Original Article

Association of Serum High Sensitivity C-Reactive Protein with Insulin Resistance in Patients with Type-II Diabetes Mellitus

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Abstract

Background: *Insulin resistance (IR) is the major event in type-II diabetes mellitus followed by an increasing degree of β-cell dysfunction. Chronic inflammation is the driving force for IR and type-II DM. The process of inflammation induces hepatic synthesis of high sensitivity C-reactive protein (an acute-phase protein) which plays a role in IR. The study was undertaken to find out the association of serum hs-CRP with insulin resistance in patients with type-II diabetes mellitus.* **Methodology:** *A hospital-based observational study was carried out in the Outpatient Department of Endocrinology, Chittagong Medical College Hospital and Department of Biochemistry, Chittagong Medical College. A total of 126 patients with type-II diabetes mellitus aged 40-64 years were included by non-probability consecutive sampling technique. Serum hs-CRP, fasting serum insulin was estimated. IR was calculated by using the Homeostasis Model Assessment of Insulin Resistance index (HOMA-IR).* **Results:** *The mean serum hs-CRP level was 9.1 ± 0.36 mg/L in patients with type-II DM. Serum hs-CRP was significantly associated with insulin resistance and positively correlated with HOMA-IR in type-II diabetics. Body mass index (BMI) as one of the key indicators of insulin resistance was also associated with serum hs-CRP in this study population. Additionally serum hs-CRP was correlated positively with BMI and fasting serum insulin in patients with type-II DM.* **Conclusion:** *The results of this study concluded that increased serum hs-CRP was well associated and positively correlated with insulin resistance and its parameters signifying a possible role of subclinical inflammation in IR among patients with type-II diabetes mellitus.*

Key words: Type-II DM, Serum hs-CRP, IR, HOMA-IR, BMI

Introduction

Type-II diabetes mellitus (DM) is a combination of resistance to the actions of insulin in the liver and muscle together with impaired pancreatic β-cell function leading to insulin sensitivity[1]. Basic research studies are in progress with the hypothesis that chronic subclinical inflammation is the main cause of insulin resistance leading to the development of type-II diabetes mellitus[2-4]. Type-II DM may be precipitated or accelerated by an acute phase reaction as part of the innate immune response, in which large amounts of cytokines are released from adipose tissue, creating a low-grade inflammatory milieu[5]. Inflammatory cytokines secreted by adipose tissue exert an endocrine effect conferring insulin resistance in the liver, skeletal muscle and vascular endothelial tissue ultimately leading to the clinical expression of type-II DM[6]. Such systemic and subclinical inflammatory processes can be characterized by elevated circulating levels of inflammatory cytokines including CRP or hs-CRP[7].

Among several markers of inflammation C-reactive protein is found to be significant in people with type-II diabetes mellitus[8]. The recent emphasis on high sensitivity or 'highly sensitive' CRP abbreviated as so-called hs-CRP, seems to have created a false impression in some quarters that this is somehow a different analyte from 'conventional' CRP[9]. hs-CRP is the same exquisitely sensitive and systemic marker of infection, inflammation, tissue damage, and/or almost any form of adverse non-physiological stress as the CRP, which has been extensively studied and used clinically for over 75 years[10]. A new method of enzyme-linked immunosorbent assay was established to evaluate the level of hs-CRP, which has much higher sensitivity than

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classic methods used previously[11]. The American Heart Association Centers for Disease Control has classified serum hs-CRP levels <1, 1-3 and >3 mg/L as low, intermediate and high-risk groups for global cardiovascular disease respectively[11].

Elevated C-reactive protein concentrations may reflect not only local inflammation at atherosclerotic lesions but also systemic abnormalities related to insulin resistance such as an increase in fasting insulin, body mass index, systolic blood pressure and triglyceride as well as a decrease in high-density lipoprotein cholesterol[12-13]. Insulin resistance (IR) was calculated by using the Homeostatic Model Assessment of Insulin Resistance index (HOMA-IR)[14] i.e. HOMA-IR=Fasting serum insulin (mU/L) x Fasting plasma glucose (mmol/L) ÷ 22.5 and value more than 2.6 is considered as insulin resistance[15].

Serum hs-CRP is one of the simply available inflammatory markers and can be done in routine clinical practice. We therefore planned to find out the association of serum hs-CRP with insulin resistance estimated by the HOMA-IR in patients with type-II diabetes mellitus.

Materials and Methods
This hospital-based observational study was carried out in the Outpatient Department of Endocrinology, Chittagong Medical College Hospital in collaboration with the Department of Biochemistry, Chittagong Medical College. The study was conducted from January 2016 to December 2016. The study was undertaken after approval by the Ethical Review Committee of Chittagong Medical College and the concerned departments.

Patients with acute infection/systemic diseases with known serum C-reactive protein levels ≥6 mg/L were excluded from the study. Based on OGTT, 126 patients with type-II DM aged between 40-64 years were selected by non-probability consecutive sampling. A questionnaire regarding the variables of interest was also noted. Weight and height were measured. Body mass index was calculated.

Before the screening procedures all the participants gave informed written consent. Upon arrival in the morning, a 5 ml of fasting venous blood sample was obtained from each participant under all aseptic precautions. Subjects were then allowed to drink 75 gm of oral glucose in 300 ml of water within 3-4 minutes. They had not been asked to eat. After 2 hours of oral glucose consumption another 3 ml venous blood sample was taken. For the measurement of plasma glucose, venous blood samples were collected into the NaF containing test tube. Serum was separated by centrifugation for 10

min at 3000 rpm. Plasma glucose was determined by the glucose oxidase method using the multichannel autoanalyzer. Serum hs-CRP was measured by nephelometry in Siemens BN proSpec system. Fasting serum insulin was measured by Siemens ADVIA Centaur autoanalyzer, which is a direct chemiluminescence technology utilizing constant amounts of two antibodies.

All the data were processed and analyzed using Microsoft excel and IBM-SPSS v22.0 for Windows. Statistical inference was based on 95% confidence interval and p value ≤0.05 was considered statistically significant. Quantitative data were expressed as mean±SEM. Qualitative data were expressed in frequency and percentage. Chi-squared (χ^2) test and odds ratios were used to measure the significance of association between categorical variables. The Pearson's correlation coefficient observed a correlation analysis between variables. In the relevant presentations tables and diagrams were produced where necessary.

Results
Based on the oral glucose tolerance test, we have taken a total of one hundred and twenty-six (126) individuals with type-II DM. Out of 126 patients 63 were males and 63 were females. Mean age were 48.75 ± 0.56 years in the study.
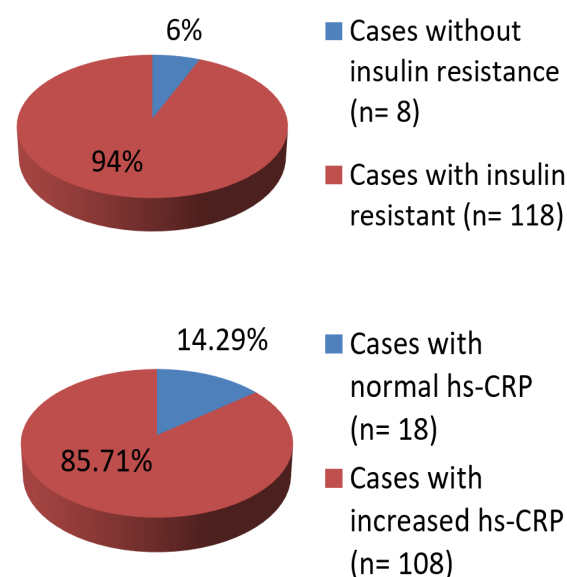

Figure-1: Pie diagram shows 94% of patients with type-II DM were insulin resistant. The trend of elevated hs-CRP was 85.71% among the type-II diabetics.

Table-I shows that mean serum hs-CRP level of type-II diabetic patients was 9.1±0.36 mg/L. The mean value of HOMA-IR was 9.94±0.51. The mean BMI of the participants was 25.12±0.18, which for Asians is regarded to be pre-obese.

Table-I: Baseline characteristics of the study cases (n=126)

Variables	Mean±SEM	Range
Age (Years)	48.75±0.56	40-64
BMI (kg/m^2)	25.12±0.18	21.09-32.81
FPG (mmol/L)	12.1±0.33	05.33-20.94
2 HPG (mmol/L)	18.55±0.41	11.12-29.44
Serum hs-CRP (mg/L)	9.1±0.36	03.09-29.9
Fasting serum Insulin (mU/L)	18.49±0.87	01.69-64.4
HOMA-IR	9.94±0.51	0.93-26.37
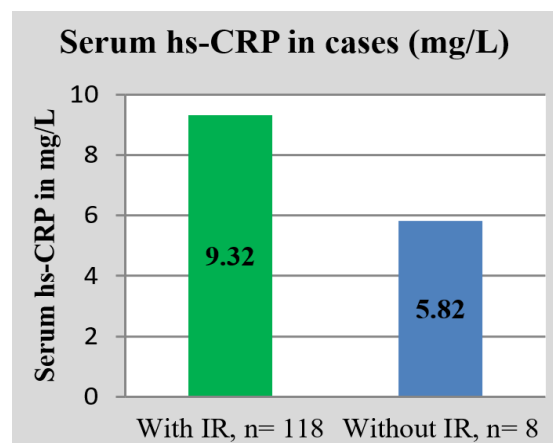
Figure-2: Bar diagram showing mean serum hs-CRP in cases with insulin resistance (9.32 mg/L) and cases without insulin resistance (5.82 mg/L).

Table-II: Association between increased serum hs-CRP and insulin resistance in cases (n = 126)

Groups	hs-CRP status		Total
	Increased hs-CRP (Serum hs-CRP ≥ 6 mg/L)	**Normal hs-CRP (Serum hs-CRP < 6 mg/L)**	
With insulin resistance	104 (88.14%)	14 (11.86%)	118 (100%)
Without insulin resistance	04 (50%)	04 (50%)	08 (100%)
Total	108 (85.71%)	18 (14.29%)	126 (100%)
$\chi 2$ value = 8.90; p<0.05 (Significant)			
Odds ratio = 7.43; p<0.05 (Significant)			

Table-III: Association between BMI and serum hs-CRP in cases (n = 126)

BMI groups	hs-CRP status		Total
	Increased (Serum hs-CRP ≥ 6 mg/L)	**Normal (Serum hs-CRP < 6 mg/L)**	
Upper tertile (BMI > 25.53 kg/m^2)	40 (95.24%)	02 (4.76%)	42 (33%)
Lower two tertiles (BMI < 25.53 kg/m^2)	68 (80.95%)	16 (19.05%)	84 (67%)
	108 (85.71%)	18 (14.29%)	126 (100%)
Odds ratio = 4.71; p<0.05 (Significant)			

Table-IV: Pearson's correlation coefficient among serum hs-CRP with parameters of insulin resistance in cases (n = 126)

Correlations between variables	Pearson's Correlation coefficient (r)	p value
Serum hs-CRP with BMI	+ 0.53	p<0.05 (Significant)
Serum hs-CRP with Fasting insulin	+ 0.68	p<0.05 (Significant)
Serum hs-CRP with HOMA-IR	+ 0.78	p<0.05 (Significant)

Table-II indicates an increased/normal level of hs-CRP depending on insulin resistance for the distribution of patients with type-II DM. In contrast to just 4 cases without insulin resistance, serum hs-CRP rose in 104 patients with IR. In patients with type-II DM, a statistically significant association between increased serum hs-CRP with insulin resistance was found. Table-III reveals that BMI in the upper tertile resulted in an odds ratio of 4.71 for elevated serum hs-CRP compared to both the lower tertiles. This was statistically significant i.e. increased BMI in the patients with type-II DM was significantly associated with increased serum hs-CRP. Table-IV shows that there were positive significant correlation of serum hs-CRP with BMI fasting serum insulin and HOMA-IR in cases.

Discussion

Subclinical inflammation and insulin resistance forerunners to coronary heart disease and type-II diabetes mellitus may be pathophysiologically interlinked[16]. Experimental evidence and some cross-sectional data revealed C-reactive protein as a sensitive physiological marker of systemic subclinical inflammation and associated with hyperglycemia, insulin resistance and overt type-II diabetes[17]. In this study, 85.71% (n=108) of patients with type-II DM experienced an elevated level of serum hs-CRP. This frequency is somewhat greater than that of a prospective multi-ethnic Hispanic-American atherosclerosis study (40%) with incident type-II DM[18]. Persistent and moderate increases of serum hs-CRP may be caused by increased exposure to recurrent infection in Bangladesh.

In the present study, we attempted to evaluate the association of serum hs-CRP, the most commonly measured inflammatory marker with IR. The mean value of serum hs-CRP in type-II DM was observed to be 9.1±0.36 mg/L in this study. Rekha Bhagwat, et al. found raised levels of serum hs-CRP three times higher in type-II diabetes mellitus than control[19]. As shown in Figure-2, cases with IR had higher serum hs-CRP levels (9.32 mg/L) than those without IR (5.82 mg/L) which was consistent with the CURES-105 study on the South Indian population[20]. A population-based research showed high levels of serum hs-CRP amongst persons with both insulin resistance syndrome and clinically overt type-II diabetic[21].

It was also observed from table-I that mean values of fasting serum insulin (18.49±0.87 mU/L) and HOMA-IR (9.94±0.51) among the type-II diabetics were above the cut-off value for defining insulin resistance[14]. In this study it was shown that serum hs-CRP concentrations increased by 88.14% in patients with insulin resistance, while serum hs-CRP concentrations rose by 50% in cases without insulin resistance. The difference was cross-tabulated in table-II (χ2 value=8.90, Odds ratio=7.43, p<0.05) and it was found that there was a significant association of increased serum hs-CRP with insulin resistance in type-II DM. The above-mentioned observation is similar to observations carried out by other researchers[17,20-22]. Previous studies have shown the positive correlation of HOMA-IR with serum hs-CRP and similar results were reported also in our study in table-IV (r=+0.78, p=<0.05) suggesting that subclinical inflammations may have a possible involvement in insulin resistance and glucose intolerance[23-24].

The association between serum hs-CRP and insulin resistance was explained by different possible mechanisms. Adipose tissue is an active endocrine organ, releasing a variety of hormones and cytokines that contributes to CRP elevation[25]. Synthesis of C-reactive protein primarily in the liver is regulated by the pro-inflammatory cytokine IL-6 and tumor necrosis factor-alpha (TNF-α) in adipocytes[26]. In addition C-reactive protein provides downstream integration to overall cytokines activation as well as it bounds to the membranes of damaged vascular cells where it activates complement proteins[27-28]. This enhances the production of thrombogenic agents. These instances of vascular inflammation may contribute to the development of insulin resistance[27-28].

We also look at the association of serum hs-CRP and the body mass index, one of the most significant IR-related factors, to comprehend the relation between serum hs-CRP and IR. BMI in the upper tertile compared with the lower two tertiles resulted in an odds ratio of 4.71 for increased serum hs-CRP. From table-III, we found that elevated serum hs-CRP levels were significantly associated with increased BMI (odds ratio: 4.71, p<0.05). This result is consistent with the findings from earlier studies that have demonstrated a good association between serum hs-CRP and obesity[29].

Further table-IV showed a positive correlation between serum hs-CRP and BMI (r= +0.53, p<0.05) which supported earlier studies[30]. In obesity, the accumulation of free fatty acid intermediates activates proinflammatory serine kinase cascades, such as IkB kinase and c-Jun N-terminal kinase. These cascades promote the secretion of IL-6 which in turn triggers the hepatic synthesis of C-reactive protein[31]. A very recent study has found gene polymorphisms to explain the inter-individual variability in C-reactive protein in severely obese patients[32].

Earlier studies have been shown the relationship between systemic abnormalities of IR such as increase in fasting serum insulin, BMI and elevated level of serum C-reactive protein[12-13]. The existence of a strong and positive correlation between serum hs-CRP and fasting serum insulin has been placed into table-IV (r= +0.68, p<0.05) which is a remarkable observation of this study. As with our observation, several cross-sectional studies among type-II DM patients have shown the positive correlation of acute-phase reactants with measures of insulin resistance or plasma insulin concentration, BMI or waist circumference[23].

Studies regarding serum hs-CRP levels in type-II DM are limited in Bangladesh. This study revealed that increased serum hs-CRP was well associated with insulin resistance in type-II DM and strongly correlated with parameters of IR such as BMI, fasting serum insulin and HOMA-IR. In this study serum hs-CRP has been shown in patients with type-

II DM as a convenient marker of insulin resistance. The results of our study suggest that serum hs-CRP might be a further predictor for coronary artery diseases in such cases.

Conclusion
The present study demonstrated an association of elevated serum hs-CRP with insulin resistance in patients with type-II DM. The estimation of serum hs-CRP in the early stage of type-II DM may be a helpful enthusiastic intervention in the detection of future diabetic complications. Further research may propose the addition of serum hs-CRP as an effective marker of type-II DM in clinical practice.

Conflict of interest
The authors declare that they have no conflict of interest.

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