Activin a and follistatin in chronic heart failure

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ABSTRACT
Activin A a member of TGF-β superfamily has been involved in several pathologic processes. It is also accused to have a pathognomonic role in atherogenesis and the development of heart failure. Its activity is regulated by a glycoprotein called follistatin that bind activin preventing its function. uPA is a serine protease that activates plasminogen thus initiating a cascade of fibrinolysis and extra cellular proteolysis. The aim of this study is to assess the role of activin A, follistatin and uPA in patients with chronic heart failure and to find if there is any correlation among their levels. The present study was conducted on 30 patients with chronic heart failure as a result of cardiomyopathy or ischemic heart diseases (group I). There were 20 healthy subjects of matched age and sex involved in the study as a control group (group II). In both groups serum activin A, follistatin, uPA and lipid profile that included serum T.G, total cholesterol, LDLc and HDLc were estimated. Results: there was a significant increase in serum activin A and follistatin and a significant decrease of uPA in group I as compared to controls. As regard to lipid profile there was a significant increase in serum T.G, serum total cholesterol and serum LDLc in group I than group II while there was a significant decrease in patients than the controls regarding HDLc. There was significant positive correlation between activin A and urokinase plasminogen activator (uPA) in group I. Conclusion: activin A/follistatin system may play a role in the pathogenesis of heart failure; also uPA could be suggested to have an important role in atherosclerosis and ischemic vascular disease that predisposes to heart failure due to the possible role of activin A cytokine in the fibrinolytic activity of uPA.

INTRODUCTION
Activins are members of the TGF-β superfamily. They are dimeric proteins composed of two β subunits, which are linked by a single covalent disulfide bond; allowing for the formation of three forms of activin: A, AB, and B.(1) Activin signaling is mediated by cell- surface type I and type II activin receptors.(2) Activin A, a homodimer of activin βA subunits, although originally described as an inducer of a follicle- stimulating hormone release, activin A has been recognized as a multifunctional cytokine expressed in a wide range of tissues and cells with roles in regulation of wound repair, cell differentiation, apoptosis, embryogenesis, and inflammation.(1)
Moreover, a role of activin A has been proposed in several pathological processes such as carcinogenesis and fibrosis, and that cytokine may also be involved in the pathogenesis of
various inflammatory disorders such as inflammatory bowel disease and rheumatoid arthritis.\(^{(3, 4)}\) In addition, activin A seems to be involved in atherogenesis by inhibiting foam cell formation and neointimal hyperplasia.\(^{(5, 6)}\) It is widely recognized that inflammatory mechanisms play a pathogenic role in coronary artery disease (CAD). In fact, some researches have suggested that inflammatory mediators play a causal role in several steps involved in the progression of atherosclerosis from local inflammation through plaque formation and rupture.\(^{(6, 7)}\) However, these inflammatory mediators exert several biologic functions and their relative importance, are not fulfilled.

The activity of activin is regulated by follistatin, a 34-kDa glycoprotein of 288 amino acids which binds activin with high affinity in equimolar complexes that are unable to bind and activate the activin receptors.\(^{(11, 12)}\) Later it has been reported that follistatin accelerate endocytosis and degradation of activin.\(^{(13, 14)}\)

The plasminogen activator (PA) system is an important protective mechanism against stable thrombus formation, two forms of PA have been identified, namely, tissue type plasminogen activator (tPA) and urokinase type plasminogen activator (uPA).\(^{(15)}\) Urokinase-type plasminogen activator is a serine protease that activates the zymogen plasminogen, potentially initiating a cascade of fibrinolysis and extracellular proteolysis.\(^{(16)}\) Abundant data suggest that uPA may play roles in the vessel wall other than initiating fibrinolysis.\(^{(17)}\) It is expressed by endothelial cells and smooth muscle cells in normal human arteries.\(^{(17)}\) Its expression could contribute to vascular lesion formation by facilitating smooth muscle cell migration and proliferation.\(^{(18)}\) On the basis that persistent inflammation involves increased levels of inflammatory cytokines that seems to play a pathogenic role in chronic heart failure by influencing heart contractility. The aim of the present work was to assess the role of activin A, follistatin and urokinase-type plasminogen activator in patients with chronic heart failure and to find if there is any correlation among their levels.

**SUBJECTS & METHODS**

The present study was conducted on 30 patients with chronic heart failure as group I and 20 healthy control subjects as group II matched for age and sex.

Chronic heart failure in these patients was developed as a result of dilated cardiomyopathy in 20% of them (six patients), the rest were the outcome of ischemic heart disease diagnosed by ECG findings. None of the patients had concomitant diseases such as infections, malignancies, autoimmune disorders, diabetes mellitus, hypertensions or chest diseases. All patients and controls were subjected to:

- Thorough history taking.
- Complete clinical examinations.
- Routine investigations.
- Abdominal U/S and x-ray chest.
- ECG.
• After fasting far more than 12 hours, and while fasting peripheral blood samples were taken for the determination of the following laboratory investigations:
  - Lipid profile including serum cholesterol, serum triglycerides, serum LDL-c and HDL-c by spectrophotometry.
  - Serum level of both activin A and serum level of follistatin were determined by sandwich-type enzyme-linked immunosorbent assay (ELISA).
  - Serum urokinase type of plasminogen activator (uPA) by colorimetric method of the chemicon uPA activity assay kit.
• Body Mass index for each person of both groups were also calculated by dividing the body weight in Kg over the height in meters square.

RESULTS

Body mass index (BMI) and lipid profile for the studied groups are shown in table I, where there was no significant difference between the two studied groups as regard BMI or the serum HDL-c while there was a highly significant difference as regard the rest of lipid profile among the studied groups (p<0.001).

Table II shows the statistical difference between the two groups as regard the studied parameters, serum activin A, serum follistatin and serum urokinase plasminogen activator where there was a highly significant increase in serum activin A and follistatin in group I than group II while a statistical significant decrease was detected as regard serum urokinase plasminogen activator in group I as compared to group II (p<0.001).

Table III shows the correlation between the studied parameters among the chronic heart failure patients (group I). There was significant positive correlation between Activin A and urokinase plasminogen activator, follistatin and activin A (p<0.001) while there was a significant negative correlation between follistatin and urokinase plasminogen activator (p<0.05).

As regards to the BMI, there was a significant positive correlation with activin A (p<0.01), also a positive correlation with follistatin and urokinase plasminogen activator was observed (p<0.05). Lipid profile showed that there was a significant positive correlation between serum total cholesterol, LDL-c and activin A (p<0.01) while a significant negative correlation was shown with uPA (p<0.05), (p<0.01) respectively.

The LDL fraction showed significant positive correlation with serum cholesterol (p<0.001). While the HDL fraction of lipid profile showed a significant negative correlation with activin A (p<0.05) and a significant positive correlation with uPA (p<0.05). A significant negative correlation were with both serum cholesterol and LDL was observed (p<0.001).
Table I. Body mass index (BMI) and lipid profile for the two studied groups.

<table>
<thead>
<tr>
<th>Studied groups</th>
<th>BMI kg/m²</th>
<th>Serum T.G mg/dl</th>
<th>Serum cholesterol mg/dl</th>
<th>Serum LDL-c mg/dl</th>
<th>Serum HDL-c mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>25.697 ± 1.348</td>
<td>201.0 ± 39.448</td>
<td>230.4 ± 45.503</td>
<td>177.3 ± 41.382</td>
<td>44.6 ± 8.633</td>
</tr>
<tr>
<td>Group II</td>
<td>25.205 ± 1.460</td>
<td>137.65 ± 8.75</td>
<td>176.7 ± 13.436</td>
<td>141.9 ± 10.867</td>
<td>51.2 ± 9.833</td>
</tr>
<tr>
<td>P</td>
<td>&gt;0.05 &lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Table II. Serum levels of Activin A (ng/ml), Follistatin (pg/ml) and Urokinase plasminogen activator (IU/ml) of the two studied groups.

<table>
<thead>
<tr>
<th>Studied groups</th>
<th>Activin A (ng/ml)</th>
<th>Follistatin (pg/ml)</th>
<th>Urokinase Plasminogen activator (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>8.007± 2.877</td>
<td>2086.563± 768.930</td>
<td>1.457± 0.546</td>
</tr>
<tr>
<td>Group II</td>
<td>2.725± 0.433</td>
<td>1361.985± 464.255</td>
<td>3.975± 1.268</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table III Correlation between the studied parameters among the chronic heart failure patients (group I).

<table>
<thead>
<tr>
<th></th>
<th>Activin A</th>
<th>Follistatin</th>
<th>uPA</th>
<th>BMI</th>
<th>Serum T.G</th>
<th>Serum total cholesterol</th>
<th>Serum LDL-c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follistatin</td>
<td>0.770***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uPA</td>
<td>0.650***</td>
<td>-0.418*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>0.510**</td>
<td>0.410*</td>
<td>0.359*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum T.G</td>
<td>0.420*</td>
<td>0.081</td>
<td>-0.410*</td>
<td>0.510**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum total cholesterol</td>
<td>0.450**</td>
<td>0.020</td>
<td>-0.440*</td>
<td>0.480**</td>
<td>0.181</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum LDL-c</td>
<td>0.450**</td>
<td>0.028</td>
<td>-0.480**</td>
<td>0.550**</td>
<td>0.060</td>
<td>0.692***</td>
<td></td>
</tr>
<tr>
<td>Serum HDL-c</td>
<td>-0.440*</td>
<td>0.059</td>
<td>0.390*</td>
<td>-0.006</td>
<td>-0.268</td>
<td>-0.591***</td>
<td>-0.563***</td>
</tr>
</tbody>
</table>

Data are presented as r (correlation coefficient)
*Significant at the 0.05 level (2-tailed)
**Significant at the 0.01 level (2-tailed)
***Significant at the 0.001 level (2-tailed)
DISCUSSION

A growing body of evidence links inflammation to the pathogenesis of heart failure. On the basis of its potential role in inflammation, fibrosis and wound repair – activin A was thought that it might be involved in the pathogenesis of heart failure.\(^{(23,24)}\)

In the current study, heart failure patients had markedly elevated serum level of activin A compared to healthy control subjects, a finding which might suggest a pathogenic role for activin A in the development of heart failure.

Such finding is in agreement with the result of Yndestad et al.\(^{(23)}\) who found high levels of activin A in heart failure patients with high levels according to disease severity.\(^{(23)}\) They suggested that, the failing myocardium itself may contribute to the enhanced activin A levels during heart failure with cardiomyocytes as the primary cellular source.\(^{(23)}\) Also, Smith et al.\(^{(25)}\) have reported high levels of activin A as assessed by protein serum level and mRNA levels in PMNCs in patients with stable angina than healthy controls, they suggested that activin A promote plaque stabilization by inhibiting foam cell formation and by inducing a contractile non-proliferative phenotype in cultured smooth muscle cells.\(^{(25)}\)

Waard et al.\(^{(26)}\) found that activin A is a potent inducer of cardiac ankyrin repeat protein expression in smooth muscle cells which play a key role during atherosclerosis.\(^{(7,26)}\)

Engelse and his colleagues\(^{(6)}\) experiments revealed the presence of activin A in normal media of blood vessels with a higher level of expression in the atherosclerotic vessels. They also found that follistatin was expressed at similar levels to that of activin A.\(^{(6)}\)

Their findings concerning follistatin are coinciding with the present findings that there is high follistatin level in heart failure patients as compared to the controls.

Although Smith et al.\(^{(25)}\) found no significant difference in plasma levels of follistatin between patients with stable and unstable angina as compared to the control group as well as its gene expression among the three groups.\(^{(25)}\)

The results of the present study showed a significant decrease in serum level of uPA among the heart failure patients than the normal control group. Such finding is coinciding with the fact that plasminogen activation system is an important protective mechanism against thrombus formation as it is responsible for breakdown of plasminogen into plasmin which is able to breakdown the fibrin polymer of blood clots.\(^{(27,28)}\)

Ljungner and Bergqvist \(^{(29)}\) have demonstrated diminished plasminogen activation activity in atherosclerotic compared with normal blood vessels.\(^{(29)}\)

Bjorkerud\(^{(30)}\) has characterized the expression of the overall fibrinolytic capacity of cultured vascular smooth muscle cells from normal media and atherosclerotic intima and suggested that impaired fibrinolytic capacity existed from the later source.\(^{(30)}\)
Raghunath et al.\textsuperscript{(28)} showed that altered expression of plasminogen activator system components may predispose to thrombosis as they found by immuno histochemistry that the degree of staining showed the following order PAI.1 > tPA > uPAR > uPA either in fibrointimal proliferative human coronary arteries or those who developed atherosclerotic plaques.\textsuperscript{(28)} In other investigations it was found that uPA deficient mice have marked fibrin deposition and shortened life span.\textsuperscript{(31)}

Labarrere et al.\textsuperscript{(32)} concluded that depletion of tPA from vascular smooth muscle cells is accompanied by the loss of antithrombin natural anticoagulant pathway from arteries and arterioles and considered as early sign of the development of coronary artery disease.\textsuperscript{(32)}

Bochaton-Piallat et al.\textsuperscript{(33)} demonstrated that uPA increases in cultured smooth muscle cell from intimal thickening 15 days after endothelial injury.\textsuperscript{(33)} As proved by other investigators who showed that both uPA and its receptors have been detected in human atherosclerotic lesions.\textsuperscript{(34)}

Carmeliet and colleagues\textsuperscript{(35)} have demonstrated that neointimal formation is reduced in uPA deficient mice suggesting its role in that process.\textsuperscript{(35)}

The current results showed a positive significant correlation between activin A and uPA suggesting a possible role of the former cytokine in fibrinolytic activity of uPA. Bochaton-Piallat et al.\textsuperscript{(35)} showed that TGF-B generally didn’t affect uPA activity in smooth muscle cell type but surprisingly only to spindle clones exhibited an increase in uPA activity in response to TGF-\beta, that finding indicated that the effects of TGF-B depend on several parameters.\textsuperscript{(33)}

Generation of plasmin can activate TGF-B release that are autocrinally regulate the cell growth in vessel walls.\textsuperscript{(34)}

Also, there was a significant negative correlation between uPA ,serum total cholesterol and serum LDL-c which can be explained by the work of Zhang and his colleagues who showed that although uPA was involved in the release and disaggregation of LDL in macrophages as the resulted plasmin is protected from the action of the serum inhibitors,it doesn t cause degradation of native (monomeric) LDL owing to limited expression of LDL receptor on macrophages.\textsuperscript{(36)}

A positive significant correlation was noticed in the present study between uPA and HDL-c. However, some investigators suggested that over expression of Apo A in mice (a component of HDL) increases susceptibility to diet-induced atherosclerosis by decreasing cell-associated plasminogen activation of the vessel wall.\textsuperscript{(28)}

A positive significant correlation was observed between serum activin and both serum cholesterol and serum T.G. kozaki et al.\textsuperscript{(5)} who treated macrophages with activin A and found a dose dependant decrease in cholesterol ester accumulation which was paralleled by a reduction in cell association and degradation of acetylated LDL-c. Results of follistatin showed an opposite effect.\textsuperscript{(5)}
In conclusion, the present study could suggest the possible pathogenic role of activin A/follistatin system in the development of heart failure as well as the role of urokinase plasminogen activator in the pathogenesis of atherosclerosis that predisposes to heart failure. Further investigations are needed to identify the role of different cytokines and fibrinolytic system in the progression of atherosclerosis and myocardial failure.

REFERENCES


مستوى الأكتيفين A والبوليساتين في حالات هبوط القلب المزمن

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أكتيفين A عضو في عائلة عامل النمو التحولوي - بيتا له دور في عدة أمراض. منها مرض تصلب الشرايين وتطور هبوط القلب. نشاطه يُنظّم من قبل بروتين سكري هو البوليساتين الذي يرتبط بالأكتيفين فيمنع وظيفته. من مشتقة البوليفينز بلازمينوز ينتمي إلى أنه محل البروتين السريرى الذي ينشط البلازمينوز من خلال مشاركة الجهاز الخلوي.

هدف هذا الدراسة تقييم دور الأكتيفين A والبوليساتين في مشتقة البوليفينز بلازمينوز في المرضى هبوط القلب. أجريت هذه الدراسة على 30 مرضاً هبوط القلب المزمن كنتيجة لأعتلا عضلة القلب أو أسكيميا القلب (مجموعة 1) و20 شخص أصح ات ونافوق في العمر والجنس كمجموعة ضابطة (مجموعة 2) لأفراد المجموعتان، تم قياس مستوى الأكتيفين A والبوليساتين في مشتقة البوليفينز بلازمينوز في مصل الدم، وأيضاً قياس مستوى الدهون الثلاثية والكوليسترول والدهون مختصرة الكثافة والدهون عالية الكثافة في مصل الدم.

النتائج: أظهرت زيادة ذات دالة إحصائية في مستوى الأكتيفين A والبوليساتين في مصل الدم للمجموعة الأولى بالمقارنة بالجموعة الضابطة، أيضاً كان هناك نقص ذو دالة إحصائية في مستوى مختصرة البوليفينز بلازمينوز في المرضى مقارنة بالمجموعة الضابطة بالنسبة لمستوى الدهون. وجدت زيادة ذات دالة إحصائية في مصل الدم لكل من الدهون الثلاثية والكوليسترول والكوليسترول بالبولي يز في المجموعة الأولى بالمقارنة بالسياسة الضابطة بالنسبة لمستوى الدهون عند مقارنة المجموعتين.

الخاتمة: الأكتيفين A والبوليساتين قد يكون له دور في مرض هبوط القلب المزمن ومن المتفق أن يكون مشتقة البوليفينز بلازمينوز دور هام في تصلب الشرايين وأسكيميا الأوعية الدموية مما يؤدي إلى هبوط القلب. من الممكن أن يكون الأكتيفين A دور في تنشيط تحلل الألياف لمشتقة البوليفينز بلازمينوز.