Hepatocyte growth factor and the risk of pulmonary embolism

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Abstract Background: Diagnosis of pulmonary embolism (PE) in early stages by conventional laboratory methods is difficult because the currently available tests lack sufficient sensitivity and specificity. Hepatocyte growth factor (HGF) was originally regarded as specific to hepatocytes, but has been found to be identical to the scatter factor affecting a wide range of tissues including the lungs.

The aim of this work is to study the relationship between HGF and PE.

Patients and methods: This study included 40 patients with PE, 40 stable angina (SA) patients, and 10 healthy controls. HGF and D-dimer were measured in all patients of this study.

Results: Mean HGF was significantly higher in the PE group (788.8 ± 361.5 pg/ml) compared to the SA group (262.4 ± 158.1 pg/ml) and control group (215.5 ± 18.5 pg/ml) (P = 0.0001). The predictive values of D-dimer in the diagnosis of PE were as follows: 100% sensitivity and negative predictive value, 80% specificity, 83.3% positive predictive value and 90% accuracy, while those of HGF were: 97.5% sensitivity, 97.4% negative predictive value, 92.5% specificity, 92.9% positive predictive value and 95% accuracy. When used both D-dimer and HGF together the values improved to: 100% sensitivity and negative predictive value, 97.6% positive predictive value and 98.8% accuracy.

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Introduction

Pulmonary embolism (PE) is a common and often fatal post-operative complication. Late diagnosis and delayed management of this condition are associated with pulmonary infarction. The common clinical manifestations in pulmonary embolisms are frequently inconsistent and often vague. Routine laboratory examinations are also nonspecific. A chest film, arterial saturation, and electrocardiogram may be helpful in excluding other cardiorespiratory diseases, but are frequently unreliable in establishing an objective diagnosis of pulmonary embolism. Although pulmonary arteriography is the gold standard for the diagnosis of pulmonary embolism, it requires expensive equipment and trained radiologists, and the patient could show sensitivity to the contrast agents used. Therefore, it is necessary to use a more convenient and reliable method of diagnosing pulmonary embolism [1].

Hepatocyte growth factor (HGF) was initially thought to be liver-specific, but it has become clear that HGF acts on a variety of epithelial cells and organs such as mitogen, motogen and morphogen [2,3]. HGF may also be involved in vascular proliferation and regeneration, as it is a principal mediator of mesenchymal, epithelial, and endothelial interactions that contribute to wound healing and angiogenesis. It is likely that HGF plays an important role in ischemic lung injury [4].

It is unclear whether high HGF levels are a risk factor for ischemic pulmonary disease or a result of it. To address these issues, we conducted this study to evaluate the association between circulating HGF levels and pulmonary embolism and whether it could serve as an indicator of this disease in the early period of pulmonary ischemia.

Patients & methods

This study included 40 patients with pulmonary embolism (PE group 1), 40 stable angina patients (SA group 2), and 10 healthy controls (group 3).

All patients and controls were recruited from patients attending Intensive Care unit, Menoufia University Hospital, Shebin El-Kom and Critical Care Unit, Alexandria University Hospital. All studied subjects underwent a detailed clinical history and physical examination. An informed consent was obtained from all subjects enrolled in the study. This study was approved by our Ethics Committee of Faculty of Medicine.

Exclusion criteria were patients whose medical history was consistent with systemic metabolic disorders (other than DM), systemic vasculitis, or apparent liver disease.

The control group consists of 10 examinees of the same age and sex.

Laboratory assessment:

1- Liver enzymes (SGOT, SGPT) were done on autoanalyser SYNCHRON CX5 from Beckman.
2- Lipid profile.
3- D-Dimer: was considered positive if > 500 ng/ml FEU (fibrinogen expressed unit by using latex agglutination test).
4- Hepatocyte growth factor (HGF) was measured in all patients and controls within 24 h of onset of pulmonary embolism and before giving heparin. HGF is relatively stable and that a single measurement at baseline may reflect an individual’s long-term exposure to this growth factor. It was estimated by ELISA kit based on standard sandwich enzyme-linked immune-sorbent assay provided by biorbyt. We did not research the time course of HGF concentration in patients with pulmonary thromboembolism who received heparin treatment for 3–7 days after the admission, because Matsumori et al. found that serum HGF concentration increased immediately after heparin use [5].

Pulmonary embolisms were diagnosed by using computed tomography pulmonary angiography.

Statistical analysis

Data input to the computer was done followed by tabulation and analysis. Analysis was done using SPSS-9 (Statistical Package for Social Sciences version 12). We represent the data in arithmetic mean, standard deviation, frequency and percentage. The following tests were used to analyze the results: analysis of variance (ANOVA), least significant difference, Student ‘‘t’’ test, Chi square test and correlation coefficient test. Statistical analysis was done at level of significance of $P \leq 0.05$.

Results

There were no significant differences between the 3 studied groups as regards age, gender and body mass index (Table 1). There were no significant differences between both patient groups as regards systolic and diastolic blood pressure ($P = 0.321$ and $= 0.167$, respectively).

There were no significant differences between the PE group and SA group as regards liver enzymes (ALT, AST), renal
Hepatocyte growth factor and the risk of pulmonary embolism

Table 1  Comparison among the studied groups as regards demographic data.

<table>
<thead>
<tr>
<th></th>
<th>PE group (n = 40)</th>
<th>SA group (n = 40)</th>
<th>Control group (n = 10)</th>
<th>F-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td>P = 0.192NS</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>62.5 ± 9.3</td>
<td>63.8 ± 7.7</td>
<td>61.2 ± 7.1</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>22 (55.0%)</td>
<td>20 (50.0%)</td>
<td>5 (50.0%)</td>
<td></td>
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<tr>
<td>Females</td>
<td>18 (45.0%)</td>
<td>20 (50.0%)</td>
<td>5 (50.0%)</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>27.4 ± 5.12</td>
<td>27.3 ± 4.7</td>
<td>26.9 ± 3.8</td>
<td></td>
</tr>
</tbody>
</table>
| PE group, pulmonary embolism group; SA group, stable angina group; n, number; NS, non-significant.

Table 2  Comparison among the studied groups as regards laboratory investigations.

<table>
<thead>
<tr>
<th></th>
<th>PE group (n = 40)</th>
<th>SA group (n = 40)</th>
<th>t-Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Mean ± SD</td>
<td>25.5 ± 12.3</td>
<td>27.8 ± 11.9</td>
<td>P = 0.571NS</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td></td>
<td></td>
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<tr>
<td>Mean ± SD</td>
<td>22.5 ± 13.4</td>
<td>23.9 ± 11.5</td>
<td>P = 0.356NS</td>
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<tr>
<td>Blood urea (mg/dl)</td>
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<tr>
<td>Mean ± SD</td>
<td>41.5 ± 10.7</td>
<td>37.0 ± 7.4</td>
<td>P = 0.915NS</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.03 ± 0.2</td>
<td>1.02 ± 0.3</td>
<td>P = 0.176NS</td>
</tr>
<tr>
<td>Serum cholesterol (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>241.6 ± 43.5</td>
<td>231.9 ± 44.1</td>
<td>P = 0.091NS</td>
</tr>
<tr>
<td>Serum triglycerides (mg/dl)</td>
<td></td>
<td></td>
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<tr>
<td>Mean ± SD</td>
<td>171.4 ± 56.6</td>
<td>169.9 ± 53.8</td>
<td>P = 0.211NS</td>
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<tr>
<td>Serum LDL (mg/dl)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mean ± SD</td>
<td>173.9 ± 39.1</td>
<td>170.6 ± 41.7</td>
<td>P = 0.331NS</td>
</tr>
<tr>
<td>Serum HDL (mg/dl)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mean ± SD</td>
<td>39.1 ± 9.2</td>
<td>41.7 ± 14.1</td>
<td>P = 0.156NS</td>
</tr>
<tr>
<td>Serum D-dimer (ng/ml)</td>
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<td></td>
<td></td>
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<tr>
<td>Mean ± SD</td>
<td>788.8 ± 361.5</td>
<td>262.4 ± 158.1</td>
<td>P = 0.0001*</td>
</tr>
</tbody>
</table>

PE group, pulmonary embolism group; SA group, stable angina group; n, number; NS, non-significant.

Table 3  Comparison among the studied groups as regards hepatocyte growth factor.

<table>
<thead>
<tr>
<th></th>
<th>PE group (n = 40)</th>
<th>SA group (n = 40)</th>
<th>Control group (n = 20)</th>
<th>F-test</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatocyte growth factor (pg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>788.8 ± 361.5</td>
<td>262.4 ± 158.1</td>
<td>215.5 ± 18.5</td>
<td>P = 0.0001*</td>
<td>Group I vs II, III</td>
</tr>
</tbody>
</table>

PE group, pulmonary embolism group; SA Group, stable angina group; n, number.
* Significant (P < 0.05).

HGF is a mesenchymal-derived pleiotropic factor that regulates growth, motility, and morphogenesis of various cells. Although HGF was known initially as a potent mitogen for hepatocytes, it has recently been shown to have effects on other cells, including epithelial and endothelial cells. It is expressed in several tissues including lung, kidney, heart and brain [6].

In lung regeneration, several polypeptide growth factors, such as endothelial growth factor (EGF), insulin like growth factor-I (IGF-I), and hepatocyte growth factor (HGF) were reported to elicit mitogenic activity for alveolar type II epithelial cells and bronchial epithelial cells [7]. Compensatory proliferation and subsequent differentiation of alveolar type II epithelial cells and bronchial epithelial cells are essential for lung regeneration. In case of alveolar injury, alveolar type I epithelial cells are predominantly damaged, and alveolar type II cells proliferate and differentiate into alveolar type I epithelial cells. In case of bronchial injury, the remaining bronchial epithelial cells, Clara cells, and basal cells are thought to proliferate and differentiate into multipotent progenitor cells, and bronchial epithelial cells are regenerated by the differentiation of those cells [8-10].

Several studies have demonstrated that human HGF may play a role in endothelial cell growth, arterial vascularization, pathogenesis of coronary artery diseases, and cerebral infarction. Endothelial dysfunction and thrombosis play an important role in cardiovascular diseases including acute coronary syndromes, pulmonary embolism, and acute aortic dissection [11-15]. Gordon et al. [16] and Ueda et al. [17] reported the presence of HGF in coronary artery plaques and thrombi. Matsumori et al. [13] found that increased HGF had relationship with thrombotic diseases.

function (blood urea and serum creatinine), serum cholesterol, serum triglycerides, high density lipoprotein cholesterol, and serum low density lipoprotein cholesterol. The number of patients with positive D-dimer (>500 ng/ml) was significantly higher in the PE group compared to the SA group (Table 2).

The mean hepatocyte growth factor was significantly higher in the PE group (788.8 ± 361.5 pg/ml) compared to the SA group (262.4 ± 298.1 pg/ml) and group 3 (215.5 ± 18.5 pg/ml) (P = 0.0001) (Table 3).

The predictive values of D-dimer in the diagnosis of PE were as follows: 100% sensitivity and negative predictive value, 80% specificity, 93.3% positive predictive value and 90% accuracy, while those of HGF were: 97.5% sensitivity, 97.4% negative predictive value, 92.5% specificity, 92.9% positive predictive value and 95% accuracy. When used both D-dimer and HGF together the values improved to: 100% sensitivity and negative predictive value, 97.5% specificity, 97.6% positive predictive value and 98.8% accuracy (Table 4).
The aim of this work is to study the relationship between hepatocyte growth factor and pulmonary embolism.

In the present study, HGF was measured in all patients and controls within 24 h of onset of chest pain and before giving heparin. HGF levels were significantly higher in the pulmonary embolism group compared to the stable angina group and control group.

Similarly, Hata et al. [11] showed that increased concentrations of circulating HGF were seen in the early stage of patients with acute pulmonary thromboembolism, but not in those with stable angina. High concentrations of serum HGF were specifically noted in patients with thrombi demonstrated by angiography, angioscopy, ultrasonography, and tomography. These results suggest that an increased concentration of circulating HGF is a marker of the presence and synthesis of thrombi in pulmonary arteries.

Also, Li et al. [18] concluded that acute PE was associated with a significant increase in plasma HGF. Moreover, acute PE was also associated with an enhanced HGF expression in the lungs, the right ventricle, and the liver.

Nagai et al. [1] found that the first increase of the plasma HGF level was noted 30 min after pulmonary ischemia, and reached a peak at 12 h. Real-time reverse transcription polymerase chain reaction (Real-time RT-PCR) revealed that the HGF messenger RNA (mRNA) expression in the injured lung was markedly increased at 1, 6, and 12 h after pulmonary ischemia. The interleukin-1β (IL-1β) mRNA expression, one of the inflammatory cytokines which induces HGF expression, was markedly increased at 1 h in the injured left lung. Therefore, they considered that HGF might be mainly induced by paracrine mechanisms in pulmonary ischemia.

Two sequence elements, an interleukin-6 (IL-6) response element and a potential binding site for nuclear factor IL-6, are located near the transcription initiation site of the human HGF gene, and might be involved in the regulation of HGF gene expression. It has also been reported that inflammatory cytokines such as IL-1β stimulate HGF production. IL-1 is a multifunctional factor produced by macrophages, monocytes, neutrophils, vascular endothelial cells, fibroblasts, keratinocytes, and Kupffer cells. Among various biological activities, IL-1 plays an important role as an inflammatory cytokine to mediate acute phase reactions, such as the induction of acute protein synthesis. IL-1β also stimulates nitric oxide (NO) synthesis in vascular cells. NO, an important regulator of vessel tone and vascular homeostasis through its effect on platelets and smooth muscle cell function inhibits platelet adhesion and aggregation in pulmonary thrombosis. In addition, inhaled NO is a selective pulmonary vasodilator and effectively reduces pulmonary hypertension [3,4].

Therefore, it was hypothesized that IL-1β is thought to be released into the circulation and to act in turn on pulmonary ischemia with subsequent induction of HGF expression. IL-1β mRNA was rapidly up-regulated in the ischemic lung, and precedes HGF mRNA, which began at 6 h after the pulmonary ischemia. Therefore, these studies suggested that IL-1β might be released in pulmonary ischemia which acts by regulating HGF gene expression, and is an important regulator of the vessel. It was shown that the expression of HGF was induced in the early stages of pulmonary ischemia, and was rapidly up-regulated compared with in other cardiorespiratory diseases [3,4].

From the previous data we can concluded that the expression of HGF was induced in pulmonary ischemia, and may be a useful biological marker for the early diagnosis.

Another important marker for diagnosis of PE is D-dimer. D-Dimer, which is fragment specific to the degradation of fibrin, has been reported to be useful in the diagnosis of thrombosis. In the present study, D-dimer showed slightly better sensitivity while, HGF showed markedly better specificity. The predictive values of D-dimer in the diagnosis of PE were as follows: 100% sensitivity and negative predictive value, 80% specificity, 83.3% positive predictive value and 90% accuracy, while those of HGF were: 97.5% sensitivity, 97.4% negative predictive value, 92.5% specificity, 92.9% positive predictive value and 95% accuracy. When used both D-dimer and HGF together the values improved to: 100% sensitivity and negative predictive value, 97.5% specificity, 97.6% positive predictive value and 98.8% accuracy.

Matsumori et al. showed that increased levels of D-dimer were found in 6 of 11 patients (55%) with high HGF values, although patients with increased HGF concentration did not consistently have elevated D-dimer levels. Because the mechanisms of increase in the concentrations of HGF and D-dimer may be different, combining the 2 measurements detected cerebral thrombosis more reliably on admission (18 of 23 patients) than either measurement alone [19].

The important question is: how can we use the biological significant elevation of HGF in treatment or adjuvant treatment to the currently used drugs in treatment of PE or as an adjuvant treatment to the currently used drugs in treatment of PE?

Yamada et al. [20] concluded that HGF neutralizing treatment with an anti-HGF antibody dramatically reduced DNA synthesis of alveolar epithelial cells in the reperfused lung and aggravated lung injury. These findings suggest that HGF may play an important role in regeneration of an injured lung after pulmonary ischemia reperfusion.

Morishita et al. [21] have demonstrated the potential application of HGF to treat cardiovascular diseases such as peripheral vascular disease, myocardial infarction, pulmonary embolism and cerebrovascular disease.

Kinosita et al. [22] reported that heparin rapidly induces the release of HGF into the circulation, and HGF is a major factor involved in heparin-induced angiogenesis. Furthermore,
the activation of mast cells by thrombus formation releases HGF into the circulation. This new pathway, thrombus formation-mast cell activation-degranulation-heparin-HGF-angiogenesis, may be both diagnostically useful and a therapeutic target in PE.

Drug development using growth factors including HGF remains a challenge, Chung et al. [23] found that HGF, a pleiotropic factor regulating development and wound healing, is secreted as inactive pro-HGF and is converted into active HGF by coagulation serine proteases. HGF receptor overexpression can cause massive venous thrombi, and factor Xa is reported to release soluble HGF from granulocytes. It is hypothesized that a hypercoagulable condition, such as disseminated intravascular coagulation (DIC), may increase circulating HGF through active cleavage by coagulation serine proteases. These findings suggest that circulating HGF is a potential laboratory marker reflecting coagulation activity and DIC prognosis in non-cancer patients and that HGF may play a role in a vicious cycle of hypercoagulability [23].

Conclusions

Our observations suggest that the plasma HGF level may be a useful biological marker of pulmonary ischemia, and a valuable tool for early diagnosis of PE. Clarification of the mechanisms, characteristics, and biological significance of HGF elevation is important for clinical use in diagnosing and treating pulmonary ischemia. The use of both dimer and HGF increases the predictive power of both tests when used together.

The clinical significance of the role of HGF in the pulmonary embolism opens a new therapeutic area in treating acute ischemic pulmonary disease that would be able to prolong the time frame for the application of reperfusion-thrombolytic therapy.

Conflict of interest

None.

References