DIFFERENTIAL EFFECTS OF STATINS ON ANNEXIN A1 SERUM LEVEL IN PATIENTS WITH ACUTE CORONARY SYNDROME: A PLEIOTROPIC UPDATE

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ABSTRACT

Uncontrolled inflammatory response is considered as an important triggering factor in the pathogenesis of atherosclerosis in patients with acute coronary syndrome (ACS), and numerous studies demonstrating the protective effect of statins therapy via its anti-inflammatory action.

The aim of the present study is to estimate the protective role of statins on Annexin A1 in patients with ACS.

A total number of 63 patients with ACS were recruited compared with 25 healthy control subjects. The enrollments were divided into; Group (A): Patients with ACS on atorvastatin (n=20), Group (B): Patients with ACS on rosuvastatin (n=20), Group(C): Patients with ACS were not on statins therapy (n=23), and Group(D): Healthy controls (n=25). Body mass index (BMI) and both systolic (SBP) and diastolic blood pressures (DBP) were measured. Lipid profile, atherogenic index (AI), cardiac risk ratio (CRR), cardiovascular risk index (CVRI) and human AnxA1 level were estimated.

There was significant dyslipidemic status in patients with ACS not on statins therapy as compared to patients with ACS on statins therapy and healthy controls. TC, TG, VLDL, LDL, AI, CRR and CVRI were higher in patients with ACS not on statins therapy. Higher AnxA1 levels (3.35±0.84) was obtained from patients on statins therapy as compared with controls (1.51±0.91) and non-statins using patients (2.08±0.76), (P=0.0001). AnxA1 was significantly higher in rosuvastatin (3.69±0.92) than in atorvastatin (3.00±0.60) (P=0.008).

AnxA1 serum level is higher in patients with ACS as compared with healthy controls. Patients with ACS on statins therapy mainly rosuvastatin showed higher level of AnxA1 compared with patients with ACS on atorvastatin.

Keywords: Annexin A1, Acute coronary syndrome, rosuvastatin, atorvastatin.

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INTRODUCTION

Acute coronary syndrome (ACS) involves a number of clinical conditions, including myocardial infarction, unstable angina and sudden cardiac death, as the blood supply to the cardiac muscle is suddenly reduced by partial or complete thrombosis, which is frequently related to atherosclerotic plaque rupture. Signs and symptoms of ACS start abruptly, including burning chest pain with shortness of breath, sweating, nausea and vomiting. Several factors can contribute to ACS, including; advancing age, male gender,
ethnicity, family history of premature coronary heart disease (CHD), smoking, obesity, hypertension, diabetes mellitus, physical inactivity and hyperlipidemia \(^{(3)}\).

Statins have an important role in reducing both morbidity and mortality in patients with ACS and apart from cholesterol lowering influence, statins are known to have multiple effects, which are independent of cholesterol lowering effect known as pleiotropic effects, which are antioxidant properties, enhance endothelial function, promote atherosclerotic plaque stability, reduction of platelets aggregation and coagulation process, and have anti-inflammatory effect \(^{(4)}\).

Annexin A1 (AnxA1) is a cytoplasmic, calcium and phospholipid binding protein, characterized by its ability to suppress eicosanoid generation through inhibition the activity of phospholipase A2 (PLA2). AnxA1 is expressed mainly in immune, epithelial and endothelial cells \(^{(5)}\). Studies show that endogenous AnxA1 can protect the myocardium from acute ischemia. Endogenous AnxA1 has anti-inflammatory effects that able to prevent excessive cardiac necrosis, inflammation and fibrosis following myocardial infarction \(^{(6)}\).

AnxA1 insufficiency impairs the restoration of ventricular function after ischemia. While exogenous AnxA1 preserves fiber organization, decrease TNF-\(\alpha\) and macrophage in myocardial tissues \(^{(7)}\). Since neutrophils play a major role in ACS by the production of inflammatory mediators, Neutrophil recruitment and adherence to endothelial cell were shown to be diminished by AnxA1.Furthermore, AnxA1 mimetic peptide, Ac2-26 can evoke cardioprotective measures directly by maintenance of cardiovascular contractile function and viability of cardiac cells, as AnxA1 reduces the infarct size by about 50\% \(^{(8)}\).

Therefore, the aim of the present study was to investigate the effect of statins therapy mainly atorvastatin and rosuvastatin on AnxA1 level in patients with ACS.

**MATERIALS AND METHODS**

In this case-control study, 63 patients aged between 45-70 years were recruited from the coronary care unit (CCU) of Al-Yarmouk Teaching Hospital and compared with 25 healthy controls. According to the statins therapy, patients with ACS and healthy controls were divided into:

- **Group (A):** Patients with ACS on atorvastatin \((n=20)\)
- **Group (B):** Patients with ACS on rosuvastatin \((n=20)\)
- **Group (C):** Patients with ACS not were on statins therapy \((n=23)\)
- **Group (D):** Healthy controls \((n=25)\)

**Inclusion criteria:** any patients with ACS with age > 45 years with or without statins therapy (atorvastatin or rosuvastatin) were included.

**Exclusion criteria:** any patients with severe or morbid obesity, end-stage kidney disease, liver failure, psychiatric disorders, severe anemia, connective tissue diseases, pregnancy, lactation and malignancy.

The study design and flow is described in figure (1).
Anthropometric measurements

Body mass index (BMI) was obtained from measuring the weight in kilograms & the height in meters, then BMI was calculated by specific equation: \( \text{BMI} = \text{weight (kg)} / (\text{Height (m)}^2) \).

Blood pressure in (mmHg) was measured by using mercury sphygmomanometer device (MDF/Germany), in supine position, for each patient two blood pressure readings were taken.

Biochemical measurements

Estimation of lipid profile: total cholesterol (TC), triglyceride(TG) and high density lipoprotein (HDL) were measured by auto-analyzer (ERBE diagnostic Manheim, Germany). Low density lipoprotein (LDL) was estimated by Friedewald equation. \([9]\) \(\text{VLDL} = \text{TG} / 5\), atherogenic index (AI) =log TG/HDL, cardiac risk ratio (CRR) =TC/HDL, cardiovascular risk index (CVRI), TG/HDL\([10]\). Determination of human AnxA1 level was done by using ELISA kit method (Human ANXA1, MyBioSource /USA) which was expressed as ng/ml (figure 2).

Statistical analysis

Analysis of data was carried out using the available statistical package of SPSS-24 (Statistical Packages for Social Sciences, version 24). Data were presented in simple measures of percentage, numbers, mean and standard deviation. Moreover, the significance of difference of different means were tested using unpaired-t-test for difference between two independent means, or ANOVA test for difference among more than two independent means. Statistical significance was considered whenever the P value was less than 0.05.
RESULTS

Demographic characteristics of the present study

The allocation of patients and control groups seem to be equally matched in respect to age, gender, weight, height and body mass index (BMI). The study showed that there was no significant difference in body mass index (BMI) among patients with or without statins therapy and controls (P=0.52). The study revealed that, 44.4% were smokers while controls 60% were smoker. Patients gave a history of other associated comorbidities that seem to increase the risk of ACS, include 31 patients with hypertension, 1 patient with familial dyslipidemia, 28 patients with ischemic heart disease and 7 patients with cerebrovascular accident. Patients were sub classified according to their lifestyle; 38% of patients had a sedentary lifestyle, 26.9% were of moderate activities and 34.9% were physically active, while controls group were 80% with good physical activity and 20% with moderate activities (Table 1).

Table 1: Demographic characteristics of ACS patients and controls

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls (n=25)</th>
<th>Patients (n=63)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>62.64±13.6</td>
<td>62.8±9.85</td>
<td>0.52</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.30±5.52</td>
<td>27.66±5.39</td>
<td>0.44</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>18 (72%)</td>
<td>46 (73%)</td>
<td>0.32</td>
</tr>
<tr>
<td>female</td>
<td>7 (28%)</td>
<td>17 (27%)</td>
<td>0.52</td>
</tr>
<tr>
<td>Smoker</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>15 (60%)</td>
<td>28 (44.4%)</td>
<td>0.03</td>
</tr>
<tr>
<td>No</td>
<td>10 (40%)</td>
<td>35 (55.5%)</td>
<td>0.04</td>
</tr>
<tr>
<td>ACS subgroups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STEMI</td>
<td>n= 30 (47.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSTEMI</td>
<td>n= 22 (34.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UA</td>
<td>n= 11 (17.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>31 (49.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dyslipidemia</td>
<td>1 (1.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IHD</td>
<td>28 (44.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CVA</td>
<td>7 (11.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Life style</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>active</td>
<td>20 (80%)</td>
<td>22 (34.9%)</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>5 (20%)</td>
<td>17 (26.9%)</td>
<td></td>
</tr>
<tr>
<td>sedentary</td>
<td></td>
<td>24 (38%)</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as n, mean ±SD, %. BMI: body mass index, ACS : acute coronary syndrome, UA: unstable angina, STEMI: ST- elevation myocardial infarction, NSTEMI: non-ST elevation myocardial infarction, PMH: past medical history, CVA: cerebrovascular accident, IHD: ischemic heart disease.
Assessment of metabolic profile in patients with ACS

Regarding the metabolic profile, there was significant dyslipidemic status in patients with ACS not on statins therapy as compared to patients with ACS on statins therapy and healthy controls. TC, TG, VLDL, LDL, A1, CRR and CVRI were higher in patients with ACS not on statins therapy. Regarding HDL level; it was higher in statins as compared to non-statins and controls. As well, SBP was higher in patients with ACS compared with controls (P=0.008), however, BP was not significantly differed in patients with ACS compared with controls (P=0.57), (table 2).

Table 2: Metabolic profile in patients with ACS regarding statins therapy

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls (n=25)</th>
<th>Statins (n=40)</th>
<th>Non –statins (n=23)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dl)</td>
<td>160.4±30.7</td>
<td>141.87±40.67</td>
<td>205.78±40.6³#</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL-c (mg/dl)</td>
<td>29.5±5.4</td>
<td>33.4±9.09*</td>
<td>27.4±3.96 #</td>
<td>0.005</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>91.8±44.66</td>
<td>133.57±37.93*</td>
<td>160.65±39.9²#</td>
<td>0.001</td>
</tr>
<tr>
<td>VLDL(mg/dl)</td>
<td>18.37±8.93</td>
<td>26.71±7.58*</td>
<td>32.13±7.98 #</td>
<td>0.001</td>
</tr>
<tr>
<td>LDL-c(mg/dl)</td>
<td>112.46±32.69</td>
<td>81.76±42.42*</td>
<td>146.17±40.69 #</td>
<td>0.001</td>
</tr>
<tr>
<td>AI</td>
<td>0.45±0.2</td>
<td>0.59±0.17*</td>
<td>0.76±0.12 #</td>
<td>0.001</td>
</tr>
<tr>
<td>CRR</td>
<td>5.65±1.71</td>
<td>4.57±1.87*</td>
<td>7.66±2.08#</td>
<td>0.001</td>
</tr>
<tr>
<td>CVRI</td>
<td>3.22±1.81</td>
<td>4.24±1.45#</td>
<td>5.98±1.72#</td>
<td>0.001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>117.2±8.3</td>
<td>128.7±17.94*</td>
<td>129.0±16.51#</td>
<td>0.008</td>
</tr>
<tr>
<td>DBP(mmHg)</td>
<td>76.6±5.72</td>
<td>77.12±8.88</td>
<td>79.30±13.3</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD, ANOVA test and LSD post-hoc test, TC: total cholesterol , TG: triglyceride , HDL: high density lipoprotein , LDL: low density lipoprotein , VLDL : very low density lipoprotein, AI: atherogenic index , CRR: Cardiac risk ratio , CVRI: cardiovascular risk index
* P<0.05 compared between control with statin groups
# P<0.05 compared between statin with non-statin groups
Ⅹ P<0.05 compared between controls with non-statin groups

Effect of statins therapy on Annexin A1

Annexin A1 serum levels also displayed a significant difference among all patients and controls with (P value <0.05), higher AnxA1 level (3.35±0.84) was obtained from patients on statins therapy in comparison to the controls (1.51±0.91) and non-statins using patients (2.08±0.76) as shown in table (3). Annexin A1 was significantly higher in rosuvastatin (3.69±0.92) than in atorvastatin (3.00±0.60) (P=0.008), figure (3).
Table 3: Annexin A1 serum levels in patients with ACS and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control n=25</th>
<th>Statins n=40</th>
<th>Non –statins n=23</th>
<th>P Value ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anx A1 (ng/ml)</td>
<td>1.51±0.91</td>
<td>3.35±0.84 *</td>
<td>2.08±0.76</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

* P<0.05 compared between control with statin groups
# P<0.05 compared between statin with non-statin groups
¤ P<0.05 compared between controls with non-statin groups

Figure 3: Annexin A1 serum level patients with ACS on atorvastatin versus rosuvastatin

DISCUSSION

Regarding body mass Index (BMI), the present study showed that almost all patients with ACS were considered to be obese, that agree with several studies suggested an important role of obesity in the development of ACS.(11) Furthermore, the present data showed a lower rate of smoking among ACS patients, which is in contrast to the most studies that signifying the role of smoking in precipitating ACS.(12) This gives an evidence that causes other than smoking were more obvious as risk factors within the included patients with ACS.

Regarding lipid profile, the study reported lower level of cholesterol and triglyceride, VLDL, LDL, and higher level of HDL in patients on statins therapy as compared to non-statin using patients which is consistent with the lipid lowering effect of statins in a previous study.(13) By evaluating the atherogenic index (AI), the study showed lower level of AI in patients with ACS on statins therapy as compared to both non-statin using patients and controls, this is in agreement with a previous studies that displayed a significant reduction of AI after statins therapy.(14) Concerning cardiac risk ratio (CRR) the results revealed that there was a lower CRR in patients on statins therapy as compared to both non-statin using patients and controls.

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Previous studies reflected that CRR is a predictor of ACS and can be valuable when other lipid parameters are conflicting.\(^{(15)}\) In addition, by evaluating the cardiovascular risk index (CVRI), there was lower CVRI revealed in patients with ACS who were on statins therapy as compared to patients not on statins therapy. Several studies showed that CVRI is associated with poor cardiovascular outcomes\(^{(16)}\).

Our study showed a higher Anx A1 level was obtained from patients on statins therapy in comparison to controls and non-statins using patients. Since systemic inflammation plays an important role in atherosclerosis, in which an imbalance between pro- and anti-inflammatory activities is enhanced and mediated by hypothalamic pituitary axis (HPA) through glucocorticoids that performed its anti-inflammatory effects by an endogenous protein, AnxA1, which interacts with formyl peptide receptors like -1 / lipoxin A4 receptors FPRL-1/ALXR to inhibit the pro-inflammatory response. A previous study exhibited that there was an increased AnxA1 expression in patients with ACS in response to glucocorticoid mediated acute inflammation.\(^{(17)}\) In contrast to our study, a study on patients with CHD to evaluate the expression of AnxA1, showed that despite the anti-inflammatory effect of statins there was no change in the expression of glucocorticoids or AnxA1\(^{(18)}\).

AnxA1 provides a protective role in patients with ACS by inhibiting integrin activation and myeloid cells accumulation in the arterial wall, promotes plaque stability and reduces necrosis, induces neutrophils apoptosis and decrease endothelial cell adhesion molecules, thus reduces atherogenesis.\(^{(19)}\) The impact of statins on AnxA1 level was reported in a previous study that showed an elevation in the expression of AnxA1 in patients with statins therapy by calcium mediated effect.\(^{(20)}\) It is nice to mention that there was no published study comparing the effect of different types of statins on the AnxA1 level. A previous study demonstrated the effect of high dose atorvastatin in modifying the protein profile of monocytes in patients with ACS, and revealed an increased expression of AnxA1 in those patients.\(^{(21)}\) However, our current study highlights a significant impact of rosuvastatin on increasing AnxA1 level in patients with ACS.

The effects of rosuvastatin on enhancing AnxA1 level may be due to several indirect causes including the hydrophilic nature of the drug and its effect in improving HDL level to a greater extent than atorvastatin and since; HDL and Apo A lipoprotein has a significant role in increasing AnxA1 level through different signaling pathway. As well, rosuvastatin increases AnxA1 expression is through augmentation of endothelial cAMP level, which is involved in the synthesis and release of AnxA1\(^{(22,6)}\).

**CONCLUSION**

AnxA1 serum level is increased in patients with ACS as compared with healthy controls. Patients with ACS on statins therapy showed a higher level of AnxA1 when compared with patients with ACS not on statins therapy. As well, rosuvastatin was more effective than atorvastatin in improving AnxA1 serum level.

**ETHICAL CLEARANCE**

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.
CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES


