Association between Serum Iron with Serum TNF-α, IL-6, IGF-1 and Lipids in Both Acute Myocardial Infarction and Unstable Angina Patients

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ABSTRACT

Background: Pro-inflammatory cytokines (interleukine -6 and tumor necrotic factor-a) and Insulin-like growth factor-1 (IGF-1) play important roles in pathogenesis of acute myocardial infarction (AMI) and unstable angina (UA). In addition, serum iron overload or iron deficiency appears to be associated with atherosclerosis and ischemic myocardial damage. The aim of this investigation is to verify the role of inflammatory process and IGF-1 in pathophysiology of AMI and UA as well as to investigate the relationship of circulating IL-6, TNF-α and IGF-1 with the levels of serum iron and lipids in those patients. Methods: IL-6, TNF-α and IGF-1 were measured by ELISA assays in 18 patients with AMI and 18 patients with UA after their hospital admission, as well as in 6 healthy control subjects. Lipid profile was assessed by measuring the serum levels of total cholesterol, HDL-C, LDL-C and triglycerides. Serum iron was measured by atomic absorption flame emission spectrophotometer. Results: AMI patients with low serum iron showed significant higher levels of IL-6, TNF-α, LDL-C, cholesterol level and atherogenic index and lower levels of IGF-1 and HDL-C as compared with both low serum iron UA patients and healthy controls. On the other hand, AMI patients with high serum iron revealed non-significant differences in all previous parameters except IL-6 when compared with high serum iron UA patients. There was a significant positive correlation between serum iron with the levels of IGF-1 and HDL-C, as well as a significant negative correlation with the levels of cholesterol, triglycerides, LDL-C, TNF-α, and IL-6 in both AMI and UA patients with low serum iron. In addition, in AMI patients with high serum iron, a significant positive correlation between serum iron with IGF-1 and HDL-C and negative correlation with remaining parameters was evident. Conclusions: Both AMI and UA patients were associated with a pro-inflammatory state (increased TNF-α and IL-6), increased risky lipids (cholesterol, triglyceride, LDL-C, atherogenic index) and decreased cardiac protective factors, such as IGF-1 and HDL-C. These findings support the role of inflammation in both patients' population as well as the protective role of IGF-1 in ischemic heart disease. In addition, low serum iron in both AMI and UA patients was associated with more proinflammatory state and less cardiac protection than normal subjects or patients with either normal or high serum iron. However, the deleterious effects of low serum iron were more in AMI than UA patients.
INTRODUCTION

Ischemic heart disease is due to disparity between the oxygen supply and the metabolic demand of the myocardium\(^1\). Reduction in coronary blood flow is related to progressive atherosclerosis\(^2\).

Insulin-like growth factor I (IGF-I), which is a mitogenic peptide that circulates in plasma, has endocrine effects in numerous target tissues. Most plasma IGF-I originates from the liver, its production is stimulated by growth hormone (GH) secretion, and IGF-I mediates many of the anabolic actions of GH\(^3\). In addition to its anabolic properties, it has been suggested that IGF-I plays an important role in the development of cardiovascular diseases (CVDs)\(^4,5\). Subjects with low circulating IGF-I levels are at significantly higher risk of developing CVDs\(^6\). Because IGF-I is important for tissue repair and cell proliferation, it also involved in the pathogenesis of atherosclerosis\(^4\).

Atherosclerosis is widely accepted as a chronic inflammatory disease initiated by different vascular and extravascular sources\(^7\). The inflammatory process is involved throughout the different stages of atherosclerosis. The vulnerability of an atherosclerotic plaque is related to the presence of inflammation since it plays a crucial role in disruption of the fibrous cap, which is a major pathophysiological event for the development of the acute coronary syndromes, such as acute myocardial infarction (AMI) and unstable angina (UA)\(^8\).

The multifunctional cytokine tumour necrosis factor-alpha (TNF-\(\alpha\)) plays a central part in the inflammatory cascade that is a key feature in atherosclerosis\(^9\). TNF-\(\alpha\) levels correlate with progression of early carotid atherosclerosis and it is also a marker for recurrent coronary events after a previous myocardial infarction\(^10,11,12\). Moreover, Proinflammatory cytokines, such as interleukin (IL)-6 and TNF-\(\alpha\) play important roles in coronary arterial diseases by regulating inflammation, cellular adhesion, production of growth factors\(^13,10\).

Iron is physiologically essential, but excess iron can also be a toxic and lead to the formation of a cascade of free radicals\(^14,15\). Iron was reported as an important factor in the process of atherosclerosis\(^16\). Also, high levels of body iron, high LDL-cholesterol and antioxidant deficiency are regarded as oxidative risk factors for ischemic heart diseases\(^17\). Association between serum iron levels and pathogenesis of ischemic heart diseases is controversial. Whereas most data show no association, some have raised the possibility of a causal role, while others have suggested a protective effect of iron. Roles of serum iron and its relation with cardioprotective IGF-1, proinflamatory cytokines (TNF and IL-6) in patients with acute myocardial infarction and unstable angina are indistinctive.

The aim of this investigation was to verify the role of inflammatory process and IGF-1 in patients with AMI and UA as well as to investigate the relationship of circulating IL-6, TNF\(\alpha\), IGF-1 and lipid profile with serum iron in these patients population.
SUBJECTS & METHODS

Study population
This study was carried out throughout co-operation between departments of Physiology, Biochemistry and Internal Medicine in Assiut Faculty of Medicine. The study was approved by the Assiut University Ethics Committee and every participant signed the informed consent before enrollment.

The research enrolled 36 male patients with acute coronary syndromes (18 patients with AMI of mean age 52±8 years, and 18 patients with UA of mean age 54±7 years) and 6 control male subjects (of mean age 50±10 years). All patients were recruited from the Cardiac Clinic unit of Internal Medicine in Assiut University Hospital.

The normal volunteers had normotension (blood pressure: systolic < 140 mm Hg and diastolic < 90 mm Hg), normal resting ECG with no past history or evidence of cardiovascular disease or diabetes mellitus. The present study did not include patients or control subjects with a history of neoplastic, hepatic, thyroid, infectious, autoimmune or peripheral atherosclerotic disease, any surgical procedure in the preceding 6 months or under treatment with anti-inflammatory and/or lipid lowering drugs.

Patients with AMI were defined as presence of typical prolonged chest pain accompanied by serial changes on the standard 12-lead electrocardiogram and significant (twofold more than the upper normal range) increase in markers of myocardial damage as creatine kinase (CK), lactic dehydrogenase (LDH) or Troponine- T. Patients with UA were defined as anginal pain at rest occurring during the preceding 6 hours with transient significant ischemic ST-segment or T-wave changes or both, without significant increases in markers of myocardial damage (CK, LDH, Troponine T).

All patients were admitted to hospital within 6 hours from the onset of chest pain. All patients underwent medical treatment with combination of standard medications, including nitrates, β-adrenergic blockers, calcium antagonists, low molecular weight heparin and antiplatelet drugs such as aspirin and/or clopidogrel.

AMI and UA Patients were randomized and selected according to their serum iron into low, normal and high serum iron subdivision. Low serum iron levels were less than 38 μg/dl, normal serum iron levels were from 38 μg/dl to 158 μg/dl, and high serum iron levels were higher than 158 μg/dl[18].

The prevalence of major risk factors and medications among the normal control subjects and the two groups of patients are shown in table 1.
Table 1: Risk factors and medications used in the acute myocardial infarction (AMI), in unstable angina (UA) and control subjects involved in this study.

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>AMI Group (n=18)</th>
<th>UA Group (n=18)</th>
<th>Control Group (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>52±8</td>
<td>54±7</td>
<td>50±10</td>
</tr>
<tr>
<td>Smoking</td>
<td>15</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>Hypertension</td>
<td>15</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>10</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Hypercholestremia</td>
<td>16</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>Body mass index (≥ 25 kg/m²)</td>
<td>13</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>Family History of CAD</td>
<td>9</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

**Medications:**
- Nitrates: 18
- β-blockers: 13
- Calcium antagonists: 10
- ACE inhibitors: 10
- Aspirin: 18
- Clopidogrel: 15
- LMWH: 18
- Thrombolytic therapy: 18

ACE = Angiotensin Converting Enzyme, LMWH = Low Molecular Weight Heparin, CAD= Coronary Artery disease, n= number.

**Laboratory measurements**
- Blood sample (5 ml) was obtained from healthy controls and patients within 1 h of admission. The samples were taken slowly from an antecubital vein and were transferred to chilled sterile disposable tubes without anticoagulant. The disposable tubes were promptly centrifuged at 3000 r. p.m. for 20 min. at 4 °C within 1 h of drawing to separate serum then serum immediately stored at −70 °C until used.
- Serum iron was measured by atomic absorption/flame, emission spectrophotometer (A-A630-O2) at wave length 2483 nm and lamp current 10 mA(19).
- Enzyme-linked immunosorbent assays (ELISA) were performed for measuring concentrations of TNF-α (Cat. No. KAC 1571), IL-6 (Cat. No. M1916) and IGF-1 (Cat. No. M 1915) using Biosource Europe commercial kits with monoclonal antibodies against each substance and following the instruction supplied with each kit. The apparatus used was Ansoth, 2000 manufactured in Austria.
- Fasting levels of Lipid total cholesterol levels were measured by quantitative enzymatic colorimetric determination using Stanbio-cholesterol LiquiColor kit (procedure No. 1010), measured by spectrophotometer according to the method of Flegg, (1973)(20). HDL
levels were measured by using Stanbio-HDL Cholesterol kit (procedure No. 0599) according to the method described by Finley (1978)\(^{(21)}\). Triglyceride levels were measured by using Stanbio Liquicolor Triglycerides kit (procedure No. 2100) according to the method described by Bucolo and David (1973)\(^{(22)}\). The LDL was calculated by subtracting both levels of HDL and one fifth of triglycerides from the levels of total cholesterol\(^{(23)}\). The atherogenic index was calculated as the product of total cholesterol/ HDL\(^{(24)}\).

**Statistical analysis**

Data are expressed as mean ± SE for all parameters. The data were analyzed by using **GraphPad Prism data analysis program** (GraphPad Software, Inc., San Diego, CA, USA). For the comparison of statistical significance between patients and normal subjects, Student Newman-Keuls t-test for unpaired data was used. For multiple comparisons, one-way analysis of variance (ONE-WAY-ANOVA) test followed by least Significant Difference (LST) was used. Values were accepted as being statistically significant if a P value was less than 0.05. Correlations of serum iron with the other variables were assessed using Spearman’s non-parametric correlation coefficient \(\rho\). A value of \(P<0.05\) was considered statistically significant.

**RESULTS**

AMI and UA patients with low, normal or high serum iron revealed a strong evidence of inflammation and reduced cardioprotective agents. A summary of these results is shown in Figures 1-9 and Table 1.

**Results of demographic data:**

Baseline characteristics of the cases and controls are shown in (table 1). Risk factors (age, smoking, hypertension, and diabetes mellitus and increase body mass index above 25 kg/m\(^2\)) occurred more frequently among patients.

**Results of measured laboratory parameters:**

- **Relation to serum iron:** Estimation of serum iron (mg/dl) showed significant reduction in both AMI and UA patients with low and normal serum iron when compared with that of control value. However, it significantly increased in both patients groups with high serum iron as compared with that of control subjects. Whereas, there were non-significant differences in serum iron levels between AMI and UA patients either with low, normal or high serum iron. These results are summarized in table (2-a).

- **Relation to Pro-inflammatory cytokines:** Both measured proinflammatory cytokines as serum IL-6 and TNF-\(\alpha\) (pg/ml) increased significantly (\(P<0.001\)) in AMI and UA patients with low, normal and high serum iron when compared with control mean value as shown in table (2-a).

AMI patients with low serum iron exhibited significant higher serum levels of IL-6 (\(P<0.05\)) and TNF-\(\alpha\) (\(P<0.01\)) when compared with UA patients with low serum iron. While, AMI patients with normal serum iron displayed significant decrease of IL-6 (\(P<0.01\)) and increase TNF\(\alpha\) (\(P<0.05\)) as compared with UA patients.
with normal serum iron. Furthermore, IL-6 decreased significantly (P <0.001) in AMI patients with high serum iron in comparison to that of UA patients with high serum iron (table 2-a).

Statistic analysis showed significant increase serum levels of IL-6 (P <0.001) and TNFα, (P <0.05 and P <0.001 respectively) in AMI patients with low serum iron in relation to that of AMI patients with normal or high serum iron (figure 1-a). While in UA patients either with low, normal or high serum iron, there were non-significant changes in the levels of IL-6 and TNFα. A summary of these findings is shown in figure (2-a).

**Relation to IGF-1**: serum levels of IGF-1 (pg/ml) decreased significantly in AMI patients with low, normal and high serum iron and in UA patients with low and normal serum iron when compared with control value. Although in UA patients with high serum iron it decreased insignificantly in relation to that of control as shown in table (2-a).

AMI patients with low serum iron exhibited significant (P <0.05) lower serum levels of IGF-1 when compared with UA patients with low serum iron. While AMI patients with normal iron or high serum iron showed non-significant changes in IGF-1 levels as compared with UA patients with normal or high serum iron (table 2-a).

Moreover, IGF-1 significantly decreased (P <0.001 and P <0.01 respectively) in AMI patients with low serum iron in relation to that of AMI patients with normal and high serum iron (figure 1-b). Likewise, there was a significant reduction in the levels of IGF-1 (P <0.01) in UA patients with low serum iron in comparison to UA patients with normal or high serum iron (figure 1-b).

**Relation to risky lipid profile**: As comparable to the control subjects, assessment of risky lipids (cholesterol, triglyceride and LDL-C) and atherogenic index revealed significant increase in both patients groups (table 2-b).

AMI patients with low serum iron showed significant higher LDL-C (P <0.01), cholesterol (P <0.001) and atherogenic index (P <0.01) but non-significant increase of triglyceride level when compared with UA patients with low serum iron (2-b).

While AMI patients with normal or high serum iron displayed non-significant changes in the levels of risky lipids except (LDL-C) as comparable to that of UA patients with normal or high serum iron (table 2-b).

There was a significant increase mean values of cholesterol, LDL-C and atherogenic index in AMI patients with low serum iron as comparable to that of AMI patients with normal or high serum iron (figure 1-C). Meanwhile, LDL-C and triglyceride levels significantly (P <0.05) increased in UA patients with low serum iron in comparable to UA patients of normal or high iron (figure 2-C).

**Relation to cardioprotective lipid profile**: Serum HDL-C (mg/dl), cardioprotective lipid, diminished significantly (P <0.001) in both patients groups versus control mean value as shown in table (2-b).
AMI patients with low serum iron exhibited significant decrease serum levels of HDL-C (P <0.05) when compared with UA patients with low serum iron. However, there was non-significant change as regard HDL-C in AMI patients with normal or high serum iron when compared with that of UA patients (table 2 - b).

In addition, HDL-C significantly (P <0.01 and P <0.001 respectively) decreased in AMI patients with low serum iron as comparable to that of AMI patients with normal or high serum iron (figure 1 - c). Also, in UA patients with low serum iron, there was a significant reduction in the levels of HDL-C (P <0.05) in comparison to UA patients with normal or high serum iron. A summary of these results is shown in figure (2 - c).

**Correlation:**

In AMI patients with high serum iron, there was a statistically significant positive correlation of serum iron with HDL-C and IGF-1 as well as significant negative correlation with IL-6, TNFα, cholesterol, LDL-C, triglyceride and atherogenic index (Fig.3 and 4). Likewise, in AMI patients with low serum iron, similar significant correlations were proofed except triglyceride level (figure 5 and 6).

UA patients with low iron showed statistically positive correlation between serum iron and IGF-1 and HDL-C and negative correlation with TNFα, IL-6, atherogenic index and LDL-C (figure 7 and 8). As well, UA patients with high serum iron exhibited significant negative correlations between serum iron with TNFα, IL-6, triglyceride and atherogenic index but non significant relations with other parameters (figure 9).
**Table (2-a):** Comparison mean values of serum iron, IGF-1, IL-6, TNFα, between healthy control and both AMI & UA patients with low, normal & high serum iron.

<table>
<thead>
<tr>
<th>Items</th>
<th>Groups</th>
<th>Control (n= 6)</th>
<th>AMI Patients (low) (n= 6)</th>
<th>AMI Patients (normal) (n= 6)</th>
<th>UA Patients (normal) (n= 6)</th>
<th>AMI Patients (iron) (n= 6)</th>
<th>UA Patients (iron) (n= 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum iron (mg/dl)</td>
<td></td>
<td>114.4±1.1</td>
<td>50.9±4.8***</td>
<td>83.8±4.8***</td>
<td>131.2±5.6*</td>
<td>52.12±5.6***</td>
<td>96.7±5.2**</td>
</tr>
<tr>
<td>% change from control</td>
<td></td>
<td>Ns</td>
<td>44.4%</td>
<td>Ns</td>
<td>73.3%</td>
<td>Ns</td>
<td>45.6%</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td></td>
<td>2.87±0.3</td>
<td>17.45±0.55***□</td>
<td>9.48±0.46***□□</td>
<td>7.25±0.27***</td>
<td>15.15±0.79***</td>
<td>14.62±1.03***</td>
</tr>
<tr>
<td>% change from control</td>
<td></td>
<td>608%</td>
<td>46%</td>
<td>330%</td>
<td>252.6%</td>
<td>527.8%</td>
<td>478.3%</td>
</tr>
<tr>
<td>TNFα (pg/ml)</td>
<td></td>
<td>10.73±0.79</td>
<td>55.83±5.43***□□□</td>
<td>37.0±2.71***□</td>
<td>25.78±1.9***</td>
<td>31.68±0.87***</td>
<td>28.53±1.63***</td>
</tr>
<tr>
<td>% change from control</td>
<td></td>
<td>520.3%</td>
<td>344.8%</td>
<td>240.3%</td>
<td>295%</td>
<td>265.9%</td>
<td>276.5%</td>
</tr>
<tr>
<td>IGF-1 (pg/ml)</td>
<td></td>
<td>11430±501</td>
<td>5550±251.3***□□□</td>
<td>8432±496.9**</td>
<td>8083±627.9**</td>
<td>6877±468**</td>
<td>9453±579*</td>
</tr>
<tr>
<td>% change from control</td>
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<td>48.6%</td>
<td>Ns</td>
<td>73.8%</td>
<td>Ns</td>
<td>70.7%</td>
<td>60.2%</td>
</tr>
</tbody>
</table>

All values represent means ± SE and were analyzed by a Student Newman-Keuls t-test. AMI: acute myocardial infarction, UA: unstable angina, IGF-1: insulin like growth factors -1, TNFα: tumor necrotic factor α, IL-6: interleukin-6. HDL-C = high density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol. Ns: non significant.

* Showing comparison between all groups Vs. control (*= P <0.05, ** = P <0.01, ***= P <0.001).
□ Showing comparison between AMI patients with low iron Vs. UA patients with low iron (□ P <0.05, □□ P <0.01, □□□ P <0.001).
 exon Comparison between AMI patients with normal iron Vs. UA patients with normal iron (□ =P <0.05, □□ = P <0.01).
Ö Showing comparison between AMI patients with high iron Vs. UA patients with high iron (Ö Ö Ö = P <0.01).
Table (2-b): Comparison mean values of LDL-C, cholesterol, triglyceride, atherogenic index and HDL-C between healthy control and both AMI & UA patients with low, normal & high serum iron.

<table>
<thead>
<tr>
<th>Items</th>
<th>Control (n= 6)</th>
<th>AMI Patients (low) (n= 6)</th>
<th>AMI Patients (normal) (n= 6)</th>
<th>UA Patients (low) (n= 6)</th>
<th>UA Patients (normal) (n= 6)</th>
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<tr>
<td><strong>LDL-C (mg/dl)</strong></td>
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<tr>
<td>Serum iron</td>
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<tr>
<td>% change from control</td>
<td>85.38±1.42</td>
<td>249.0±2.48***</td>
<td>160.7±4.49***</td>
<td>145.2±2.0***</td>
<td>157.7±2.24***</td>
</tr>
<tr>
<td></td>
<td>291.6%</td>
<td>% change from control</td>
<td>1188.2%</td>
<td>1350%</td>
<td>1465%</td>
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<tr>
<td></td>
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<tr>
<td><strong>Cholesterol (mg/dl)</strong></td>
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<tr>
<td>Serum iron</td>
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<td></td>
</tr>
<tr>
<td>% change from control</td>
<td>177.7±1.54</td>
<td>316.7±13.9***</td>
<td>240.02±10.0***</td>
<td>225.8±8.0***</td>
<td>235±7.64***</td>
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<tr>
<td></td>
<td>178.2%</td>
<td>% change from control</td>
<td>135.1%</td>
<td>127.1%</td>
<td>125.7%</td>
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<tr>
<td></td>
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<tr>
<td><strong>Triglyceride (mg/dl)</strong></td>
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<tr>
<td>Serum iron</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>% change from control</td>
<td>174.7±2.34</td>
<td>203.0±4.47***</td>
<td>199.8±3.0***</td>
<td>187.3±3.8*</td>
<td>208.3±4.22***</td>
</tr>
<tr>
<td></td>
<td>116.2%</td>
<td>% change from control</td>
<td>114.4%</td>
<td>107.2%</td>
<td>109.2%</td>
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<td></td>
<td></td>
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<tr>
<td><strong>Atherogenic index</strong></td>
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<tr>
<td>(Cholesterol/ HDL-C)</td>
<td>3.65±0.09</td>
<td>6.45±0.85**</td>
<td>5.2±0.55**</td>
<td>5.28±0.39**</td>
<td>5.37±0.19***</td>
</tr>
<tr>
<td>% change from control</td>
<td>385.2%</td>
<td>% change from control</td>
<td>211.5%</td>
<td>170%</td>
<td>205.9%</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>HDL-C (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum iron</td>
<td>57.50±1.06</td>
<td>26.83±2.6***</td>
<td>39.83±1.96***</td>
<td>43.17±2.12***</td>
<td>35.50±1.52***</td>
</tr>
<tr>
<td>% change from control</td>
<td>46.7%</td>
<td>% change from control</td>
<td>69.3%</td>
<td>75.1%</td>
<td>61.7%</td>
</tr>
</tbody>
</table>

All values represent means ± SE and were analyzed by A Student Newman-Keuls t-test. AMI: acute myocardial infarction, UA: unstable angina, IGF-1: insulin like growth factors -1, TNFα: tumor necrotic factor α, IL-6: interleukin-6. HDL-C = high density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol. Ns: non significant.

* Showing comparison between all groups Vs. control (*= P <0.05, ** = P <0.01, ***= P <0.001).
¤ Showing comparison between AMI patients with low iron Vs. UA patients with low iron (¤ P <0.05, ¢¤ P <0.01, ¢¢¤ P <0.001).
◘ Showing comparison between AMI patients with normal iron Vs. UA patients with normal iron (◘ =P <0.05, ◘◘= P <0.01).
Ö Showing comparison between AMI patients with high iron Vs. UA patients with high iron (Ö Ö Ö = P <0.001).
Fig. (1-a, b and c): Mean values of IL-6 and TNFα (1-a), IGF-1 (1-b), cholesterol, Triglyceride, LDL-C, HDL-C and atherogenic index (1-c) of AMI patients with low serum iron versus AMI patients with normal and high serum iron. (***= P <0.001, **= P <0.01 and *= P <0.05, NS = non significant). HDL-C = high density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol.
Fig. (2- a, b and c): Mean values of IL-6 and TNFα, (2-a), IGF-1 (2-b), cholesterol, Triglyceride, LDL-C, HDL-C and atherogenic index (2-c) of UA patients with low serum iron versus UA patients with normal and high serum iron.

NS= non significant. (*= P <0.05), (** =P <0.01). HDL-C = high density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol.
Fig. (3-a and b): Correlation and linear regression of serum iron with HDL-C and IGF-1 in AMI patients with high serum iron.

Fig. (4-a and b): Correlation and linear regression of serum iron with TNFα and IL-6, cholesterol, LDL-C, triglyceride and atherogenic index (4-b) in AMI patients with high serum iron.
Fig. (5-a and b): Correlation and linear regression of serum iron with insulin like growth factor 1 (IGF-1) and HDL-C in AMI patients with low serum iron.

Fig. (6-a and b): Correlation and linear regression of serum iron and Tumor necrotic factor (TNFα) & interleukin-6 (IL-6), cholesterol, LDL-C, triglyceride and atherogenic index in AMI patients with low serum iron.
Fig. (7-a and b): Correlation and linear regression of serum iron with insulin-like growth factor 1 (IGF-1) and HDL-C in UA patients with low serum iron.

Fig. (8-a and b): Correlation and linear regression of serum iron and Tumor necrotic factor (TNFα), interleukin-6 (IL-6), cholesterol, LDL-C, triglyceride and atherogenic index in UA patients with low serum iron.
DISCUSSION

Whereas a large body of evidence exists to confirm the pathophysiological role of inflammation in atherosclerosis and in rupture of atherosclerotic plaques, minimal informations are available regarding its role in ischemic heart disease and the ‘protective’ and ‘counterbalancing’ role of IGF-1 in this cases. Also, iron is known as a physiologically essential agent, but the association between serum iron level and pathogenesis of ischemic heart diseases is controversial. Whereas most data show no association, some have raised the possibility of a causal role, while others have suggested a protective effect of iron. Our knowledge about the association between serum iron and inflammatory markers (TNF-α and IL-6) and IGF-1 is still incomplete. In this report, we hypothesized that inflammatory cytokines, IGF-1, serum lipid and iron may influence on pathogenesis of AMI and UA. To illustrate these influence and to test our hypothesis, we carried out this investigation. To accomplish our goals, we set up three subgroups of AMI and UA patients with low, normal or high serum iron as well as control subjects. The serum levels of IL-6, TNF-α, IGF-1 in control subjects and patients with AMI and UA, as well as the potential correlations of these parameters with serum iron and lipids in different studied groups were estimated.
The present study showed predominance of smoking, hypertension, diabetes, hypercholesteremia and increase body mass index in both AMI and UA patients that coincide with previous investigators\(^{25,26}\).

Both AMI and UA patients with low, normal or high serum iron were characterized by increasing pro-inflammatory cytokines as TNF-\(\alpha\) and IL-6.

In this study, the elevated levels of inflammatory cytokines, TNF-\(\alpha\) and IL-6, in AMI and UA goes in line with other previous studies\(^{10,9,27,28}\). The inflammatory process is involved throughout the different stages of atherosclerosis. The mechanisms of actions and roles of TNF \(\alpha\) in pathogenesis of AMI and UA are supported by several observations. TNF \(\alpha\) plays a central part in the inflammatory cascade and correlates with the progression and recurrence of myocardial infarction\(^9,28\). In addition, the vulnerability of an atherosclerotic plaque is related to the presence of inflammation since it plays a vital role in disruption of the fibrous cap, which is a major pathophysiological event for the development of AMI and UA\(^8\). Furthermore, Kai et al., (1998)\(^{29}\) and Mallat et al. (2001)\(^{30}\) reported that pro-inflammatory cytokines produced by lymphocytes and macrophages, inhibit the collagen synthesis by smooth muscle cells, and enhance the expression of proteases (matrix metalloproteinases, MMPs) that lead to proteolytic destruction of connective tissue matrix in vulnerable atherosclerotic plaques.

The association between TNF-\(\alpha\) and ischemic heart diseases are supported by many investigations. TNF-\(\alpha\) is related to several common risk factors for ischemic heart diseases\(^{25}\). Smoking is a strong risk factor for the development of cardiovascular diseases and also contributes to variation of TNF-\(\alpha\) protein levels in healthy subjects\(^{31}\). In addition, TNF-\(\alpha\) is synthesized by adipocytes and increased adiposity (obesity) leads to raised levels of TNF-\(\alpha\)\(^{32,33}\) as well as elevated risk for cardiovascular diseases. So that, the increased levels of TNF-\(\alpha\) seen in association with ischemic heart may be attributable to environmental stimuli of inflammatory processes (as smoking and obesity), or genetic variations that contribute to individual differences in TNF-\(\alpha\) levels\(^{25}\).

The elevated level of IL-6 in both AMI and UA patients in the current study and its role in ischemic heart disease coincide with the other studies\(^{34,35}\). Interleukin-6 is the main regulators of the acute-phase response of inflammation, often acting in synergistic and cascade-like reactions and is capable of inducing the synthesis of the whole spectrum of acute-phase proteins\(^{46}\). It is produced by several different cell types such as fibroblasts, monocytes, macrophages, T and B lymphocytes, endothelial cells and myocytes\(^{47}\). Furthermore, IL-6 protein and gene has been shown to be present in the human atherosclerotic vessel wall and is believed to play a role in the atherosclerotic process\(^{58}\) and acute coronary disease such as AMI and UA\(^{39,34,35}\).
Insulin like growth factor-1 (IGF-1) decreased in AMI and UA patients with low, normal or high serum iron.

In our study, the significant reduction of serum level of IGF-1 in both AMI and UA patients in relation to controls is in agreement with previous reports and suggests the cardioprotective role of IGF-1 in selected patients' populations. The association between IGF-1 and ischemic heart disease is studied by other investigators\(^{(40,26)}\). IGF-1 has insulin-like metabolic effects and originates from the liver under stimulation of growth hormone (GH). It mediates many of the GH anabolic and growth actions\(^{(41)}\). IGF binding protein-3 (IGFBP-3) inhibits the bioactivity of IGF-1 by sequestering IGF-1 into a circulating reservoir, thereby reducing the free active fraction of IGF-1 in the circulation. Therefore, decreased IGF-1 and increased IGFBP-3 were associated with increasing risk of developing ischemic heart disease\(^{(40)}\). Because IGF-1 is important for tissue repair and cell proliferation, it has been implicated in the pathogenesis of atherosclerosis\(^{(3)}\), progression of focal coronary atherosclerosis\(^{(42)}\), ischemic heart diseases\(^{(6)}\) and increasing mortality of AMI\(^{(26)}\). Furthermore, Hong et al., 1996\(^{(43)}\), Vaessen et al., 2001\(^{(44)}\) and Moigian et al., 2006\(^{(26)}\) reported that absence of 192- and 194-bp alleles of the IGF-1 promoter was associated with declining IGF-1 production and increased risk of myocardial infarction. These special effects of IGF-1 may be attributed to its ability to regulate myocardial structure and function and promote cardiac muscle growth, differentiation, promoting tissue remodeling and survival during myocardial ischemia\(^{(6)}\).

One of the most important property of IGF-1 is its ability to suppresses myocardial apoptosis and improves myocardial function\(^{(5,45)}\). IGF-1 may also increase cardiac output and myocardial contractility, which may contribute to improved survival after a AMI\(^{(6)}\). Taken collectively, these findings imply important roles for IGF-1 in pathophysiology of ischemic heart diseases and decrease the incidence of these cases. Potential therapeutic and prophylactic situation of IGF-1 in these cases need further studies.

Riskful lipids (cholesterol, triglyceride, LDL-C) and atherogenic index increased whereas cardio-protective lipid, HDL-C declined in both AMI and UA patients.

Alteration of serum lipid profile (significant increase of cholesterol, triglyceride, LDL-C, atherogenic index and highly significant decrease, \(P <0.001\), of HDL-C) in both AMI and UA patients evident in this report harmonizes with other investigations. High triglyceride and low HDL-C, were at least as powerful a predictor of ischemic heart diseases (IHD) as isolated high LDL-C\(^{(46,47,48)}\). In support of this role, triglyceride appears to be closely related to a series of potentially atherogenic and thrombogenic changes, such as small, dense LDL particles\(^{(49)}\), prolonged postprandial hypertriglyceridemia\(^{(50)}\). Also, triglyceride increases accumulation of chylomicron remnants\(^{(50)}\), higher concentrations of
plasminogen activator inhibitor-1 (51,52) and higher concentrations of coagulation factor VI (53). So, it seemed more meaningful to focus on the combined lipid profile, high TG–low HDL-C, as a risk factor of IHD than on high TG and low HDL-C as individual risk factors. Therefore, scientific efforts to prevent IHD should include intervention against high TG–low HDL-C, and not just against hypercholesterolemia.

A significant increase of atherogenic index (Cholesterol/HDL-C ratio) in both AMI and UA patients in this study coincides with other studies. Isabelle Lemieux et al., (2001) (54) reported that total cholesterol/HDL-C ratio may be associated with more substantial alterations in metabolic indices predictive of ischemic heart disease risk than variation in the LDL-C/HDL-C ratio. This may propose the possibility of using atherogenic index as an indicator for ischemic heart risk.

The current study indicates that increase in risky lipids and reduction of HDL-C were especially in AMI patients with low serum iron than AMI patients with normal or high serum iron or UA patients with low serum iron that is in agreement with other studies. The concentration of LDL-C was regarded as oxidative risk factors for ischemic heart diseases (57). In addition, in AMI patients, both interleukin 10 and HDL-cholesterol decreased which confirmed their protective roles in coronary artery disease (58). Controversy, Lee et al. (2006) (18) reported that serum TG and HDL-C were not change significantly in ischemic heart disease (IHD) patients with low, normal, or high serum iron, whereas LDL-C were increased in IHD patients with low serum iron, compared with the normal subjects. Collectively, we can propose that lipid profile is an important pathophysiological parameter associated with atherosclerotic inflammatory process take place in ischemic heart diseases.

In the existing work, a significant positive correlation between serum iron with cardio protective agents IGF-1 and HDL-C and a negative correlation with IL-6, TNFα, cholesterol, LDL-C, triglyceride and atherogenic index was evident in AMI patients with high or low serum iron. Likewise, a positive correlation between serum iron with IGF-1 and HDL-C and negative correlation with TNFα, IL-6, atherogenic index and LDL-C were manifested in this investigation among UA patients with low serum iron. However, UA patients with high serum iron exhibited only significant negative correlation between serum iron with inflammatory cytokines (TNFα and IL-6), triglyceride and atherogenic index. These proofed correlations in this study concur with other investigators (56&18). Besides, Tziakas et al., (2003) (55) reported a strong positive correlation between Interleukin 10 (IL-10) and HDL-cholesterol in AMI patients, which might be an indication of a common synergistic anti-atherogenic action of two molecules in coronary artery disease.

These findings may propose that AMI and UA patients with low serum iron were associated with less cardiac protection and more pro-inflammatory states and risky lipids as
well as may have a higher cardiovascular risk. In addition, Maria-Chiara et al. (1997)\(^{(56)}\) found that low serum iron levels were associated with an increased risk of ischemic heart disease and all-cause mortality. Also, high serum iron in both AMI and UA patients appeared to be associated with increasing cardioprotection and reducing proinflammatory cytokines and risky lipids.

The association between low serum iron and risk of AMI and UA are supported by several observations. The role of reduced oxygenation due to decreasing hemoglobin and hematocrit on the myocardial performance has been proved, especially on the physiologic level, so that anemia is a known risk factor for ischemic heart disease\(^{(57)}\). When hemoglobin concentration is reduced to less than half the normal level, ventricular function is impaired, presumably because the coronary flow has approached its maximum\(^{(58)}\). Based on knowledge of the physiologic role of oxygen delivery to the myocardium, anemia may be a cause of more severe cardiovascular diseases as IHD, congestive heart failure, rhythm disturbance and higher mortality rate\(^{(59)}\).

Moreover, IHD patients with low serum iron appear to be associated with increased myocardial damage markers as lactic dehydrogenase and aspartate amino transferase suggesting that myocardial anaerobic metabolic activity may be higher in IHD patients with low iron\(^{(18)}\). Bilirubin, an antioxidant derived from biliverdin\(^{(60)}\) was significantly lower in the blood of IHD patients with low serum iron which suggest that bilirubin had a protective role in the cardiovascular events\(^{(61,62,18)}\).

Iron is physiologically essential; contradictory, dietary intake of iron elevates LDL-C that was regarded as oxidative risk factor and decreases plasma antioxidant levels\(^{(17)}\). In addition, excess iron can be a toxic and lead to the formation of a cascade of free radicals and lipid peroxidation\(^{(14,15)}\) and accelerate the process of atherosclerosis\(^{(16)}\). This effect may be due to the ease with which iron is reversibly oxidized and reduced and thus able to participate in the generation of numerous powerful oxidant species\(^{(14)}\).

AMI patients with especially low serum iron incorporated in this study revealed significant increase the measured proinflammatory cytokines (IL-6, TNF-\(\alpha\)) as well as risky lipids and significant decrease cardioprotective, HDL-C and IGF-1 as comparable to that of AMI patients with normal or high serum iron and UA patients with low serum iron. Whereas in UA patients with low serum iron, triglyceride significantly increased and cardioprotective agents (IGF-1 and HDL-C) decreased significantly in comparison with UA patients with normal or high iron. These outcomes are in agreement with previous reports of Maria et al., 1997\(^{(56)}\), Lee et al., (2006)\(^{(18)}\) and Shin-Da et al., 2006\(^{(63)}\) who found that IHD patients with low serum iron were associated with a pro-inflammatory state, such as increased TNF-\(\alpha\), IL-6, and C-reactive protein and decreased cardiac protective factor, such as decreased IGF-1.
Subsequently, low serum iron in AMI patients selected in this study induced more injurious effects than UA patients. These effects may be consistent because measured serum ion levels were much reduced in AMI (50.9±4.8 mg/dl) than UA patients (52.12±5.6 mg/dl) which produced more damaging effects. Beside, these effects may be reasoned to more damage in AMI, similar to chronic inflammatory diseases, may be accompanied by a significant decrease of iron and total iron binding capacity, especially during the first 3 days. In addition, potential mechanisms include direct damage of the vessels or an immunologic inflammatory response were especially in AMI patients. An increased uptake of iron in the reticulo-endothelial system for synthesis of ferritin may account for the lowered Fe level and iron saturation of transferrin and TIBC in cases of AMI. Moreover, these findings could be the result of the intensity and the duration of the inflammatory stimulus, which characterize AMI, as well as perhaps being the consequence of the different patterns of immunological reactions and pathophysiological processes that underlie AMI cases.

**Conclusions:**

The outcomes of this study can be summarized as following: Both AMI and UA patients were associated with a pro-inflammatory state, such as increased TNF-α and IL-6, increased risky lipids (cholesterol, triglyceride, LDL-C, atherogenic index) and decreased cardiac protective factors, such as IGF-1 and HDL-C. These findings support the pivotal role of inflammation in pathophysiology of both patients' population as well as the protective role of IGF-1 in ischemic heart disease. In addition, low serum iron in both AMI and UA patients was associated with more proinflammatory state and less cardiac protection than normal subjects or patients with either normal or high serum iron. However, the deleterious effects of low serum iron were more in AMI than UA patients. Also, a positive correlation was evident between serum iron and cardioprotective agents, IGF-1 and HDL-C with negative correlation with proinflammatory cytokines and risky lipids. The underlying mechanisms of these effects are open for further investigations. Of course, further extensive studies are required to clarify the detailed mechanism of low iron in heart diseases.

**Clinical application:**

For clinical application, the low levels of serum iron adjoined with other cardiac risk factors might be used as a set of prognostic markers for predicting possible occurrence and recurrence of ischemic heart diseases or possible sudden death. We may further propose that IHD patients should regularly monitor their serum iron. In addition, strong therapeutic and preventive approaches of anemia and low serum iron might improve the outcome of IHD.

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العلاقة بين العامل المماثل للانسولين - 1 و عامل التحلل الخلوي الفا والانترلوكين - 6 ودهون مع مستوى الحديد في مصل مرضى الاحتباء القلبي الحاد و الدمية الصدرية غير مستقرة

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المقدمة: ينتج الاحتباء القلبي الحاد و الدمية الصدرية غير مستقرة، عن صعوبة نقل الدم و ضيق الشرايين التاجية و ما يتبناها من أمراض القلب المختلفة. كما أن زيادة أو نقص مستوى الحديد في الدم قد يكون له دور في زيادة معدل الإصابة بنقص الشرايين و قصور الدورة الدموية الناتجة.

الهدف: تأكيد دور الألتهاب و العامل المماثل للانسولين، وفي حدوث مرض الاحتباء القلبي الحاد، و الدمية الصدرية غير مستقرة وكذلك دراسة العلاقة بين العامل المماثل للانسولين و الفا، والانترلوكين - 6 ودهون مع مستوى الحديد في مصل الدم للمرضى.

الآراء والنتائج: تم البدء بالعمل بين قسم الفسيولوجي و الكيمياء الحيوية والأمراض الداخلية بكلية الطب جامعة أسيوط و 18 مريض حيث تم قصاص لدمهم و تشخيصهم كمرضى، ورفع عيانهم من الأفراد الذين لم يعانون من أمراض القلب أو ارتفاع ضغط الدم كمجموعة ضابطة، و تم تحضير المرضى أكلينياً و عمل تخطيط كهربائي للقلب، ثم حساب عيان من الدم الوريدي وفصل المصل منها لكل من المشاركين في البحث.

النتائج: وقد أثبتت النتائج أن هناك زيادة ذات دالة إحصائية في حالات الالتهاب مثل عامل التحلل الخلوي الفا والانترلوكين - 6 ودهون ضارة في عوامل الحماية للقلب مثل العامل المماثل للانسولين - 1، وللبروتين الدهني مرجع الكثافة، في مرض الاحتباء القلبي الحاد و yansıمة الدمية الصدرية غير مستقرة بالمقارنة بالعمومية. و نشأت هذه الدراسات في حدوث التأثر الضار على مرضى الاحتباء مشابهًا للمرضى الذين يعانون من أمراض القلب غير مستقرة، و هناك اتجاه نحو زيادة في مرضى الاحتباء القلبي الحاد، و برأيك أن هناك نقص في نقل الدم، و مع ذلك، يمكن استخدام الحديد و عنا في مستوى الحديد في الدم، وعلاج أي نقص في أولاً و أخيرًا.

و قد وجدت علاقة موجبة ذات دالة إحصائية بين مستوى الحديد و عوامل الحماية للقلب مثل العامل المماثل للانسولين، و البروتين الدهني مرجع الكثافة، وكذلك علاقة سلبية ذات دالة إحصائية بين و بين حالات الالتهاب، وكذلك الدم و ممارسة الكثافة الحرارية في مرضى الاحتباء القلبي الحاد، و يمكن استخدام مقياس كثافة الدم من هذه النتائج، بأن ضبط نقص مستوى الحديد في الدم إلى العوامل الضارة التي يجب تقديرها في مرضى قصور الدورة الدموية الناتجة للقلب و نقصهم في المتابعة المنتظمة للمرضى المصابين في الدم وعلاج أي نقص فيه أو لا أول.