ESTIMATION OF LEVEL OF CARDIAC BIOMARKERS IN TYPE II DIABETES PATIENTS WITH AND WITHOUT CORONARY ARTERY DISEASE

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ABSTRACT

INTRODUCTION: Cardiovascular diseases account for serious health issues, which results in loss of quality of life and high mortality and morbidity. Cardiovascular disease risk in India is greater than US and Europe. Among various causative factors, Type 2 diabetes mellitus (T2DM) is one of the most prevalent metabolic diseases associated with increased risk for CAD. METHODOLOGY: In this study, we assess the clinical and demographic parameters along with hsCRP levels between the group of participants with 1) T2DM  2) T2DM with CAD compared with healthy controls. All participants were subjected to basic clinical laboratory test like Blood pressures (systolic and diastolic), lipid profile, HbA1c level (glycosylated hemoglobin), FBS and PPBS. Additionally, the enzyme-linked immune absorbent assay was used to determine the levels of serum hs-CRP in all the participants recruited for this study. RESULTS: Analysis of clinical characteristics showed significant association of lipid, HbA1c, and hsCRP levels in the participants with T2DM with CAD contributing towards cardiovascular disease. Particularly, the level of hsCRP along with HbA1c may contribute detecting early CVD event in participants with type 2 diabetic mellitus. CONCLUSION: Overall, our study provides significant contribution in predication of early CVD event in T2DM.
which helps reduce CVD related morbidity and mortality in T2DM.

Keywords: Coronary Artery diseases, Diabetes Mellitus, Biomarkers


Introduction: Cardiovascular diseases are one of the leading causes of death world-wide. Globally, the prevalence of cardiovascular disease is noticed high in India than US and Europe. Although, the ‘major constitutional risk factors’, such as age, gender and genetic factors play a vital role in CVD, there are acquired risk factors like hypertension, smoking, diabetes, obesity and hypercholesterolemia contribute significantly to this disease. Importantly, the Type 2 diabetes mellitus (T2DM) is most contributing factor associated with increased risk for CVD. Study suggests T2DM patients have two to four time higher CAD risk compared to non-diabetic individuals. Poor glycemic control on the basis HbA1c level reports increased HbA1c by one percentage result in 25 percentage increased risk of cardiac diseases. It has been demonstrated the diabetes accelerate the chance of atherosclerosis formation that causing unstable plaque in arteries. Any biomarkers that determine the progression of atherosclerosis events in patients with the diabetes will be useful in early management of cardiovascular disease incidences in T2DM patients can reduce the morbidity and mortality [1]. Generally, the occurrence and progression of CVD has been evaluated by the measure of biomolecules such as C-reactive protein (CRP), cystatin C, B-type natriuretic peptide (BNP), myeloperoxidase (MPO), N-terminal prohormone BNP (NT-proBNP), fibrinogen and lipoprotein-associated phospholipase A2 in biofluids and tissues. Although, these markers have significant diagnosis value, only a few are useful in clinical setup. Among them, C-reactive protein (CRP), is an inflammatory protein belongs to pentraxin family that express mainly by the liver. The elevated Serum CRP are noticed in response to infections, inflammatory, psychological conditions and trauma. In certain conditions, the rapid increase of serum CRP levels are noticed beyond 10 mg/l that are relatively stable with the half-life period of more than 18 h. Also, the CRP levels do not exhibit diurnal variations in relation to intake of food. Based on multiple epidemiological and intervention studies, increased concentrations of high-sensitivity C-reactive protein (hs-CRP), a marker for systemic inflammation, are found to be associated with future cardiovascular risk [2-4]. Similarly, the HbA1c (hemoglobin A1c) is considered as a potential marker to determine the glycaemic index of an individual with diabetes. As HbA1c is the indicator of glycaemic control, it is well proven that association of improved glycaemic control showed significant reduction vascular risk of cardiovascular disease, whereas the interdependence between the inflammatory cardiovascular marker C-reactive protein (CRP) with the diabetic clinical parameter is less clear.

In this study, we investigate the association of C-reactive protein (CRP) with the diabetic associated parameters in diabetic population with and without coronary artery disease and compared with healthy control. Analysis of CRP with the physical exercise, gender and age shows significant change between the groups. Particularly, the change in levels of CRP and HbA1c suggest involvement in the diabetes participants with coronary artery disease. Overall, our results show the significant association between CRP and diabetic associated parameters will be helpful in detecting and managing the diabetic completion that progress towards coronary artery disease.

Materials and method: This study was approved by the Institutional Ethics Committee of Chettinad Academy of
Research and Education (CARE), Tamil Nadu, India. All participants in this study were voluntarily contribute the samples for the estimation of biochemical parameters. Participants were informed with details of the study both verbally and written sheet. Personal information obtained from the participants were kept secure, computerized database, maintained by the investigator. In addition, participants were free to withdraw from the study, if desired so. Also, the participants are allowed to contact the investigator, if they required further information about the project. The population of this study was attending the outpatient (OP) setting of Cardiology and Diabetology Departments, Chettinad Hospital and Research Institute, CARE. This study was approved by the Human Institutional Ethics Committee of CARE. Participants were approached by the investigator, and information sheets containing the details of the study were given to them. The participant’s consent was obtained. The target population was the patients diagnosed to have the type 2 diabetes mellitus attending the cardiac OP department. Healthy controls were selected from those attending the master health check up clinic of CHRI. The target population was investigated to select the participants for this study based on the following criteria. The study involves 30 participants who are attending the cardiac and diabetic OP at Chettinad academy of research and education. Questionnaire was created containing three items (1) past history, (2) social demographic information, and (3) clinical parameters. Among 30 patients, 10 were healthy controls, 10 were diagnosed as T2DM with CAD (with angiogram proven) collected from the cardiology department, and remaining 10 were T2DM without CAD were selected from the diabetic out-patients. All participants were ranging between 35 to 65 years. The participants gave informed consent to participate in this study. The inclusion criteria for this study, For T2DM patient attending OP department of CHRI, patients who are willing to give blood for the study, T2DM patients were selected based on the criteria of fasting blood glucose level (FBG) ≥ 140 mg/dl and postprandial blood glucose level > 200 mg/dl. Patients were excluded with T1DM, gestational diabetes mellitus, diabetic retinopathy and nephropathy, chronic inflammatory disease because they may have a different inflammatory response that will affect their bio-marker levels. The Anthropometric measurements include Body height (cm) and weight (kg) were measured in all participant to calculate BMI based on their height and weight (kg/m²). BMI (body mass index) calculated by using the formula BMI = Weight in kilograms / (Height in meters). The demographic data like age, gender, employment, and domicile were collected. Also, information relating cardiovascular risk factors such as hypertension, smoking, diabetes mellitus, family history were collected.

The clinical and biochemical parameters such as lipid profile, HbA1c level (glycosylated hemoglobin), FBS and PPBS were recorded for all the participants in excel format. Blood pressures (systolic and diastolic) are also included in assessment. Blood sugar levels were presented in mg/dL units. The enzyme-linked immune absorbent assay was used to estimate the levels of serum hs-CRP following the manufacture (Bioassay technology) 5 ml of blood drawn from the participants using vacationer tube by a qualified technician and placed in ice packs. The collected blood was centrifuged at 3000 rpm for 10 minutes. Serum was separated from other cellular material and transferred to a polypropylene tube and stored it at -20°C until for further use. Measure the level of Hs-CRP by using ELISA technique [15]. Human High Sensitivity C-reactive Protein ELISA Kit is used for this examination of antigen-antibody binding. Assay ranges of this kit for hs CRP are 12.8 mg/L with less cross reactivity with available and other molecules for better sensitivity and specificity. In 96 well plate is coated with hs-CRP antibody, 100µl of serum sample was added and kept at 4°C overnight. Wash it with PBS for 3 times, 1% of BSA added to all 96 well plates and incubate it for 1 hour, wash it with 1X PBST 3 times. After the incubation, TMB (substrate solution)
added to it, left it for 15 minutes for color development. Once the color appears, measure the OD value of each well at 450nm within 10 minutes by using ELISA reader.

**Statistical Analysis:** Data stored in Microsoft Excel format was used for descriptive and comparative statistical analysis using PrismGraph Pad (Version 5.0, USA) and. Student’s T-test and Chi-Square were used to analyze continuous variables and discontinuous variables and chi-square respectively. Statistical analysis of outcome measures performed by using paired t-test within-group and unpaired t-test between groups. Outcome measures are presented as means ± standard error (SE) with 95% confidence intervals. A comparison of these 3 groups’ variables performed using the chi2 test. A two-tailed p-value with equal variances < 0.05 considered as statistically significant. Potential factors such as age, sex, smoking, alcoholic, systolic and diastolic BP, FBS, PPBS, HbA1c were associated with atherosclerosis identified by univariate analysis (ANOVA). The statistical analyses were carried out to measure the level of hsCRP protein between 3 groups: control, Type 2 diabetes mellitus without CAD and Type 2 diabetes mellitus with CAD patients. Calculate the correlation coefficient between the hsCRP and other groups.

**Results:** All collected data were analyzed using appropriate statistical method and represented as figures and tables. The demographic details such as age, education; employment and income were analyzed using statistical method. Analyses these data shows no significant changes in demographics and anthropometric measurements between the control and intervention groups. The mean age of controls was 53.2±3.8 years. Also, the Type 2 diabetic subjects without CAD complications was 52.8±8.8 years (men: 51.5±9.1 years, women: 54.1±8.5 years) and the mean age of the diabetic patients with CVD 54.7±7.0 years (men: 54.8±7.2 years, women: 54.7±7.1 years). Similarly, the analysis of gender, Domicile, Family history, Employment, and Dietary patterns showed no significant changes between the grouped confirming that demographics and anthropometric measurements are matched between the analyzed groups.

**Levels of hsCRP between the groups:** The analyzed hsCRP levels between the groups were presented as box-plot. A box-and-whisker plot provides a graphical summary of a set of data based on the quartiles: The data were split into three groups, each containing 25% of the measurements. By combining the box-and-whisker plot with a display of each data point as a scatter plot, a most informative data display can be obtained. This graph is box and whisker plot, it is used for graphical method of displaying variation in a set of data. Figure 1 shows hs-CRP level associated with three groups.

**Figure 1: hsCRP level in serum of the participants**

Association between makers levels with gender: The bar plot represents hsCRP levels between the gender. The Figure 2A representing the comparison of hsCRP levels between male and female in control. Similarly, figure 2B representing the comparison of hsCRP levels between male and female in T2DM without cardiac complication. Whereas the figure 2C, representing the comparison of hsCRP levels between male and female in T2DM without cardiac complication. Comparative data showed increase in hsCRP levels in male between the groups.

Figure 2A: Association between hs-CRP level and gender in control

Figure 2B: Association between hs-CRP level (x-axis) and gender in T2DM

Figure 2C: Association between hs-CRP level (x-axis) and gender in T2DM with CAD
Comparative analysis of hsCRP with other clinical parameter between the groups: In addition to gender, we assess the role of self management like physical exercise contributing the levels hsCRP between the groups. The analysis showed variation in hsCRP levels between the individual involved in regular and irregular exercise. In control (figure 3A), representing the comparison of hsCRP levels between regular and irregular exercise. Similarly, figure 3B representing the comparison of hsCRP levels between regular and irregular exercise in T2DM without cardiac complication. Whereas figure 3C, representing the comparison of hsCRP levels between regular and irregular exercise in T2DM without cardiac complication. Comparative data showed increase in hsCRP levels in regular exercise compared to irregular exercise between the groups.

Figure 3A: Association between hs-CRP level (x-axis) and physical exercise (y-axis) in control.

![Figure 3A: Association between hs-CRP level (x-axis) and physical exercise (y-axis) in control.](image1)

Figure 3B: Association between hs-CRP level (x-axis) and physical exercise (y-axis) in T2DM.

![Figure 3B: Association between hs-CRP level (x-axis) and physical exercise (y-axis) in T2DM.](image2)

Figure 3C: Association between hs-CRP level (x-axis) and physical exercise (y-axis) in T2DM with CAD.

Analysis of clinical parameters between the groups: In addition, the attempt was made to find out whether there are any changes between the demographic and clinical variables between the groups. These results are presented in

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The ANOVA between the groups shows the hs-CRP, HbA1c and cholesterol were significantly altered between the groups, $p < 0.05$ (Table 1). Particularly, the analysis of clinical parameters between each group provides their involvement in pathogenesis. For instance, the clinical characteristics between control and T2DM have shown the HbA1C play a vital role differentiating control and T2DM with $p$-value 0.0001 (Table 2). The Table 3 representing the significance of clinical characteristics between Control and T2DM-CAD. For example, the clinical characteristics such as hs-CRP, BMI, HbA1C, CHO altered significantly and play a vital role in T2DM-CAD with $p < 0.05$. Furthermore, the comparative analysis (Table 4) of T2DM and T2DM-CAD showed significant variation in hs-CRP, cholesterol and VLDL.

Table 1: Analysis of hs-CRP, HbA1c and cholesterol levels between the groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Healthy (n=10)</th>
<th>T2DM (n=10)</th>
<th>T2DM-CAD (n=10)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-CRP (mg/ml)</td>
<td>1.2 ± 0.4</td>
<td>1.3 ± 0.5</td>
<td>1.8 ± 0.4</td>
<td>0.0049</td>
</tr>
<tr>
<td>HbA1c(%)</td>
<td>5.3 ± 0.2</td>
<td>8.3 ± 1.3</td>
<td>7.2 ± 1.9</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>189.7 ± 29.3</td>
<td>159.8 ± 50.1</td>
<td>233.8 ± 11.2</td>
<td>0.041</td>
</tr>
</tbody>
</table>

Table 2: Clinical characteristics between Control and T2DM

<table>
<thead>
<tr>
<th>S. No</th>
<th>Variables</th>
<th>Control (n=10)</th>
<th>T2DM (n=10)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>hs-CRP (mg/ml)</td>
<td>1.2±0.4</td>
<td>1.3±0.5</td>
<td>0.525</td>
</tr>
<tr>
<td>2</td>
<td>BMI</td>
<td>23.4±4.4</td>
<td>26.2±4.2</td>
<td>0.147</td>
</tr>
<tr>
<td>3</td>
<td>HbA1C (%)</td>
<td>5.3±0.2</td>
<td>8.3±1.3</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>4</td>
<td>CHO (mg/dL)</td>
<td>190±29.3</td>
<td>160±50.1</td>
<td>0.185</td>
</tr>
<tr>
<td>5</td>
<td>TG (mg/dL)</td>
<td>137 ± 92</td>
<td>140.3 ± 73.1</td>
<td>0.791</td>
</tr>
</tbody>
</table>

Table 3: Clinical characteristics between Control and T2DM-CAD

<table>
<thead>
<tr>
<th>S. No</th>
<th>Variables</th>
<th>Control (n=10)</th>
<th>T2DM-CAD (n=10)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>hs-CRP (mg/ml)</td>
<td>1.2±0.4</td>
<td>1.8 ± 0.4</td>
<td>0.0016</td>
</tr>
<tr>
<td>2.</td>
<td>BMI</td>
<td>23.4±4.4</td>
<td>27 ± 2.4</td>
<td>0.0346</td>
</tr>
</tbody>
</table>
3. HbA1C (%) & 5.3±0.2 & 7.2 ± 1.9 & 0.0048 
4. CHO (mg/dL) & 190± 29.3 & 234 ± 11.2 & 0.0369 
5. TG (mg/dL) & 137 ± 92 & 232 ± 57.9 & 0.15 

Table 4: clinical characteristics between T2DM and T2DM-CAD

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Variables</th>
<th>T2DM (n=10)</th>
<th>T2DM-CAD (n=10)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>hs-CRP (mg/ml)</td>
<td>1.3±0.5</td>
<td>1.8 ± 0.4</td>
<td>0.0195</td>
</tr>
<tr>
<td>2.</td>
<td>CHO (mg/dL)</td>
<td>160 ± 50.1</td>
<td>234 ± 11.2</td>
<td>0.0216</td>
</tr>
<tr>
<td>3.</td>
<td>VLDL(mg/dL)</td>
<td>28 ± 15</td>
<td>51.3 ± 13</td>
<td>0.0326</td>
</tr>
<tr>
<td>4.</td>
<td>TG (mg/dL)</td>
<td>140.3 ± 73.1</td>
<td>232 ± 57.9</td>
<td>0.0696</td>
</tr>
<tr>
<td>5.</td>
<td>HbA1C (%)</td>
<td>8.3±1.3</td>
<td>7.2 ± 1.9</td>
<td>0.251</td>
</tr>
</tbody>
</table>
DISCUSSION: The exponential growth of diabetes mellitus burden in India is likely to affect the poverty alienation programs of the country. Novel strategies need to be developed to overcome this huge burden. Early and intensive intervention could reduce the risk of disease progression and complications [5]. One of the major complications of T2DM is the risk of developing cardiovascular diseases. The diabetic patients face 4 time higher risk than healthy individuals in developing cardiovascular diseases. Serum based biomarkers that can help in CVD risk stratification in diabetic patients may help to minimize cardiac events in T2DM patients. There are many studies were aim to achieve this nevertheless they have limited application in the clinical setup [6]. One of the widely used markers is hs-CRP but its level is higher in other inflammatory diseases also. Hence based on system biological integrated approach the new biomarkers are being explode for potential in risk stratification. In the study the other groups is being compared with hs-CRP. The present study demonstrated that the hsCRPlevels were higher in Type 2 diabetic and diabetic CVD patients than non-diabetic controls [7, 8]. There may be a significant relationship between hs-CRP and complications of Type 2 diabetes mellitus through the acute phase response. A relationship between inflammation, Type 2 diabetes mellitus and its complications has been established [9]. A high level of inflammatory plasma proteins leads to the increased incidence of cardiovascular risk especially in Type 2 diabetic patients. An increased incidence of vascular complications of Type 2 diabetes mellitus has been observed among subjects with high levels of inflammatory markers [10,11]. Measurement of markers of inflammation might be helpful for the assessment of the vascular risk in Type 2 diabetic patients. Inflammation is linked to the pathogenesis of Type 2 diabetes mellitus. Insulin resistance and hyperglycemia are also promoting inflammation by increased oxidative stress and that may link diabetes mellitus to the development of atherosclerosis. Serum hs-CRP is one of the factors as a biomarker for predicting cardiovascular events as serum hs-CRP is correlated with the cardiovascular risk and elevations of other biomarkers are found to be significantly associated with vascular events[12]. It was found that study subjects with high CRP and low LDL-C were at higher risk than those in the low CRP and high LDL-C group. Even after adjustment for age, smoking, blood pressure, Type 2 diabetes mellitus, HDL-C and LDL-C levels, CRP remain as an independent prognostic risk factor. These observations indicate the need for physicians to consider CRP in one of the biomarkers. A relationship between C-reactive protein and hyperglycemia absorbed in urban Northern Indian Type 2 diabetic patients is an agreement with our study [13]. This paper makes one such attempt. We performed comparative study of the various demographic and clinical parameters of patients attending Chettinad Hospital and Research Institute is attempted. Further these patients are classified into three groups which include healthy control, Type2 diabetic patients without CVD and Type2 diabetic patients with CVD [14]. The serum levels of hsCRP proteins in these patients are measured by ELISA technique Descriptive and inferential statistical analysis of these data made to arrive at the potential of these markers for CVD risk stratification in Type2 DM patients. The goal of our study to estimate the level of cardiac biomarkers in type 2 diabetic patients with and without coronary artery disease. To recruit healthy and diabetic patients attending cardiology OP departments of CHRI for this study. To analyze the demographic level and clinical profile of those participants involved in 3 groups. To measure serum levels of hs-CRP levels in patients by ELISA technique. Statistical analyze results to find out the risk factors and also to compare the efficacy of hs-CRP in other groups. Comparative study is more essential to validate the utility of hsCRP as a specific biomarker for T2DM patients. Measurement and comparative analysis of hsCRP levels may
contribute to improved early prediction of CVD events in T2DM patients, thereby it can reduce CVD related morbidity and mortality [15].

CONCLUSION: This study showed significant association of lipid, HbA1c, and hsCRP levels in the participants with T2DM with CAD contributing towards cardiovascular disease. Particularly, the level of hs-CRP along with HbA1c may contribute detecting early CVD event in participants with type 2 diabetic mellitus. Overall, our study provides significant contribution in predication of early CVD event in T2DM which helps reduce CVD related morbidity and mortality in T2DM.

REFERENCES


