Relationship between duration, fatality rate and severity of disease and serum epidermal growth factor in human acute lung injury

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Aim: The present study was undertaken to clarify whether serum concentrations of epidermal growth factor (EGF) are changed during the first week after the onset of acute lung injury (ALI) and to determine whether the change of EGF concentration was specific for ALI by including a control subject. **Methods:** We enrolled 30 consecutive patients with ALI, prospectively identified on admission to the intensive care unit, and ten patients in the same unit with chronic interstitial disease. The serum EGF concentration was measured on days 1 to 7 after the onset of ALI. **Results:** At each day tested, the mean EGF level of the patients with ALI was not significantly higher than that of the non-ALI controls and normal volunteers. In a univariate analysis, the mean EGF level in nonsurvivors was not higher at different days ($p > 0.05$). The overall fatality was not associated with increased serum EGF levels. **Conclusion:** It is concluded that the concentration of EGF in the serum of ALI patients does not change significantly.

The present study was undertaken to clarify whether serum concentrations of epidermal growth factor (EGF) are changed during the first week after the onset of acute lung injury (ALI) and to determine whether the change of EGF concentration was specific for ALI by including a control subject. Whether it is caused by direct assault from pneumonia or indirectly through sepsis or trauma, ALI represents a significant healthcare burden [1,2]. Fatality and morbidity associated with ALI are considerable, with a significant impact on public health [3]. The clinical course of patients with ALI is variable and influenced by different factors. One of the most important mechanisms that determines the severity of lung injury is the magnitude of injury to the alveolar epithelial barrier. The possibility of repairing epithelial injury at an early stage is a major determinant of recovery. Specific treatments to accelerate alveolar epithelial repair do not exist, although progress in studies with experimental models of ALI suggest that specific treatments may be possible in the future. The majority of treatment modalities tested recently were based on diminution of the inflammatory response in the lung in order to minimize the initial injury. However, an alternative therapeutic approach is to accelerate the repair process in the alveolar epithelium in the early stages of ALI in order to enhance resolution of pulmonary edema and improve outcomes in these patients. Little is known at present about the cellular and molecular mechanisms of alveolar epithelial repair in ALI. In particular, soluble mediators, which play a key role in alveolar epithelial repair in these patients must be identified and characterized if novel therapeutic strategies are to be developed [4]. Although the extracellular matrix, in particular fibronectin, more than likely plays an important role in the alveolar repair process [5], growth factors such as EGF have also been shown to augment alveolar epithelial repair *in vivo* and *in vitro* [4,6]. For example, it has been demonstrated that in a monolayer of mammary epithelial cells, the addition of EGF or transforming growth factor (TGF)-α result in accelerated wound closure that was associated with an upregulation of several integrin molecules [6]. Moreover, elevated levels of EGF were reported in the fluid of skin wounds in humans [7]. EGF can upregulate sodium transport and markedly increase net alveolar fluid clearance in rats [8]. Furthermore, studies in bleomycin-injured rats and transgenic mice strongly suggest that EGF plays a role in alveolar repair and remodeling after lung injury [9]. Given the mounting evidence implicating growth factors in lung homeostasis and disease, there are experimental data that support the role of growth factors in preventing damage or facilitate recovery by restoring or inducing an optimal balance of signals in the lung. A number of studies have shown that administration of a growth factor prior to ALI may be protective [10]. However, EGF concentrations in the serum of patients with established ALI and its association with clinical outcomes of the disease has not been yet evaluated.

Keywords: acute lung injury, epidermal growth factor

future medicine part of fsg

In the present study, our goals were to test the following hypothesis

- That EGF concentrations are increased in patient serum during the first week of ALI
- That changes in EGF levels are associated with fatality rates in established ALI
- Whether or not the effect of EGF on fatality rates is dependent on the severity of lung injury

Methods

All patients between the ages of 18–80 years who were admitted to the Intensive Care Unit (ICU) of an educational Hospital (Tehran, Iran), belonging to the Tehran University of Medical Sciences (TUMS) were screened prospectively for the onset of ALI. Patients were diagnosed with ALI according to the North American–European Consensus Conference definition with demonstrations of acute onset of illness, PaO_2/FIO_2 of less than 300, bilateral infiltrates on chest radiograph, and no clinical evidence of left arterial hypertension. This study was approved by the Ethics Committee of TUMS. Patients with severe hypotension (systolic blood pressure <90 mmHg) or cardiac dysrhythmias (heart rare >140 beats/min or complex ventricular ectopy) were excluded from the study for safety reasons. Prior to serum collections, levels of FIO_2, PaO_2, and positive end-expiratory pressure were recorded. Blood samples were collected serially at days 1, 3 and 7 after the onset of ALI unless the patient died or was excluded. Risk factors associated with the development of ALI were identified as previously described [11] and identified prospectively when the patient entered into the study. For this analysis, three risk categories were included: sepsis syndrome, trauma, and other risks. Trauma risk was defined as the presence of multiple long bone or pelvic fractures, pulmonary contusion, or trauma associated with multiple transfusions (≥15 units in 24 h of emergency resuscitation). The category entitled other risks included aspiration of gastric contents, drug overdose and multiple transfusions. Serum samples collected from patients in ICU with chronic interstitial disease were analyzed as a control for comparison purposes. All of the patients were admitted into the ICU because of respiratory failure associated with idiopathic pulmonary fibrosis. None of the patients complicated with sepsis. Overall samples were obtained from 30 patients with ALI, 10 with chronic interstitial lung disease as control group and 10 normal volunteers. Samples were first centrifuged and then stored at -70°C until evaluated.

Table 1. Clinical characteristics (age data as mean values).

	Acute lung injury group (n = 30)	Chronic interstitial disease group (n = 10)	Healthy group (n = 10)
Mean age (years)	45	48	47
Sex (male/female)	18/12	4/6	5/5

Quantification of EGF in serum

Quantikine human EGF kit was used. The assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for EGF has been precoated onto a microplate. Standards and samples were pipetted into the wells and any EGF present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for EGF was added to the wells. Following a wash to remove any unbound antibody enzyme reagent, a substance solution was added to the wells and color developed in correlation with the amount of EGF bound in the initial step. Color development was ceased and the intensity of the color measured.

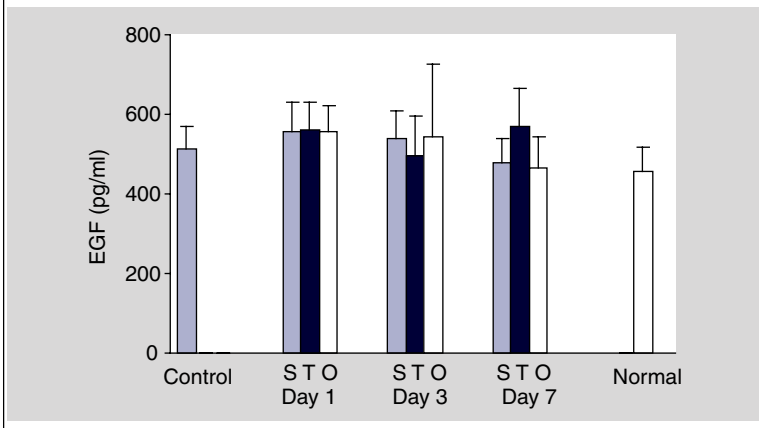
Statistical analysis

Serum EGF concentrations in patients, control and normal groups were compared initially using the one-way ANalysis Of VAriance (ANOVA) test. Similarly, the difference between EGF concentrations among the three ALI risk groups at each day was compared using the one-way ANOVA test. The association between serum EGF levels and outcome was analyzed at day 3 and 7 by independent sample t-test. The relative risk (RR) for fatality in patients with a serum EGF concentration level of 534.42 pg/ml (the mean value) or more was compared with those with values less than 534.42 pg/ml, by independent sample t-test. We also performed a stratified analysis to determine whether the relation between EGF concentration and fatality differed as a function of lung injury, using the PaO_2/FIO_2 ratio by two-way ANOVA.

Results

Serum samples were available from 30 of 30 (100%) patients prospectively identified as having ALI from January 2002–2004. Based on our review of clinical data, as well as ALI criteria, 30 patients had ALI and 10 had chronic interstitial disease. Clinical characteristics of the study

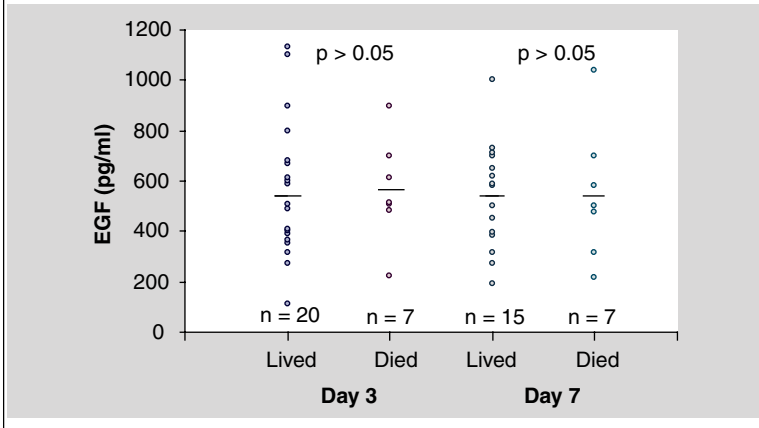
Figure 1. Serum EGF concentrations in patients with ALI segregated by risk group.



Serum epidermal growth factor (EGF) levels for normal subjects were assigned a value of 458.1 pg/ml. For each day, the mean serum EGF levels of patients with acute lung injury (ALI) and control patients were not significantly higher than that of normal subjects ($p > 0.05$). The mean serum EGF levels of patients with ALI were not significantly different among risk groups (O: Other; S: Sepsis; T: Trauma) at each day ($p > 0.05$). The mean serum EGF level of control patients was not significantly different from those of patients with ALI in each risk group at each day ($p > 0.05$). Data are reported as mean ± SEM.

population are shown in **Table 1**. Sepsis and trauma were risk factors for ALI in most of our study patients (n = 24). Other risk factors were present in six patients. Impairments of gas exchange and lung mechanics were served as indicated by PaO_2/FIO_2 ratio. EGF was detected in the serum of patients within the first 7 days after the onset of ALI. The mean EGF value for the patients with ALI was 534.42 pg/ml (range 107–1040 pg/ml). EGF was also detected in serum samples from ten of ten (100%) patients in the ICU without ALI with a mean EGF concentration of 513.8 (range 250–731 pg/ml). EGF concentrations in serum samples from ten normal volunteers were 458.1 (range 205–780 pg/ml).

Figure 2. EGF concentrations in patients with ALI who lived or died.



Mean epidermal growth factor (EGF) levels were not higher in patients who died than in those who survived at 3 and ($p = 0.852$) and 7 days ($p = 0.938$). Horizontal lines indicate the mean value. Individual patient serum EGF concentration in pg/ml for survivors and nonsurvivors.

At each day tested, the mean serum EGF level of patients with ALI was not significantly different from the normal group ($p > 0.05$) (**Figure 1**). There was no significant difference in mean serum EGF levels among the three ALI risk groups at each day. Similarly, there was no significant difference in mean serum EGF levels between control patients compared with ALI for the three risk groups at each day. The association between serum EGF levels and outcome was analyzed at each day (**Figure 2**). There were no higher mean serum EGF levels in patients who died compared to those who survived.

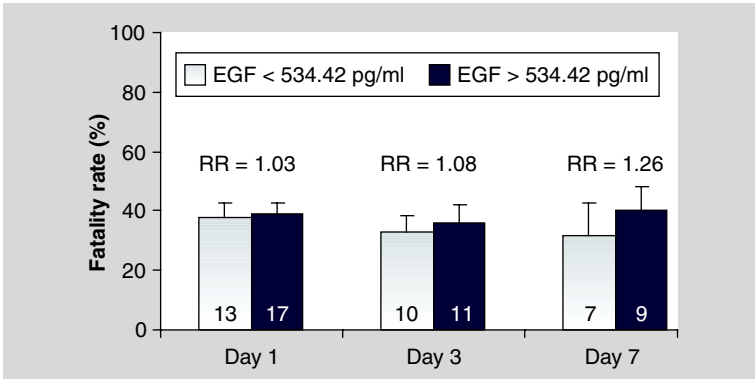
Serum EGF concentration was analyzed as a dichotamous variable in order to determine the RR for fatality after the onset of ALI. The cutoff value for EGF (534.42 pg/ml) used in this categorical analysis was the mean value of our study population. At 1, 3 and 7 days after onset, the RR for fatality was approximately 1.08, 1.08 and 1.26 times in patients with elevated serum EGF concentrations or 534.42 or more compared with those with concentrations less than 534.42 respectively (**Figure 3**).

To investigate the relationship between serum EGF levels and severity of lung injury, patient populations were stratified on each day by $PaO2/FIO_2$ (<200 compared with ≥200) (**Figure 4**). At day 1 after onset, patients with decreased $PaO2/FiO_2$ and elevated EGF levels had a fatality rate of 51.86% compared with 45.66% for patients with decreased PaO_2/FIO_2 alone, representing a RR for fatality of approximately 1.14. The fatality rate for patients with both decreases in PaO_2/FIO_2 and elevated serum EGF levels was 1.3 times more than that of patients with only low PaO_2/FIO_2 at day 3. Similarly, at 7 days after onset, patients with low PaO_2/FIO_2 and high EGF concentrations had an approximately 1.25-fold increase in the RR for fatality compared with increased lung injury scores alone.

Discussion

The major goal of this study was to investigate the relationship between EGF concentrations in serum and the course of patients with established ALI. We found that EGF levels in serum are not significantly elevated in patients with established

Figure 3. Fatality rates in patients with ALI according to serum EGF concentration.

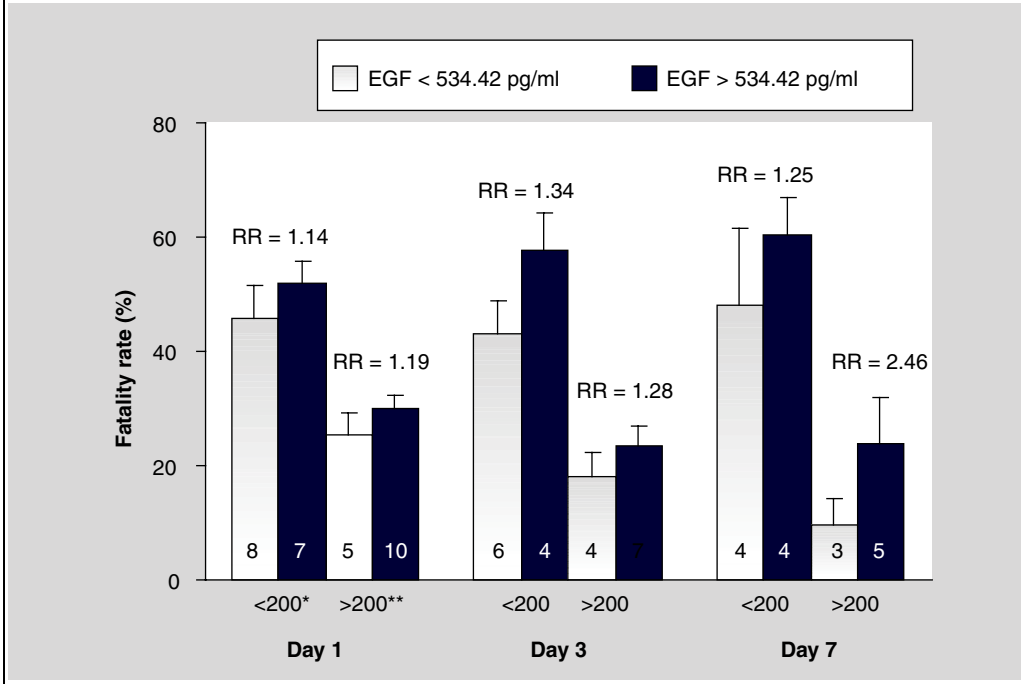


Fatality rates for patients with epidermal growth factor (EGF) less than 534.42 pg/ml compared with levels of 534.4 pg/ml or more. There were no significant differences in fatality between the groups on the various days. The number of patients in each category is indicated within the bar. RR: Relative risk.

ALI. At each day, the median serum EGF levels of patients with ALI are not significantly higher than that normal. Also in patients with sustained ALI, there is not any trend toward increased fatality when serum EGF levels are changed.

There is evidence that pulmonary edema fluid from ALI patients can increase alveolar epithelial repair *in vitro* by an interleukin(IL)-1β-dependent mechanism. Further studies revealed that IL-1β induces alveolar epithelial repair by an epidermal growth factor-dependent pathway. Neutralizing antibodies to EGF were found to decrease the IL-1β-induced alveolar epithelial repair. In addition, blocking the EGF receptor or its intracellular signaling pathway by inhibitors of the mitogen-activated protein kinase specifically inhibited the effect of IL-1β [4]. These data indicate that IL-1β enhances alveolar epithelial repair by activating the epithelial EGF/TGF-α pathway. Our data are consistent with a previous report in which pulmonary edema fluid and plasma from patients with ALI was added to a mechanically wounded monolayer of alveolar epithelial cells and the rate of wound closure over time determined by means of a digital imaging system connected to the microscope and appropriate image analysis software. Surprisingly, alveolar epithelial repair activity induced by pulmonary edema fluid from patients with ALI was markedly increased compared with plasma

Figure 4. Fatality rates in patients with ALI according to serum EGF concentration and PaO_2/FIO_2.



Fatality rates for patients with epidermal growth factor (EGF) level less than 534.2 or more stratified for PaO_2/FIO_2 of less than 200 and PaO_2/FIO_2 200 or more. The number of patients in each category is indicated within the bar.
RR: Relative risk.

Executive Summary

- It seems unlikely that epidermal growth factor (EGF) extravasated from the alveolar compartment to the vascular space in acute lung injury (ALI) patients.
- EGF levels in serum are not significantly elevated in patients with established ALI.
- There is no correlation between fatality rate and serum EGF level during the first week in ALI patients
- Perhaps the biologically significant EGF concentration in the alveolar fluid of ALI patients reveals some relationship with fatality rate.

obtained from the same patients or pulmonary edema fluid from patients with hydrostatic edema [4]. Therefore, it seems unlikely that EGF extravasated from the alveolar compartment into the vascular space.

Expert opinion

The relationship between EGF and fatality rate might have been underestimated due to the fact that we tested EGF in the serum of patients with ALI. Perhaps the biologically significant EGF concentration in the alveolar fluid of ALI patients reveals some relationship with fatality rate. It should be noted that our study is limited from the small sample size for different pathophysiology of ALI as that seen clinically with sepsis, trauma or others, and also from the number of patients in normal and control groups. Performing additional research with larger groups of patients seems to be necessary. As reported previously elevated lavage TGF-α concentrations may be associated with delayed resolution of ALI [12]. However, to our knowledge, there is no study demonstrating a direct relationship between any single mediator of ALI early in the course of disease and fatality rates.

Outlook

Based on the present results, we hypothesize that biologically active mediators capable of enhancing alveolar epithelial repair might be released into the alveolar space in patients with ALI. We suggest that this growth factor should be measured in the alveolar fluid of patients with ALI to establish more sophisticated *in vivo* models to improve our understanding of the mechanism involved in the alveolar repair process.

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