 0.142$ and 0.604 respectively). This may be explained by the small number of patients who had severe sepsis in the present study.

Also, the circulating admission VEGF levels were significantly higher in patients who needed organ supportive measures (MV, inotropic/vasopressor support and hemodialysis) during their ICU stay ($P = 0.002$, 0.006 and 0.008 respectively).

In the current study, with increasing values of APACHE IV scores reflecting the severity of sepsis and higher risk of mortality, higher level of admission VEGF was observed ($r = 0.397$, $p < 0.001$). Also, admission VEGF level was significantly positively correlated with APACHE IV score ($r = 0.397$, $p < 0.001$).

Though APACHE IV was used in this study, this observation goes with the results obtained by Nathan et al. [28] who reported that with increasing values of APACHE II scores, higher VEGF levels were observed. Also, this observation goes with the results obtained by Liu et al. [29] who found that the VEGF level showed obvious positive correlation with APACHE II score ($r = 0.510$, $P < 0.001$).

However, there was no significant correlation between the SOFA scoring system and the levels of admission VEGF and VEGF 2 ($r = 0.153$ and 0.291 respectively); this observation is in agreement with Yang et al. [30], who studied the predictive value of the VEGF level for the disease severity and organ dysfunction of patients with pneumonia-related sepsis, reporting that the role of the initial plasma VEGF level in predicting organ dysfunction was insignificant.

Also, the current study demonstrated that admission VEGF level was significantly correlated with CRP and Microalbuminuria ($r = 0.475$ with $p = 0.002$ and 0.623 with $p < 0.001$ respectively). In contrast, Basu et al. [31] reported that no significant correlation was found between level of microalbuminuria and serum VEGF-A in septic patients ($p = 0.396$). Further larger studies are needed to study these correlations in the septic patients.

In the current study, we demonstrated that ICU mortality rate was 60%. The admission VEGF level in non survivors was significantly higher than that in survivors ($P = 0.001$). However, there was no significant difference between VEGF 2 level in non survivors and in survivors ($P = 0.268$).

In agreement with the current results, Flier et al. [32] reported that maximum VEGF levels during severe sepsis were higher in non survivors than in survivors (313 ± 53 ng/l vs. 147 ± 21 ng/l; $P = 0.018$). In contrast, Yang et al. [30], reported that there was no statistically significant difference in the plasma VEGF levels between the survivors and non-survivors of pneumonia-related septic shock (219.9 ± 232.1 ng/l vs 386.5 ± 524.1 ng/l, $P = 0.455$); in addition, Karlsson et al. [27] reported that the main finding of their study was that although VEGF level is increased in severe sepsis, low VEGF level is associated with more severe forms of organ dysfunction and mortality. These observations indicate that the complex effect of VEGF in sepsis remains unclear.

ROC curve was calculated for the use of plasma VEGF level as a predictor of ICU mortality. The AUROC curve for plasma admission VEGF to predict ICU mortality was 0.746 (95% confidence interval, 0.594–.898, $p = 0.009$), with an optimal cutoff value of 410 ng/l; this value resulted in a sensitivity of 85.15% and a specificity of 92.3% for ICU mortality (Fig 1).

In contrast with these results, Karlsson et al. [27] reported that the ROC curve for hospital mortality at the time day 0 when the VEGF samples were taken showed an AUROC curve of 0.58 (95% confidence limits 0.48–0.68, $P = 0.1$). However, the ROC curve for hospital mortality and SOFA score at the time day 0 when the VEGF samples were taken showed AUROC curve of 0.73 (95% confidence limits 0.65–0.82, $P < 0.001$) (see Fig. 2).

**Limitation of the study:** (1) The relatively small number of cases may lead to a wide range of standard deviations of biomarker levels. (2) The samples were taken only at two time points; therefore, the variation trend of this biomarker in the course of sepsis could not be evaluated in the present study. The half-life of VEGF has been reported to be short, (33.7 ± 13 min) based on pharmacokinetic studies with recombinant VEGF. Nonetheless, previous studies have shown increased VEGF levels up to 29 days, indicating sustained VEGF production in patients with severe sepsis. [33] (3) Our VEGF levels were measured in serum samples. Plasma samples have been preferred by some investigators because platelet-mediated secretion of VEGF during the clotting process could interfere with the results. [34,35] However, it has been shown recently that VEGF levels were correlated between plasma and serum in paired samples in otherwise healthy women having controlled ovarian hyperstimulation. [36]

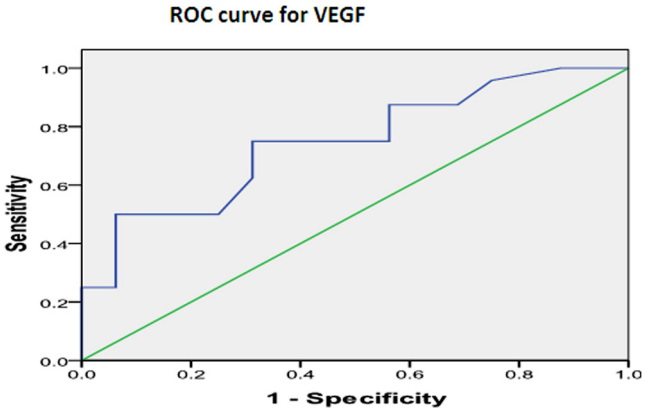


**Figure 1** ROC curve analysis of VGEF concentrations for prediction of mortality.

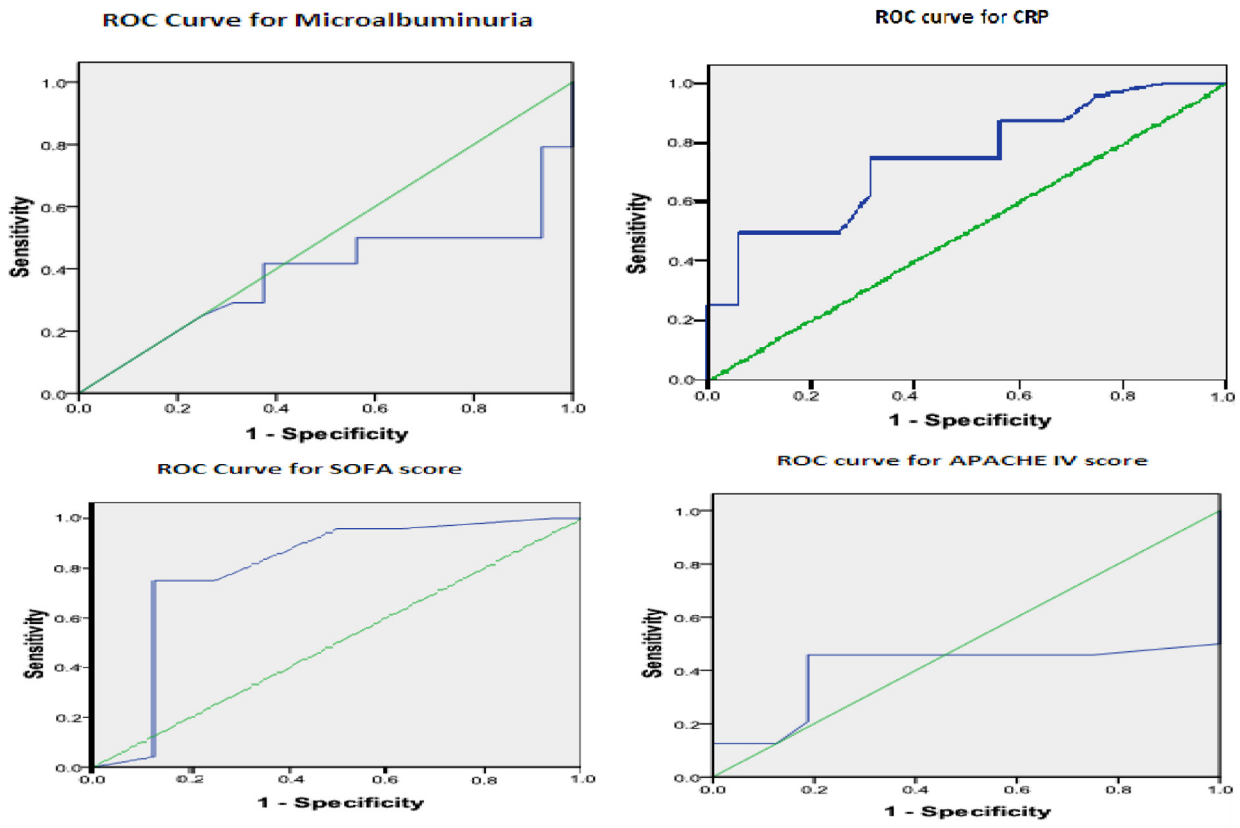


**Figure 2** ROC curve analysis of CRP concentrations, Microalbuminuria levels, APACHE IV and SOFA scores for prediction of mortality.

## 5. Conclusion

Admission VEGF (measured by ELISA) may be used as a rapid, simple and easy to perform and interpret test for the early prognosis and prediction of adverse outcome of septic patients at their ICU admission. It correlates with the severity of septic condition, mortality and needs for mechanical ventilator, hemodynamic support and hemodialysis.

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