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## RESEARCH ARTICLE

# HIGH SENSITIVITY C-REACTIVE PROTEIN in TYPE-2 DIABETES MELLITUS

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**ABSTRACT**

**Background:** Type 2 diabetes mellitus is associated with chronic low grade inflammation. (Pickup *et al.,* 1998) C-reactive protein is a classic marker for inflammation. (Naveed *et al*., 2009)
**Materials and Methods:** Study was conducted on 96 diabetic patients attending the diabetic clinic of government medical college, Trivandrum and on 96 non-diabetic controls. Serum hs-CRP, HbA1c, FBS, lipid profile, Waist: Hip ratio, systolic and diastolic BP was measured in cases and controls and the results compared.
**Results:** Mean serum hs-CRP was significantly higher in cases than in controls (p=0.009).
**Conclusion:** The new pathogenetic vision of diabetes mellitus includes inflammatory pathways playing pivotal roles its development and progression.



## INTRODUCTION

Alterations in human behavior and lifestyle associated with globalization have resulted in a dramatic increase in the prevalence and incidence of type 2 diabetes globally. (Zimmet, 2011) The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 60 million in India and this is further set to rise to 69.9 million by the year 2025. (Kumar *et al.,* 2013) Activation of the innate immune system and low-grade inflammation has been proposed to be one of the factors involved in the pathogenesis of type 2 diabetes. (Ole *et al*., 2005) The emerging paradigm is that metabolic imbalance leads to immune imbalance. (Chandra *et al*., 1996) Even in the prediabetes stage, people destined to develop type 2 diabetes have immune systems that are over active. Diabetics and prediabetics have been proved to have persistent, subclinical elevations of molecules of inflammation, such as C-reactive protein and IL-6 (Smitha *et al*., 2014)

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Circulating CRP is present in only trace amounts in healthy individuals, and is hardly detectable by the standard clinical tests, which typically have a lower detection limit of 3–8 mg/L. (Ridker *et al*., 2001)With the recent development of high-sensitivity CRP assays (hs-CRP), the lower detection limit is as low as approximately 0.04 mg/L, which permits assessment of low-grade inflammation. (Jaye *et al*., 1997) This study was primarily undertaken to observe the low grade inflammation in diabetic patients when compared to non-diabetic controls in the general population.

## MATERIALS AND METHODS

This case-control study was done among 96 type 2 diabetes patients from the diabetic clinic of Government Medical College, Trivandrum for a period of one year beginning from June 2010 to June 2011in the age group 30-55 yrs and on 96 non-diabetic controls in the same age group among patient's attenders. Obese (BMI>35), Smokers, hypertensives, persons suffering from acute infections or chronic inflammatory

diseases or if on any treatment for any of these conditions in the last 4 weeks, recent trauma, previous history of ischemic heart disease, myocardial infarction, any other cardiac problem, gestational diabetes mellitus, postmenopausal hormone usage, and subjects suffering from malignancy or having evidence of severe hepatic or renal disease were excluded from both the case and control groups

After obtaining an informed consent, and after ensuring that the subject is in fasting condition, blood sample for hematologic tests (hs-CRP, HbA1c, lipid profile, FBS and PPBS) were collected. Anthropometric parameters (height, weight, waist circumference, waist-hip ratio, body mass index) and hemodynamic parameters (systolic and diastolic Blood pressure) were also measured. Body weight was taken by using a standard weighing machine available in the diabetic clinic. Height was measured with a wall mounted scale. Patient stands straight, bare-foot with heels together, feet angled at about 60 degrees. Buttocks and Back should touch the wall. Head is positioned in Frankfort position, (ie, inferior border of the bony orbit and the groove at the top of the tragus of the ear lie in the same horizontal plane). Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Waist circumference was measured at the midpoint between the lowest part of the costal margin and superior border of iliac crest. Hip circumference was measured at the maximum protrusion point of the buttocks around the pelvis, at the level of the greater trochanters. The waist-to-hip ratio, calculated as waist circumference divided by hip circumference. Systolic and diastolic blood pressures were measured using a standard mercury sphygmomanometer.

Hs-CRP(high-sensitivity C-reactive protein) assay was done by ERBA (Economic Reliable Biochemistry Analysis) turbidimetric immunoassay kit on fully automated EM200 (ERBA Mannheim-200) analyzer.

HbA1c (glycosylated hemoglobin) was estimated by ion-exchange resin method. Estimation of fasting blood glucose, total cholesterol, triglyceride and HDL (high density lipoprotein) cholesterol using standard enzymatic techniques on ERBACHEM5 (Economic Reliable Biochemistry Analysis chemical analyser-5) was done. LDL (Low density lipoprotein) cholesterol was estimated by using the Friedewald equation, ie, [LDL-cholesterol] = [Total cholesterol] - [HDL-cholesterol] - ([Triglycerides]/5) where all concentrations are taken in mg/dl.

**Statistical analysis**

The data was entered into the computer catalogue. The response frequencies were calculated and analyzed by using statistical software SPSS software version 14. Mean values of all parameters were compared between cases and controls and student's T-test was done for ascertaining significance. The probability value $P<0.05$ was considered as significant, $P<0.01$ and $P<0.001$ were considered as highly significant

## RESULTS

The number of males was 39 (40.6%) and number of females was 57(59.4%) among the cases, whereas the number of males was 41(42.7%) and number of females was 55 (57.3%) in the control group.

**Table 1. Distribution of gender in cases and controls**

| | Diabetics | | Non-diabetics | |
|---|---|---|---|---|
| | Number | Percentage | Number | Percentage |
| Male | 39 | 40.6 % | 41 | 42.7% |
| Female | 57 | 59.4 % | 55 | 57.3 % |
| Total | 96 | 100% | 96 | 100% |

**Table 2. Mean age of cases and controls**

| | | N | mean | SD | t | p |
|---|---|---|---|---|---|---|
| AGE | Cases | 96 | 46.2 | 6.8 | 1.854 | 0.065 |
| | Controls | 96 | 44.4 | 6.5 | | |

The mean age of the cases was 46.2 and that of controls was 44.4. There is no statistically significant difference in the ages of cases and controls

**Table 3. Mean serum hs-CRP (mg/L) in cases and controls**

| | | N | mean | sd | t | p |
|---|---|---|---|---|---|---|
| hs-CRP | cases | 96 | 0.679 | 0.69 | 2.657 | 0.009 |
| | Control | 96 | 0.487 | 0.24 | | |

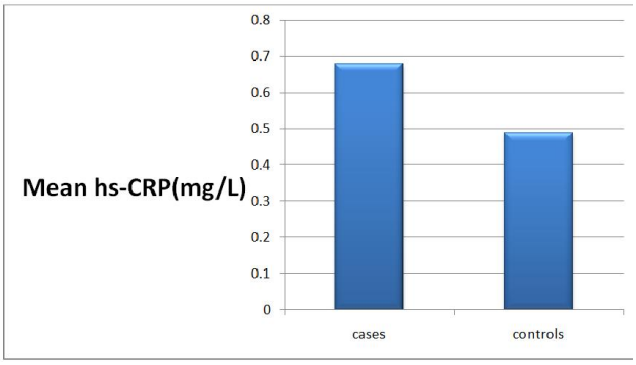


**Figure 1. Diagram showing the mean hs-CRP in cases and**

Table 3 and Figure 1 shows that mean serum high-sensitivity C-reactive protein is significantly higher in cases (0.679mg/L) than in controls (0.487mg/L). (p value =0.009)

BMI (body mass index), WC (waist circumference), HC (hip circumference) WHR (waist-hip ratio), SBP (systolic blood pressure), DBP (diastolic blood pressure), HbA1c (glycosylated hemoglobin), hs-CRP (high-sensitivity C-reactive protein), FBS (fasting blood sugar), HDL (high density lipoprotein), LDL (low density lipoprotein), TG (triglycerides)

Table – 4 shows Comparison of different parameters between cases and controls. Mean values of body mass index ($p=0.002$), waist circumference($p=0.001$), systolic blood pressure (0.030), total cholesterol ($p=0.002$) and that of low-density lipoprotein cholesterol (LDL) ($p=0.022$) were significantly higher in diabetic patients when compared with non-diabetic subjects.

As commonly expected, the mean values of fasting blood glucose ($p<0.001$) and glycosylated haemoglobin ($p<0.001$) were significantly higher in diabetics when compared with non-diabetics.

**Table 4. Comparison of other different parameters between cases and controls**

| | | N | mean | sd | t | p |
|---|---|---|---|---|---|---|
| BMI | Cases | 96 | 26.24 | 3.95 | 3.204 | 0.002 |
| | Controls | 96 | 24.67 | 2.70 | | |
| WC | Cases | 96 | 96.10 | 9.53 | 4.669 | 0.001 |
| | Controls | 96 | 90.29 | 7.61 | | |
| WHR | Cases | 96 | 0.97 | 0.07 | -0.639 | 0.523 |
| | Controls | 96 | 0.95 | 0.07 | | |
| SBP | Cases | 96 | 136.47 | 15.96 | 2.185 | 0.030 |
| | Controls | 96 | 131.15 | 17.75 | | |
| DBP | Cases | 96 | 84.77 | 7.38 | 1.517 | 0.131 |
| | Controls | 96 | 83.00 | 8.74 | | |
| HbA1C | Cases | 96 | 7.15 | 1.05 | 16.881 | <0.001 |
| | Controls | 96 | 5.11 | 0.55 | | |
| hs-CRP | Cases | 96 | 0.679 | 0.69 | 2.657 | 0.009 |
| | Controls | 96 | 0.487 | 0.24 | | |
| FBS | Cases | 96 | 152.80 | 47.82 | 13.842 | <0.001 |
| | Controls | 96 | 83.10 | 12.14 | | |
| PPBS | Cases | 96 | 251.83 | 45.15 | 26.199 | <0.001 |
| | Controls | 96 | 128.61 | 9.23 | | |
| Total cholesterol | Cases | 96 | 210.66 | 51.61 | 3.111 | 0.002 |
| | Controls | 96 | 191.47 | 31.43 | | |
| HDL | Cases | 96 | 40.61 | 8.38 | -0.320 | 0.750 |
| | Controls | 96 | 41.97 | 6.90 | | |
| LDL | Cases | 96 | 135.544 | 44.74 | 3.157 | 0.002 |
| | Controls | 96 | 118.06 | 30.68 | | |
| TG | Cases | 96 | 167.49 | 75.94 | 1.071 | 0.285 |
| | Controls | 96 | 157.20 | 55.60 | | |

However, although the mean values of, high-density lipoprotein cholesterol (HDL) (p=0.75) was lower and that of Waist-Hip Ratio (WHR) (p=0.52), triglycerides (TG) (p=0.28) and diastolic Blood pressure (DBP) (p=0.131) were higher in diabetics than in controls, these differences were not statistically significant.

## DISCUSSION

Type 2 diabetes is the type of diabetes that occurs relatively later in life and is characterized by the combination of peripheral insulin resistance and inadequate insulin secretion by pancreatic β-cells. The development of type 2 diabetes is caused by a combination of lifestyle and environmental factors particularly associated with urbanization (obesity, diet consisting of more refined foods, physical inactivity), increasing age and genetic factors. (Mather *et al*., 1987) While some are under personal control such as diet and obesity, others such as increasing age, and genetics are not.

Chronic low-grade inflammation and acute phase response initiated by the innate immune system, is most probably the cause of elevated levels of CRP in diabetes patients, when compared to non-diabetic individuals. It is perceived that chronic low-grade inflammation as evidenced by elevated hs-CRP might potentially be an underlying etiology of type 2 diabetes, although the exact mechanisms are still not well understood. (Pradhan *et al*., 2001) Accumulating evidence implicates inflammation as a potential pathway in the pathogenesis of type 2 diabetes. There is now a wealth of evidence indicating close ties between metabolic and immune systems. (Khovidhunkit *et al*., 2004) C-reactive protein, an acute-phase reactant, is a critical component of the immune system and an extremely sensitive marker of systemic inflammation.

CRP is an acute-phase reactant produced primarily in the liver under the stimulation of adipocyte-derived pro-inflammatory cytokines, including IL-6 and TNF-α. Guidelines from the Center for Disease Control and Prevention, and the American Heart Association were developed in 2003 to guide how hs-CRP should be integrated within healthcare practice. The guidelines endorse the following average hs-CRP values that correspond to a relative risk category for Cardiovascular disease risk: low risk is less than 1 mg/L, average risk is 1.0 to 3.0 mg/L and high risk is greater than 3.0 mg/L. (Pearson *et al*., 2003)

In our study mean serum hs-CRP was significantly higher in cases (0.679mg/L) than in controls (0.487mg/L) p value =0.009. This is in accordance with a number of other studies which had described the association between circulating hs-CRP levels and Type 2 diabetes (Thorand *et al*., 2003), (Duncan *et al*., 2003). The Casale Monferrato Study (Bruno *et al*., 2009) reported that CRP was related to risk of all-cause and CVD mortality in their 5-year follow-up of type 2 diabetes patients. The present study shows significant dyslipidemia in diabetics only with respect to total cholesterol (p=0.002) and LDL (p=0.022) cholesterol , whereas, many other studies showed dyslipidemia in triglycerides and HDL fractions as well. (Mengesha *et al*., 2006) Also, the present study shows a significant increase in only systolic BP in diabetics when compared to non-diabetics (p= 0.030), whereas many studies have demonstrated an increase in both systolic as well as diastolic BP in diabetics than the non-diabetic subjects. (John, 1932)

### Conclusion

It can be concluded with the help of results obtained that chronic inflammation is more prevalent in diabetes patients when compared to the non-diabetic population. In accordance with the results of previous studies, this study supports the

view that type-2 diabetics have a higher cardiovascular disease risk when compared to non-diabetic subjects as assessed by higher hs-CRP levels. More work is needed to establish whether intervention targeting "high-risk" diabetic patients, identified on the basis of elevated hs-CRP, effectively lowers CVD risk. The time has come for a careful consideration of adding hs-CRP as a routine clinical investigation for all type-2 diabetic patients, to be done at least on a half yearly basis to identify those people at an increased risk for CVD. Patients falling in the intermediate and high cardiovascular disease risk categories of hs-CRP levels, may be encouraged and motivated for life-style modifications, like body weight reduction (by calorie restriction, avoidance of fatty foods, increased physical activity and exercise) and cessation of smoking. Physicians treating these patients may also aim for better control of their blood pressure, blood glucose and lipid levels by making suitable changes in drug therapy.

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**Limitations of this study**

- The study was conducted only on patients who were on regular follow-up and treatment in the diabetic clinic and may not fully represent the diabetic population of the state.
- Sample size for this study was small which might have interfered with some of the analysis.
- This study had a case-control design, which prevents us from drawing inferences concerning the directionality of associations.
- There was no follow up of patients with elevated CRP levels to find out whether they developed cardiovascular disease later on.

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