Association of circulating levels of Adiponectin and IL-6 with incidence and severity of the coronary artery disease in patients undergoing Coronary Artery Bypass Graft (CABG)

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ABSTRACT
Adipokines are the bioactive substances secreted mainly by adipose tissue. The two imperative regulators in the perspective of metabolic disorders are adipokines and cytokines. Proinflammatory adipokine-cytokine secretory capacity of inflamed adipose tissue is very high. Anti-inflammatory adiponectin is found to be reduced in various subclinical inflammatory conditions unlike IL-6 which is mainly associated with setting inflammation. The aim of this study was to compare circulatory IL-6 with adiponectin in stable and unstable Coronary artery disease (CAD) patients. In the present study serum adiponectin and IL-6 levels were estimated in CAD patients and the association between then were noted. It was observed that the adiponectin levels were reduced in CAD patients than controls [7.76 (±4.74)/ 17.76 (±9.52)] while IL-6 levels were drastically increased in CAD patients than controls [10.10(±7.10) / 2.63 (±1.51). Lowest levels of adiponectin and higher levels of IL-6 was observed in Unstable CAD patients than that of Stable CAD patients.

Conclusion: The study concluded that low circulatory levels of adiponectin and higher circulatory levels of IL-6 might be associated with CAD and severity of the disease.

Keywords: Coronary artery disease, inflammation, proinflammatory, Anti inflammatory, Adiponectin, IL-6.

INTRODUCTION
Coronary artery disease (CAD) is the major form of cardiovascular diseases (CVD). Cardiovascular epidemiology studies in India indicate that this has become an important public health problem in India. An increase in incidence of CAD was observed in Indians; especially in urban populations with sedentary lifestyles and improper food habits (1). The exact mechanism for the underline development and related pathophysiological changes is still not very clear. Inflammation plays a significant role in human health. Adipose tissue has got a new recognition as biggest endocrine organ due to its adipo-cytokine secretory capacity. Inflamed adipose tissue called adiposopathy (Sick Fat) is a pathological condition where adipose tissue secretes various proinflammatory adipokines and cytokines (2). Disturbance in pro and anti inflammatory adipokine-cytokine imbalance alter the normal scenario and proinflammatory signaling cascade gets a forward push.

Adiponectin was discovered by Meada et al, in 1996 (3). It is an anti-inflammatory adipokine which is studied by various scientists for its association with various diseases (4-10). Adiponectin plays an important role in glucose and lipid homeostasis by promoting a strong insulin-sensitizing effect.
Adiponectin has anti-oxidative and anti-inflammatory effects. Various study reported low levels of adiponectin in obesity (4-6), insulin resistance (7), type 2 diabetes (6, 8) and CAD (9, 10, and 13). It has been also observed that low levels of adiponectin are associated with endothelial dysfunction. Further it has been also reported that the presence of CAD as well severity of the CAD disease is associated with hypoadiponectinemia (11, 12). Inflamed adipose tissue (AT) secretes lesser anti inflammatory adiponectin and hence an inhibitory effect on proinflammatory signaling is lost.

The Cytokine term was coined in the 1970's and Interleukin-6 (IL-6) is the most studied cytokine. Due to its pleiotropic effects it is still in demand in the scientific studies, as its role is not completely understood. IL-6 is produced by a variety of cells and can affect various metabolic and immunological pathways. IL-6 is a common inflammatory marker and increased IL-6 concentration has been considered as a marker of high inflammatory status which is usually associated with obesity (14, 15). In obesity, an increase in circulatory levels of IL-6 was observed which showed an association with visceral adipose tissue (VAT) mass. Increased expressions of IL-6 in VAT were also observed in obese subjects. It was also reported that IL-6 may play a crucial role in path physiological changes associated with insulin resistance, type 2 Diabetes (16) and CAD (17-20). Several reports have indicated that the progression and severity of the metabolic disorders are well correlated with increased level of inflammatory parameters like Hs CRP and have shown to be associated with IL-6. It has been considered to be involved in the stimulation of these factors which play an important role in atherosclerosis and its progression (20, 22, 23).

Materials and Methods:

The study included a total 474 subjects, CAD (286), subdivided into Stable CAD (152), Unstable CAD (134) and normal controls (188). The CAD group was classified according to the pattern of Angina (Stable/ Unstable). Group I included 152 patients with stable angina, while Group II was formed from 134 patients with unstable angina. Control included 188 clinically healthy subjects with normal BMI.

Inclusion criteria: Coronary Artery disease diagnosed with Coronary Angiography. Age and sex matched healthy individuals without clinical evidence of coronary artery disease and with normal Coronary Angiography were included as controls.

Exclusion criteria: Pregnant women, pediatric population, Patient with Congenital Heart disease, acute or chronic infection, chronic liver and kidney disease.

Anthropometric measurements like height (m²), weight (Kg) and waist circumference (cm) were recorded for all the participants. The BMI was calculated according to the standard formula BMI= Height in m²/Weight in Kg. Waist circumference was measured at a central point between the last rib and iliac crest and hip was recorded at the widest area of the hip. Fasting blood samples were collected from all the study participants. The serum was separated and preserved at -80º C for further use. Biochemical parameters like lipid profile, fasting blood sugar were evaluated. Serum levels of adiponectin and IL-6 were analyzed by ELISA using commercially available kits.

All statistical analyses were performed using SPSS 20.0 version software. Data were expressed as mean ± SD. For multiple group comparisons one way ANOVA was used. Independent ‘t’ test were used for group comparison as appropriate and asymptomatic (2-tailed) P values were considered for significant differences.

Results: In the present study a statistical non significant results were obtained for BMI and other Lipid profile tests except HDL. While statistical significant results were observed for serum adiponectin and IL-6. Serum Adiponectin levels were found to be reduced in CAD patients than in controls [7.76 (± 4.74)/ 17.76 (± 9.52)], while IL-6 levels were drastically increased in CAD patients with co-morbidities than in controls [10.10 (± 7.10) / 2.63 (± 1.51)]. Further it was also observed that in unstable CAD showed lowest levels of adiponectin and higher level of IL-6 than that in Stable CAD. Low circulatory levels of adiponectin and higher circulating levels of IL-6 might be associated with severity of CAD.

Discussion

The present study observed a statistical significant association between circulatory levels of adiponectin and IL-6 in controls and CAD patients. The study
did not observe any statistically significant correlation for age, BMI, Lipid profile between controls and CAD patients. Further it has been observed that low adiponectin and higher IL-6 levels were consistent with unstable CAD patients. The present study reported lower adiponectin concentration in unstable CAD than stable CAD patients while IL-6 levels were comparatively high in unstable CAD than stable CAD patients. It was observed that Hypoadiponectinemia (decrease in anti-inflammatory adiponectin) is closely associated with inflammatory status in CAD patients. In addition to that an increase in proinflammatory IL-6 stimulates various inflammatory signaling cascades which play a crucial role in atherogenesis. IL-6 exerts its proinflammatory effects via stimulating angiogenic factors, matrix metalloproteins, LpPLA2; (22) at the same time it inhibits macrophage apoptosis and found to be associated with vulnerable plaque and play an important role in plaque rupture.

Evidence reported so far suggests that adiponectin possesses an anti inflammatory and anti atherogenic properties (3). Experimental evidence obtained from various studies suggests that adiponectin might play a protective role against atherosclerosis (3). Adiponectin suppresses the secretion of TNF-α and IL-6 (14) from macrophages residing in atherosclerotic plaque. Adiponectin also inhibits the expression of endothelial adhesion molecules and protects endothelium. Hence adiponectin acts as a suppressor of IL-6. Altogether adiponectin may exert anti atherogenic protective effects on endothelial cells and macrophages in early stages of atherogenesis. High IL-6 release in CAD patients could be involved in intensification of inflammation related to atherosclerosis and even in plaque rupture (14).

IL-6 mediates its action through a variety of signaling pathways. IL-6 induces a prothrombotic state and has important proatherogenic properties. IL-6 plays a role in the amplification of the inflammatory cascade by initiating an acute phase response. It stimulates release of HsCRP (19) which is one of the important acute phase proteins and participates in atherogenesis (21). It has been reported that HsCRP levels were found to be elevated in unstable CAD patients (20). Our findings further support the existence of an important link between IL-6 plaque rupture as IL-6 levels found to be increased in unstable CAD. Rapid progression of plaques and increased plaque rupture are more important in determining fatal events than the development of new lesions in initially healthy regions of vessels. High levels of IL-6 could promote plaque growth and rupture by increasing expression of several key genes. In present study we could not find any significant difference in serum IL-6 concentration of stable and unstable CAD patients. But at the same time significantly low levels of adiponectin were observed in unstable patients than stable, which suggest the lower inhibitory effect of adiponectin on IL-6, as a result IL-6 fabricates its inflammatory effects at a higher rate.

**Limitations of the Study:** There are some limitations of the present study that should be considered. Limited sample sizes as samples were collected from a single hospital. Multicentric studies will be more fruitful in this regard.

**Conclusion**

Hypo adiponectinemia and high levels of IL-6 was observed in CAD patients as compared to controls. In unstable CAD consistently low adiponectin levels were reported with increased circulatory IL-6 concentration than that of stable CAD.

**Disclosure:** No potential conflicts of interest were disclosed.

**References:**


20. Vidhate D, Thomas J, Thomas B, Dhadiala S, Murarka A. Association of high sensitivity C


Tables:

Table 1: Descriptive statistics for CAD and Control groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>CAD (n=286)</th>
<th>Control (n=188)</th>
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</thead>
<tbody>
<tr>
<td>Age</td>
<td>58.11(±6.46)</td>
<td>52.50(±9.5)</td>
</tr>
<tr>
<td>BMI</td>
<td>26.97(±2.57)</td>
<td>22.16(±2.63)</td>
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<tr>
<td>Triglycerides (mg%)</td>
<td>175.83(±44.1)</td>
<td>142.94(±20.2)</td>
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<tr>
<td>Cholesterol (mg%)</td>
<td>195.11(±36.18)</td>
<td>160.53(±20.1)</td>
</tr>
<tr>
<td>LDL (mg%)</td>
<td>120.36(±31.64)</td>
<td>92.61(±22.9)</td>
</tr>
<tr>
<td>HDL (mg%)</td>
<td>39.17(±6.22)</td>
<td>49.53(±6.28)</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>10.10(±7.10)</td>
<td>2.63 (±1.51)</td>
</tr>
<tr>
<td>Adiponectin (μgm/ml)</td>
<td>7.76(±4.74)</td>
<td>17.76 (±9.52)</td>
</tr>
</tbody>
</table>
### Table 2: Comparison of Variables with CAD group-Stable CAD and Unstable CAD

<table>
<thead>
<tr>
<th>Variables</th>
<th>CAD (Stable) (n=152)</th>
<th>CAD (Unstable) (n=134)</th>
<th>Control (n=188)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>59.29(±4.34)</td>
<td>57.05(±7.87)</td>
<td>52.50(±9.5)</td>
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<tr>
<td>BMI</td>
<td>26.47(±2.94)</td>
<td>27.42(±2.85)</td>
<td>22.16(±2.63)</td>
<td>0.055</td>
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<tr>
<td>TAG (mg%)</td>
<td>166.35(±22.48)</td>
<td>184.32(±36.35)</td>
<td>142.94(±20.16)</td>
<td>0.045</td>
</tr>
<tr>
<td>Chole (mg%)</td>
<td>194.59(±35.03)</td>
<td>195.58(±38.14)</td>
<td>160.53(±20.1)</td>
<td>0.021</td>
</tr>
<tr>
<td>LDL (mg%)</td>
<td>121.53(±32.55)</td>
<td>119.32(±31.67)</td>
<td>92.61(±22.9)</td>
<td>0.074</td>
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<tr>
<td>HDL (mg%)</td>
<td>39.35(±6.98)</td>
<td>39.00(±5.65)</td>
<td>49.53(±6.28)</td>
<td>0.000**</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>8.48 (±4.65)</td>
<td>11.58(±8.6)</td>
<td>2.60 (±1.51)</td>
<td>0.000**</td>
</tr>
<tr>
<td>Adiponectin (μgm/ml)</td>
<td>9.4 (±6.76)</td>
<td>6.30 (±4.74)</td>
<td>17.76 (±9.52)</td>
<td>0.000**</td>
</tr>
</tbody>
</table>

* Non significant p>0.05, ** Significant p< 0.05, ***Highly Significant p=0.000