Association of hsCRP and soluble fraction of E-selectin with diabetic and non-diabetic individuals

Subinay Datta, Mrinal Pal,* Amit Kumar Pradhan, Joydeep Ghosh, Rajarshi Rahut, Amrita Ganguly, Subhadeep Basu

Department of Biochemistry, Burdwan Medical College, Burdwan, West Bengal, India

ABSTRACT

Background and aims: It is known that endothelial dysfunction and low-grade inflammation are important element of diabetic complications. The soluble E-selectin is a marker of endothelial dysfunction and C-reactive protein is the most important inflammatory marker. The present study is done to investigate the effect of diabetes on these two markers.

Methods: The present hospital based case control study was conducted in the department of Biochemistry with the collaboration of department of Medicine of Burdwan Medical College, Burdwan, west Bengal India. All subjects were selected by simple random sampling between March 2011 and May 2013. Blood level of these soluble E-selectin and high sensitive C-reactive protein were measured in two hundred diabetic and two hundred age, sex and other non-modifiable as well as modifiable risk factors matched non-diabetic healthy controls.

Results: Compared with control value of high sensitive C-reactive protein and soluble E-selectin levels were higher in diabetic subjects (p < 0.001). Our present study shows that levels of adhesion molecules and high sensitive C-reactive protein were higher in diabetic than non-diabetic.

Conclusion: These data suggested that an increased level of inflammatory markers and adhesion molecules among diabetic compared with non-diabetic subjects. This suggests that chronic uncontrolled hyperglycemia contributes to progression of atherosclerosis associated with life-threading complications in middle-aged diabetic patients.

INTRODUCTION

Diabetes is characterized by hyperglycemia and metabolic abnormalities due to decreased insulin level or action, causing metabolic and physiological changes in various organs. [1] It is also associated with disturbances concerned with protein, carbohydrate and lipid metabolism. [2] The reduced uptake of glucose into muscle and adipose tissue leads to chronic extra cellular hyperglycemia that results in tissue damage and micro as well as macrovascular complications in both type I and II Diabetes Mellitus. [3,4] The development of endothelial dysfunction is considered an important element of diabetic microvascular disease. [5] The adherence of circulating leukocytes to dysfunctional endothelium and their subsequent transmigration into the arterial intima is the subsequent step of the complication. The recruitment of leukocytes into the tissues is dependent on a multiple cascade of events mediated by distinct adhesion molecules. [6-8] The soluble fraction of hsCRP and soluble fraction of E-selectin with diabetic and non-diabetic individuals.

*Corresponding author
Dr. Mrinal Pal
Department of Biochemistry
Burdwan Medical College, Burdwan, West Bengal, India
Email: mrinalpal77@rediffmail.com
Weight and height measurements were obtained, using Anthropometric measurements duration of smoking (years). Smoking for more than 1 month), or current smokers. Pack-being either never-smokers, ex-smokers (if they had ceased during direct interviews. [18] The subjects were classified as smoked daily and the duration of smoking were recorded on three consecutive measurements. The number of cigarettes an anti-hypertensive agent, or blood pressure > 160/95 mmHg was defined by either prior diagnosis, current treatment with diabetic subjects consisted of 56.6 ± 7.9 years. Hypertension population were from Burdwan district and adjoining areas. Declaration of Helsinki was obtained. [17] The selected study and informed consent in accordance with the principles of the study was conducted in the department of Biochemistry with the collaboration of department of Medicine of Burdwan Medical College, Burdwan, West Bengal. Two hundred out-patients having age 45-69 years suffering from uncontrolled diabetes mellitus (plasma glucose > 110 mg/dl, HbA1C > 8% of total Hb) with no history or clinical symptoms of cerebro-vascular disease or cardiovascular disease such as infarction and unstable angina, chronic and/or acute inflammatory disease, and malignancy were recruited in this study. Two hundred non-diabetic subjects, who were attended at out-patients department and matched with age, sex, blood pressure, lipid profile, smoking habit, BMI selected as controls as all these factor influence the level of hsCRP. All subjects were selected by simple random sampling between March 2011 and May 2013. All subjects were recruited consecutively and informed consent in accordance with the principles of the Declaration of Helsinki was obtained. [17] The selected study population were from Burdwan district and adjoining areas. Diabetic patients consisted of aged of 58.5 ± 6.3 years and non-diabetic subjects consisted of 56.6 ± 7.9 years. Hypertension was defined by either prior diagnosis, current treatment with an anti-hypertensive agent, or blood pressure > 160/95 mmHg on three consecutive measurements. The number of cigarettes smoked daily and the duration of smoking were recorded during direct interviews. [18] The subjects were classified as being either never-smokers, ex-smokers (if they had ceased smoking for more than 1 month), or current smokers. Pack-years were expressed as the numbers of packs per day X the duration of smoking (years).

**Material and Methods**

**Selection of subjects**

The present study was conducted in the department of Biochemistry with the collaboration of department of Medicine of Burdwan Medical College, Burdwan, West Bengal. Two hundred out-patients having age 45-69 years suffering from uncontrolled diabetes mellitus (plasma glucose > 110 mg/dl, HbA1C > 8% of total Hb) with no history or clinical symptoms of cerebro-vascular disease or cardiovascular disease such as infarction and unstable angina, chronic and/or acute inflammatory disease, and malignancy were recruited in this study. Two hundred non-diabetic subjects, who were attended at out-patients department and matched with age, sex, blood pressure, lipid profile, smoking habit, BMI selected as controls as all these factor influence the level of hsCRP. All subjects were selected by simple random sampling between March 2011 and May 2013. All subjects were recruited consecutively and informed consent in accordance with the principles of the Declaration of Helsinki was obtained. [17] The selected study population were from Burdwan district and adjoining areas. Diabetic patients consisted of aged of 58.5 ± 6.3 years and non-diabetic subjects consisted of 56.6 ± 7.9 years. Hypertension was defined by either prior diagnosis, current treatment with an anti-hypertensive agent, or blood pressure > 160/95 mmHg on three consecutive measurements. The number of cigarettes smoked daily and the duration of smoking were recorded during direct interviews. [18] The subjects were classified as being either never-smokers, ex-smokers (if they had ceased smoking for more than 1 month), or current smokers. Pack-years were expressed as the numbers of packs per day X the duration of smoking (years).

**Anthropometric measurements**

Weight and height measurements were obtained, using standardized technique. [19] BMI was calculated as the weight in kilograms divided by the square of height in meters. Blood pressure was recorded in the sitting position by using the right arm to the nearest 2mm Hg with a mercury Sphygmomanometer.

**Collection of samples**

All parameters were assayed from 12 hour fasting blood samples obtained from cases and controls. The samples were divided into three aliquots. The first one was collected in oxalate and fluoride vial for obtaining plasma for fasting glucose estimation, second one in plain vial for obtaining serum for insulin, lipid profile, hsCRP and sE-selectin estimation and third one in EDTA containing vial for HbA1c, assays. All the parameters were estimated within 24 hours.

**Parameters assay**

Quantitative estimation of plasma glucose was done by Glucose oxidase and Peroxidase method [20] from the separated plasma by using the autoanalyzer ERBA XL 600. Using commercially available Hemoglobin A1C kit supplied by Siemens Company did Hemoglobin A1C test. It implies the principle of turbidimetric inhibition immunoassay (TINIA). This company also supplied total Hb kit for estimation total Hb by alkaline hematin method. Serum insulin was assayed by ELISA kit AccuBind from Monobind Inc. USA. Both the inter- and intra-assay CVs were below 3%. [21,22] No cross reactivity with C-peptide was detected. Homeostatic model assessments (HOMA) of steady state β cell function (% B), insulin sensitivity (% S) and insulin resistance (HOMA IR) is computed with the formula that is fasting plasma glucose (mmol/l) times fasting serum insulin (mU/l) divided by 22.5. [23] Among the lipid profile serum total cholesterol, triglyceride (TG) and high density lipoprotein (HDL) were assayed by Cholesterol Oxidase–peroxidase (CHOD–PAP), glycerol-3-phosphate oxidase (GPO), polyanion precipitation methods respectively using semi-auto analyser. Serum very low-density lipoprotein (VLDL) was calculated by dividing the value of TG by 5 and serum LDL was obtained by Friedewald equation. [24] For measurement of sE-selectin, serum samples were analysed in duplicate using the single-step sandwich ELISA method. [25] The inter- and intra-assay coefficients of variation (CV) were below 5% and 7%, respectively. Serum hsCRP was measured by Sandwich enzyme linked immunosorbant assay (ELISA) [26] with a lower detection limit 0.03 mg/L. Consistent with recent recommendations from the centres for Disease control and prevention, a CRP cut point of 3 mgs/L was used to differentiate high risk and low risk groups. [2]

**Statistical analysis**

The data for biochemical analysis was subjected to standard statistical analysis using the Statistical Package for Social Science (SPSS) 11.5 software for windows.
RESULT

The personal profiles and clinical parameters of all the subjects under study are shown in Table 1.

Table 1: Personal profile and clinical details of healthy persons and patients suffering from Diabetes mellitus

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Healthy persons</th>
<th>Patients suffering from Diabetes mellitus</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>200</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>58.1± 5.4</td>
<td>57.8±6.3</td>
<td>0.175</td>
</tr>
<tr>
<td>Sex : Males / Females</td>
<td>104 / 96</td>
<td>107 / 93</td>
<td>0.152</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>23.2±2.8</td>
<td>23.4±2.6</td>
<td>0.285</td>
</tr>
<tr>
<td>Smoking habit (Current / Ex-smoker / Never-smoker)</td>
<td>61/38/101</td>
<td>59/39/102</td>
<td>0.112</td>
</tr>
<tr>
<td>Hypertension (+/-)</td>
<td>93 / 107</td>
<td>86 / 114</td>
<td>0.128</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dl)</td>
<td>83±21.56</td>
<td>198±87.45</td>
<td>0.0002</td>
</tr>
<tr>
<td>Fasting plasma insulin (pmole/L)</td>
<td>52±19.3</td>
<td>95±38.6</td>
<td>0.0017</td>
</tr>
<tr>
<td>Insulin resistance (HOMA IR)</td>
<td>1.8±1.2</td>
<td>4.3±2.7</td>
<td>0.0011</td>
</tr>
<tr>
<td>HbA₁c (%)</td>
<td>5.4±0.39</td>
<td>9.5±1.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Total cholesterol (mmole/L)</td>
<td>5.3±0.7</td>
<td>5.48±0.82</td>
<td>0.11</td>
</tr>
<tr>
<td>Triglyceride (mmole/L)</td>
<td>0.134±0.2</td>
<td>0.135±0.22</td>
<td>0.19</td>
</tr>
<tr>
<td>HDL-cholesterol (mmole/L)</td>
<td>1.52±0.45</td>
<td>1.58±0.51</td>
<td>0.09</td>
</tr>
<tr>
<td>LDL-cholesterol (mmole/L)</td>
<td>2.7±1.1</td>
<td>2.8±0.4</td>
<td>0.17</td>
</tr>
</tbody>
</table>

BMI means Body Mass Index; n = number of cases; HOMA IR means Homoeostatic model assessments (HOMA) of steady state β cell function (% B), insulin sensitivity (% S) and insulin resistance; HDL = High Density Lipoprotein; LDL = Low Density Lipoprotein; Value are mean ± SD.

Serum sE-selectin and hsCRP were found at significantly higher levels in diabetic patients when compared with non-diabetic subjects as shown in the Table 2.

Table 2: Concentration of serum sE-selectin and hsCRP in cases suffering from Diabetes mellitus and controls

<table>
<thead>
<tr>
<th>Category</th>
<th>Concentration of serum sE-selectin (µg/L)</th>
<th>Concentration of serum hsCRP (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>54±12.8</td>
<td>3.6311± 0.94</td>
</tr>
<tr>
<td>Control</td>
<td>41±16.2</td>
<td>0.6711±0.59638</td>
</tr>
<tr>
<td>Diabetes Vs Control</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Values are mean ± SD; P > 0.05 not significant; P < 0.001 highly significant.

In table it is shown that a very high percentage of diabetes mellitus cases have serum hsCRP level more than 3 mg/l (82%), hence falling in high risk group whereas only 18% percent were having lower concentration.

Table 3: Distribution of cases suffering from uncontrolled diabetes mellitus according to their serum concentration of hsCRP in serum

<table>
<thead>
<tr>
<th>Concentration of serum hsCRP (mg/l)</th>
<th>Total number of cases (n = 200)</th>
<th>% of cases in each group</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>1-3</td>
<td>32</td>
<td>16</td>
</tr>
<tr>
<td>&gt;3</td>
<td>164</td>
<td>82</td>
</tr>
</tbody>
</table>

DISCUSSION

There is several evidence that low grade systemic inflammation is associated with diabetes mellitus [15] as well as atherosclerosis. [27,28] The inflammatory cell types such as monocyte-derived macrophages and lymphocytes found in the atheroma. [29] These cells in the atherogenous plaque release several inflammatory mediators like cytokines and chemokines which in turn increase the plasma concentration of CRP. [30] C-reactive protein levels for normal adults are usually taken as <10 mg/l. To measure the CRP at such lower concentration high sensitivity assays have been developed, known as hsCRP. The hsCRP test has good standardization, precision, has a low cost and least pre-analytical variances In present study it is observed that the rise of hsCRP was highly significant in cases as compared to control group as shown in the several previous studies. [2,31] Several studies have found that hsCRP has sufficient risk prediction value for atherosclerosis. [32–34] The level for predicting low risk for atherosclerotic event has been recommended <1 mg/l, average at 1–3 mg/l and high risk at > 3 mg/l. [5] In present study it has also been shown that a very high percentage of diabetes mellitus cases have serum hsCRP level more than 3 mg/l (82%), hence falling in high risk group whereas only 18% percent were having lower concentration.

It has long been proposed that damage to the endothelium is important in the development and/or progression of atherosclerosis, [35] and increased levels of endothelial cell markers in the plasma of subjects who go onto suffer adverse cardiovascular events support this concept. [36,37] Among the most commonly used endothelial cell markers are von Willebrand factor, soluble thrombomodulin, and soluble E-selectin. [11-13] In present study it was found that an important marker of endothelial dysfunction sE-selectin, was found at significantly higher levels in diabetic patients than in non-diabetic subjects. This finding was well corroborated.
with several previous studies. [38-47] The development of endothelial dysfunction is considered an important element of diabetic microvascular disease. Endothelial dysfunction as seen in diabetes is associated with altered production of leukocyte-adhesive molecules, e.g., E-selectin and vascular cell adhesion molecule 1 (VCAM-1). These molecules are produced by the activated endothelium, and they are prerequisites for the adhesion and extravasation of leukocytes. [48] In addition, it has been suggested that E-selectin is involved in the angiogenic process. [49] Several studies have found elevated circulating levels of the soluble, shedded part of E-selectin in diabetes, [50-53] particularly among patients with microvascular disease. [54,55] Moreover, an increased expression of these molecules has been found both in the arterial wall [56,57] and in the glomeruli [58] in diabetes.

CONCLUSION

In conclusion, our present study showed increased levels of inflammatory markers and adhesion molecules among diabetic compared with non-diabetic subjects. This suggests that chronic uncontrolled hyperglycemia contributes to progression of atherosclerosis associated with life-threatening complications in middle-aged diabetic patients.

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