Association of hs-CRP levels with Obesity & Metabolic syndrome in patients with Type-2 Diabetes Mellitus: a link between inflammation, adiposity & insulin resistance

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ABSTRACT

Background: High sensitivity C-reactive protein (hs-CRP), is considered a sensitive marker of systemic inflammation and highly predictive of subsequent cardiovascular events and Diabetes mellitus. Moreover hs-CRP is also associated with Obesity, Metabolic Syndrome (MetS) and its separate components. Objective: To evaluate hs-CRP levels in normoglycemic healthy subjects, obese non-diabetic & Obese Type-2 diabetic subjects and in diabetic subjects with and without MetS & its association with individual components of MetS. Material & Methods: Hundred obese non diabetic subjects, Non-obese Type-2 diabetic individuals and 100 healthy controls were enrolled into this study. Diabetic subjects were further subdivided into Diabetic subjects with and without metabolic syndrome. Various anthropometric and biochemical parameters like hs-CRP, FBS, lipid profile, fasting insulin levels & HOMA-IR were measured. Statistical analysis was done by Medcalc v11.5.0.0. software. Results: Serum hs-CRP levels were significantly higher in obese non-diabetic subjects and non-obese Type 2 DM patients as compared to control subjects (p = 4.1 × 10−6, 7.5 × 10−10, respectively). Also There was significant difference in serum hs-CRP levels between Obese Non-diabetic subjects and non-obese Diabetic subjects (p = 0.010). hs-CRP levels correlated positively with BMI (r = 0.26, p < 0.001) & Waist circumference (r = 0.45, p < 0.001) in Obese Non-diabetic subjects. As the number of components of MetS increased, mean hs-CRP levels also increased. Conclusion: Plasma hs-CRP levels is not significantly affected by diabetes per se, and this suggest that alterations in Type 2 DM & MetS may be due to obesity and may be an important link between obesity, Insulin resistance and Type 2 Diabetes Mellitus.

1. Introduction

Diabetes mellitus, is a heterogeneous disorder characterized by metabolic abnormalities like: insulin resistance (IR) coupled with impaired β-cell function and long-term complications involving the eyes, kidneys, nerves and blood vessels. Recently it has been shown that in both developed and developing countries there is a rising epidemic of diabetes mellitus and obesity, and hence the occurrence of metabolic syndrome. The metabolic syndrome (MetS) is defined as the constellation of cardiovascular risk factors like hyperglycaemia, mild Dyslipidaemia, hypertension, and Visceral obesity and it substantially increases the risk of developing cardiovascular diseases (CVD) and Type 2 diabetes mellitus.2

In the last few decades, a hypothesis was proposed to associate the pathogenesis of Type 2 DM with a state of chronic low grade inflammation. Many studies have shown an increase in levels of inflammatory markers such as C-reactive protein (CRP), tumour necrosis factor-α (TNF-α) and Interleukin-6 (IL-6) in patients with Type 2 DM and in Metabolic syndrome (MetS).3 In recent years high sensitivity C-reactive protein (hs-CRP) - has evolved as an important marker of systemic inflammation & predictor of for cardiovascular disease and type 2 diabetes. Previous studies have shown that elevated hs-CRP levels correlate significantly with features of metabolic abnormality, including obesity, hyperinsulinaemia & insulin resistance. Moreover hs-CRP is associated with the MetS and its separate components.4 Although the mechanisms linking high hs-CRP levels to these disorders are not known, it is possible that the association may be partly mediated by adipose tissue, a main source of inflammatory cytokines.5 Considering the hypothesis that inflammation may be an important factor for causing Diabetes and metabolic syndrome and may contribute for CVD development, the present study aimed to find the relationship of plasma hs-CRP levels obesity, metabolic syndrome & type 2 diabetes mellitus and compare them to normal subjects.
Material & Methods:

Study participants: This cross sectional study was conducted from June 2014 to January 2015 at the Diabetes Clinic and Outpatient Department of Dhiraj General Hospital attached with SBKS Medical Institute and Research Centre, Gujarat. Hundred healthy non-obese, normoglycaemic subjects with a BMI < 23 kg/m² served as controls (Group A); 100 obese non-diabetic subjects with BMI ≥ 25 kg/m² and FBG < 100 mg/dl (group B); and 100 non-obese Type 2 DM patients with blood glucose > 125 mg/dl and BMI < 23 kg/m² (Group C).6 Obese Type 2 DM patients were excluded in order to examine the role of diabetes per se. In general, subjects with acute or chronic infections, severe medical conditions (malignancy, renal failure, liver cirrhosis, connective tissue disease, and chronic congestive heart failure) were excluded from the study. The study protocol was approved by the institutional ethics committee. Informed consent was obtained from all individuals after explaining the purpose and nature of the study. Metabolic syndrome was defined using the modified NCEP ATP III Definition.7

Anthropometric and laboratory measurements:

Height and weight were recorded. Waist circumference was measured using a non-elastic measuring tape at the highest level of iliac crest with the patient standing with feet 1 foot apart. BMI was computed as weight in kilograms (kg) divided by height in meters squared (m²). Hip circumference was measured at the maximum extension of the buttocks. Blood pressure (BP) measurements were taken from each patient’s right arm in the seated position by using Automatic Blood Pressure Monitor. 6 ml of blood was collected from each subject after 12 hours fast and immediately taken into vacuette, sodium fluoride (for glucose measurement) and plain tubes for other biochemical investigations.

Biochemical Analysis

Lipid profile & blood sugar levels were estimated in automated clinical chemistry analyser (Erba EM200). hs-CRP levels were measured by turbidimetric method (Agappe diagnostics) on semi-automated analyser. Serum Insulin levels were measured by AIA-IRI pack on TOOSHI AIA system analyser. Insulin resistance was calculated using the Homeostasis Model Assessment (HOMA 2) Calculator v2.2 (Oxford Centre for Diabetes, Endocrinology and Metabolism).

Statistical Analysis

The results were analyzed by Medcalc:v11.5.0.0. statistical software. Age, waist circumference, BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP), FPG, TC, HDL-c, Serum insulin, HOMA-IR and hs-CRP were log transformed because they were not normally distributed. These parameters were reported as means and 95% confidence intervals. ANOVA test was used to describe the mean differences among groups of study. To assess the differences of hs-CRP between obese non-diabetic, non-obese Type 2 DM and normal subjects Univariate analyses (ANCOVA) adjusted for age was used. The association between hs-CRP with MetS components was tested by Spearman rank correlation coefficient. A p-value less than <0.05 was considered statistically significant.

Results:

All the clinical & biochemical parameters were analysed between normal healthy subjects (Group A), obese non-diabetic subjects (Group B) & non-obese diabetic subjects (Group C). The difference between anthropometric, clinical and biochemical parameters between the study groups are described in Table 1.

Table 1: Anthropometric, clinical and biochemical parameters between the study groups Normal subjects (Group A), Non-diabetic obese subjects (Group B), Non-obese Type 2 Diabetic subjects (Group C)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal subjects (n=100)</th>
<th>Obese Non-diabetic subjects (n=100)</th>
<th>Non-obese Diabetic subjects (n=100)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>21.3 (19.2–22.4)</td>
<td>28.2 (26.7–31.7)</td>
<td>22.7 (20.1–22.6)</td>
<td>6.9 × 10⁻⁹</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>74.3 (71.3–76.5)</td>
<td>91.6 (84.0–92.2)</td>
<td>70.8 (64.8–73.7)</td>
<td>5.3 × 10⁻⁹</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>115 (107–118)</td>
<td>128 (122–132)</td>
<td>125 (118–131)</td>
<td>4.8 × 10⁻⁵</td>
</tr>
<tr>
<td>FPG (mg/dl)</td>
<td>96.3 (90.5–101.6)</td>
<td>103.2 (98.1–107.4)</td>
<td>107.5 (101.9–112.7)</td>
<td>4.1 × 10⁻⁶</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>30.6 (27.2–42.3)</td>
<td>65.4 (59.4–69.7)</td>
<td>58.6 (53.9–64.7)</td>
<td>5.1 × 10⁻⁹</td>
</tr>
<tr>
<td>HDL-c (mg/dl)</td>
<td>30.4 (24.3–34.8)</td>
<td>28.3 (22.1–33.3)</td>
<td>26.3 (21.9–26.1)</td>
<td>5.5 × 10⁻⁹</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>7.9 (6.2–10.5)</td>
<td>19.5 (17.2–22.6)</td>
<td>11.7 (9.1–15.7)</td>
<td>4.5 × 10⁻⁹</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.9 (1.0–1.4)</td>
<td>2.7 (2.3–3.1)</td>
<td>1.9 (1.4–2.4)</td>
<td>5.3 × 10⁻⁵</td>
</tr>
</tbody>
</table>

Result presented as geometric mean and 95% confidence interval of mean;

- Normal vs. Obese Non-diabetic subjects,
- Normal vs. Non-obese Diabetic subjects,
- Obese Non-diabetic subjects vs. Non-obese Diabetic subjects as evaluated by ANOVA.

Comparison of serum hs-CRP levels between Normal, Obese Non-Diabetic and Non-obese Type 2 DM groups as evaluated by Univariate analysis is shown in Table 2. Serum hs-CRP levels were significantly higher in obese non-diabetic subjects and non-obese Type 2 DM patients as compared to control subjects (p = 4.1 × 10⁻⁶ & 7.5 × 10⁻¹⁰, respectively). Also there was significant difference in serum hs-CRP levels between Obese Non-diabetic subjects and non-obese Diabetic subjects (p = 0.010)

Table 2: Comparison of serum hs-CRP levels between Normal, Obese Non-Diabetic and Non-obese Type 2 DM groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal subjects (n=100)</th>
<th>Obese Non-Diabetic subjects (n=100)</th>
<th>Non-obese Diabetic subjects (n=100)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-CRP (mg/L)</td>
<td>0.8 (0.7–0.9)</td>
<td>2.1 (1.2–2.3)</td>
<td>3.6 (2.2–4.1)</td>
<td>4.1 × 10⁻⁶</td>
</tr>
<tr>
<td></td>
<td>b. 7.5 × 10⁻¹⁰</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>c. 0.010</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results presented as geometric mean and 95% confidence interval of mean adjusted for age;

- Normal vs. Obese Non-diabetic subjects,
- Normal vs. Non-obese Diabetic subjects,
- Obese Non-diabetic subjects vs. Non-obese Diabetic subjects as evaluated by Univariate analysis.
Correlation of serum hsCRP levels with biochemical and anthropometric parameters are shown in [Table-3]. There was a significant positive correlation between hs-CRP levels & parameters of Obesity like BMI ($r = 0.26$, $p < 0.001$) & Waist circumference ($r = 0.45$, $p < 0.001$) in Obese Non-diabetic subjects (Group B). hs-CRP levels correlated significantly with BMI ($r = 0.61$, $p < 0.0001$) & waist circumference ($r = 0.42$, $p < 0.001$) in non-obese Type-2 diabetic subjects (Fig. 1). Also hs-CRP levels correlated significantly with BMI ($r = 0.26$, $p < 0.0001$) & waist circumference ($r = 0.45$, $p < 0.001$) in obese Non-diabetic Type-2 diabetic subjects (Fig. 2).

**Table 3: Spearman’s Correlation of hs-CRP with in different groups**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal subjects ($n = 100$)</th>
<th>Obese Non-diabetic subjects</th>
<th>Obese Non-diabetic subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.43 ($p&lt;0.001$)</td>
<td>0.26 ($p&lt;0.001$)</td>
<td>0.61 ($p&lt;0.001$)</td>
</tr>
<tr>
<td>FPG</td>
<td>0.38 ($p&lt;0.001$)</td>
<td>0.29 ($p&lt;0.001$)</td>
<td>0.22 ($p&lt;0.001$)</td>
</tr>
<tr>
<td>SBP</td>
<td>0.31 ($p&lt;0.001$)</td>
<td>0.15 ($p&lt;0.10$)</td>
<td>0.12 ($p&lt;0.15$)</td>
</tr>
<tr>
<td>DBP</td>
<td>0.25 ($p&lt;0.001$)</td>
<td>0.18 ($p&lt;0.002$)</td>
<td>0.16 ($p&lt;0.09$)</td>
</tr>
<tr>
<td>WC</td>
<td>0.32 ($p&lt;0.001$)</td>
<td>0.45 ($p&lt;0.001$)</td>
<td>0.42 ($p&lt;0.001$)</td>
</tr>
<tr>
<td>TG</td>
<td>0.24 ($p&lt;0.006$)</td>
<td>0.51 ($p&lt;0.001$)</td>
<td>0.56 ($p&lt;0.001$)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>-0.165 ($p=0.04$)</td>
<td>-0.412 ($p&lt;0.001$)</td>
<td>-0.381 ($p&lt;0.001$)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.396 ($p&lt;0.001$)</td>
<td>0.321 ($p&lt;0.001$)</td>
<td>0.297 ($p&lt;0.001$)</td>
</tr>
</tbody>
</table>

**Fig 1: Correlation of hs-CRP levels with BMI in non-obese Type-2 Diabetic subjects (Group C)**

**Fig 2: Correlation of hs-CRP levels with BMI in Obese Non-diabetic Subjects (Group B)**

The prevalence of Metabolic syndrome in Type-2 Diabetic Individuals according to modified NCEP ATPIII definition was 79% (79 / 100) of which prevalence rates was higher in females (41/47, 41%) compared to men (38/53, 38%). (P < 0.05). The metabolic syndrome patients were further grouped in terms of number of criteria satisfied. Out of 100 Type-2 Diabetic patients, minority 18 (18%) were only diabetic, 32 (32%) has 2 components present, 29 (29%) were satisfying 3 criteria & 21 (21%) 4 criteria (Fig 3). Mean hs-CRP levels in MetS group was significantly higher than mean hs-CRP levels in Non-MetS group (3.02 mg/L vs 1.98 mg/L respectively).

**Discussion:**

Inflammation is considered as an important causative factor in development of cardiovascular diseases in metabolic diseases such as obesity, MetS & Diabetes. Studies have revealed that a low-grade inflammation precedes and predicts the onset of diabetes in adults and inflammatory markers like hs-CRP are
The limitation of this study was that it was cross-sectional study. Hs-CRP levels were measured at only one time. hs-CRP is highly variable within subjects and thus has to be studied in prospective studies.

Conclusions:

In conclusion, plasma hs-CRP levels was not affected by diabetes per se, and this suggest that reported alterations in plasma hs-CRP levels in Type 2 DM may be due to excess adipose tissue mass/obesity. Also as there is linear increase in hs-CRP levels with increasing number of metabolic syndrome components, it can be used as a surrogate marker of chronic inflammation in patients with metabolic syndrome. Thus, the significant increase of hs-CRP in obesity and its positive correlation with parameters of obesity & insulin resistance suggest that chronic inflammation and obesity are the key players for developing diabetes & MetS.

References:


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