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Research Article

STUDY OF SERUM hs-CRP IN TYPE 2 DIABETIC PATIENTS

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ABSTRACT
Diabetes mellitus has become a leading cause of morbidity and mortality world over. hs-CRP is a marker of low-grade inflammation and its level is raised in patients with type 2 DM. The current study was undertaken in 90 subjects. 30 diabetics without complications (group I), 30 diabetics with complications (group II) and 30 non diabetics as normal control group. The aim of the study was to assess the serum levels of hs-CRP in type 2 DM patients without and with complications. hs-CRP is significantly much higher in diabetic cases with complications compared to cases without complications. The elevation is significant in both the study groups when compared to controls. TC, TG, LDL-C and VLDL-C levels where significantly elevated and HDL-C levels were significantly lower in cases of both the groups when compared to controls. Hence it is concluded that the serum levels of hs-CRP appear to be useful as markers of diabetic complications and provide valuable information for proper medical intervention.

Keywords: *High Sensitive-C Reactive Protein, Type 2 Diabetes Mellitus & Lipid Profile*

INTRODUCTION
Diabetes mellitus, the most common endocrine disease is characterized by metabolic abnormalities and by long-term complications involving the eyes, kidneys, nerves and blood vessels. With an increasing incidence worldwide. Diabetes has been recognized, since antiquity and treatment of various efficacies have known in various regions since the Middle Ages. The increase in the incidence of diabetes in developing countries follows the trend of urbanization and lifestyle changes, most importantly a western diet.

Pick up *et al.,* (2004) suggested an increasing interest in the involvement of low grade inflammation in the pathogenesis of type 2 diabetes. CRP is an inflammatory marker produced and released by the liver under the stimulation of cytokines such as tumor necrosis factor and interleukins 1 and 6.

Yeh *et al.,* (2003) has shown that hs-CRP emerged as a powerful risk marker for cardiovascular disease. Inflammation has also been postulated to play a role in the pathogenesis of type 2 diabetes.

Laaksonan *et al.,* (2004) in a recent prospective study have suggested that an elevated level of CRP is associated with an increased risk of developing type 2 diabetes.

Festa *et al.,* (2000) demonstrated that elevated levels of CRP are associated with obesity, insulin resistance and glucose intolerance, suggesting that inflammation is also involved in the etiology of type 2 diabetes.

The objective of this study was to assess the serum levels of hs-CRP in type 2 diabetic patients without and with complications.

MATERIALS AND METHODS
The study was carried out in the Department of Biochemistry, KBN Institute of Medical Sciences Gulbarga. Clearance was obtained from the institutional ethical committee. The study was carried out on 30 age and sex matched healthy controls and 60 type 2 diabetic patients who attended the outpatient and inpatient department of KBN Institute of Medical Sciences, Gulbarga. A total 60 patients of type 2 diabetes mellitus between 40 – 70 years, which were divided into following groups.

Control group: Included 30 healthy, age and sex matched individuals.

Group I*:* Included 30 patients of type 2 diabetes without complications.

Group II: Included 30 patients of type 2 diabetes with proven complications, like CAD, retinopathy and

neuropathy.

The diagnosis of type 2 diabetes mellitus was established with the recommended criteria's of American diabetes Association.

Inclusion Criteria: Patients in the age group of 40 – 70 years with type 2 diabetes without and with proven complications, like CAD, neuropathy and retinopathy were selected.

Exclusion Criteria: Patients with recent infectious disease, immunological disorders, Surgeries, Burns, renal failure, pancreatitis, alcoholism, liver diseases, tuberculosis, thyrotoxosis, Osteoarthritis, Rheumatoid arthritis and all other inflammatory disorders were excluded from the study.

Informed consent was taken from patient and control subjects. A pre-structured and pre-tested proforma was used to collect the data. Baseline data including age and sex, detailed medical history including conventional risk factors, clinical examinations and relevant investigations including ECG, echocardiogram, nerve conduction test, fundoscopy etc were included as part of the methodology.

Laboratory methods

Fasting venous blood samples were collected from cases and controls and the samples were centrifuged, serum was separated and stored at 4^0C.

Mendall *et al.*, (1996) Serum hs-CRP was measured using the immunoturbidimetric CRP assay by using auto-analyzer (A_{25}Biosystem), based on the principle of agglutination reaction. Presence of CRP in the test specimen results in the formation of an insoluble complex producing a turbidity, which is measured at wavelength between 505-578 nm. The increase in turbidity corresponds to the concentration of CRP in the test specimen. Estimation of Serum total cholesterol by COD-POD method, Serum triglycerides by Tinder's GPO-POD method and serum HDL cholesterol by Phosphotungstate method. Serum LDL cholesterol and VLDL cholesterol values were calculated by applying Friedewald's formula. Serum Creatinine estimation was carried out using Jaffe's alkaline picrate method and blood urea was measured using Specific Urease method. FBS and PPBS were measured by GOD/POD method. Urine sample was analyzed for protein and sugar by using dipsticks.

Statistical Methods

Student t test/Chi-square test has been used to find the significance of homogeneity of study characteristics between three groups of patients. Analysis of variance has been used to find the significance of study parameters between three groups.

Results were expressed as mean $\pm$ SD, p values are obtained by using the post-hoc Turkey test.Significant figures

+ Suggestive significance $0.05<p<0.10$

* Moderately significant $0.01<p \leq 0.05$

** Strongly significant $p \leq 0.01$

Statistical software: The Statistical software namely SPSS 15.0, Stata 8.0, MedCalc 9.0.1 and Systat 11.0 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.

RESULTS AND DISCUSSION

Table 1: hs-CRP in the three study groups

Study parameters	Controls	DM without complications	DM with Complications
hs-CRP mg/L	0.72±0.45	2.27±1.14	3.55±0.86

Results are presented in Mean±SD

Study parameters	**Controls Vs DM without complications**	**Controls Vs DM with complications**	**DM without Complications Vs DM with Complications**
hs-CRP mg/L	<0.001**	<0.001**	<0.001**

p values are obtained by using the Post-hoc Tukey test.

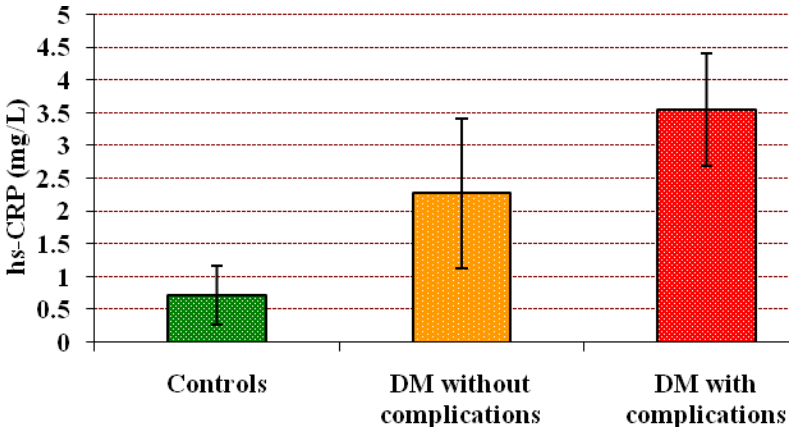

Figure 1a: hs-CRP in three study groups

Table 2: FBS and PPBS in the three study groups

Study parameters	Controls	DM without complications	DM with Complications
FBS mg/dl	88.13±18.95	142.97±12.48	187.83±29.89
PPBS mg/dl	127.03±21.42	230.70±26.84	317.00±48.32

Results are presented in Mean ± SD

Study parameters	Controls Vs DM without complications	Controls Vs DM with complications	DM without Complications Vs DM with Complications
FBS mg/dl	<0.001**	<0.001**	<0.001**
PPBS mg/dl	<0.001**	<0.001**	<0.001**

p values are obtained by using the Post-hoc Tukey test

Table 3: Lipid parameters in the three study groups

Study parameters	Controls	DM without complications	DM with Complications
TC mg/dl	179.50±44.64	239.23±36.08	267.07±31.89
TG mg/dl	121.47±28.74	186.80±58.17	257.80±71.05
HDL mg/dl	47.87±4.24	35.07±5.56	28.97±6.72
LDL-C mg/dl	103.03±33.95	164.13±37.28	187.60±28.96
VLDL mg/dl	24.40±5.71	37.33±11.60	52.90±14.12

Results are presented in Mean±SD

Study parameters	Controls Vs DM without complications	Controls Vs DM with complications	DM without Complications Vs DM with Complications
TC mg/dl	<0.001**	<0.001**	0.015*
TG mg/dl	<0.001**	<0.001**	<0.001**
HDL mg/dl	<0.001**	<0.001**	<0.001**
LDL-C mg/dl	<0.001**	<0.001**	0.022*
VLDL mg/dl	<0.001**	<0.001**	<0.001**

p values are obtained by using the Post-hoc Tukey test

Table 5: Pearson correlation of hs-CRP vs FBS, Total cholesterol, HDL and LDL

Pair	Controls	DM without complications	DM with complications
hs-CRP vs FBS	0.284	0.421	0.682
hs-CRP vs Total cholesterol	0.093	0.446	0.697
hs-CRP vs HDL	-0.035	-0.223	-0.441
hs-CRP vs LDL	0.336	0.368	0.523

Results are presented in r value

Prevention of diabetes and its associated burden primarily cardiovascular morbidity and mortality, have become major health issues worldwide. Insulin resistance and β-cell failure continue to be recognized as the central causal processes in the development of type 2 diabetes, other paradigms have also been evolved. Influenced by the findings indicating an inflammatory basis for cardiovascular diseases and following the "common soil "hypothesis of coronary heart disease and type 2 diabetes.

Bruce *et al.,* (2003) have revealed that a low-grade inflammation precedes and predicts the onset of diabetes in adults.

Malik *et al.,* (2005) in his study suggested that, High sensitive C-reactive protein (hsCRP) independently predicts cardiovascular diseases and whether it can stratify risk in those with diabetes is not well documented.

It is in this background that the current study has been undertaken to assess the serum levels of hsCRP in type 2 diabetic cases without complications and with complications.

Statistically there was no difference between the average age of controls and cases. Immaterial of the sex, cases were primarily selected on the basis of the chronicity of the disease. In the present study, mean body mass index (BMI) was found to be much higher in patients with and without complications compared to controls ($p<0.001$).

Frank *et al.,* (2004) has got similar results, BMI was higher in patients compared to control group.

Our study has revealed that hsCRP values are significantly higher in diabetics without (2.27±1.4mg/L)and with complications (3.55±0.86mg/L) as compared to controls (0.72±0.45mg/L). p value < 0.001 in both groups.

David *et al.,* (1978) and Kindmark *et al.,* (1972) studies has been demonstrated that individuals without inflammation usually have hs-CRP levels below 1mg/L.

In the present study the hsCRP values are significantly higher in diabetics without complications compared to the controls ($p<0.001$).

Yasufumi *et al.,* (2005), Simin *et al.,* (2007) and Abbas *et al.,* (2007) studies done across the world have projected similar results.

The mechanisms by which chronic inflammation can evoke type 2 diabetes are not clear. However it is known that adipose tissue can synthesize and release the main pro-inflammatory cytokines-tumor necrosis factor-alpha (TNF-α), interlelukin-1 (IL-1) and interleukin-6 (IL-6) and that inflammatory markers are associated with body fat mass. Pro-inflammatory cytokines and acute phase reactants are involved in multiple metabolic pathways relevant to insulin resistance, including regulation, reactive oxygen species, lipoprotein lipase action and adipocyte function. Therefore activated innate immunity and inflammation are relevant factors in the pathogenesis of diabetes, with convincing data that type-2 diabetes includes an inflammatory component.

In the present study, patients with diabetic complications have higher hsCRP levels compared to cases without complications and these values are far higher than in control group ($p<0.001$). Diabetic complications as suggested by clinical findings correlated with elevated hsCRP levels.

Mohan *et al.*,2005) in their study on 150 subjects selected from Chennai Urban Rural Epidemiology Study (CURES), have similar findings in that the diabetic subjects with CAD had higher CRP levels

compared to diabetic subjects without CAD and control subjects

Kang *et al.,* (2004) in their study on 105 patients with type-2 diabetes have shown that serum hsCRP level is useful to predict accelerated atherosclerotic process in type-2 diabetes.

Minna *et al.*,2006) in a large cohort study of 1045 cases of diabetes patients aged 45 to 65 years over a 7-year of follow up period, have reported that the mean hsCRP levels were significantly higher in men who died of CHD or who had a fatal or nonfatal myocardial infarction

We have evaluated the relation of hsCRP with lipid parameters. The serum total cholesterol, Triglycerides, LDL-C and VLDL Cholesterol levels were significantly higher in both the groups of cases compared to controls ($p<0.001$). The mean HDL-C was significantly lower in both the groups of cases ($p<0.001$).

Chapman *et al.,* (2006) suggested that dyslipidemia has been proved to be one of the major risk factor for CHD. Both VLDL-C and LDL-C are associated with atherogenic process and there is increasing evidence that HDL-C prevents atherogenesis

There is a positive correlation of hsCRP with FBS (r =0.421 w.o.c; r =0.682 w.c), Serum total cholesterol (r=0.446 w.o.c; r=0.697) and LDL-C (r=0.368 w.o.c; r=0.523 w.c) and negative correlation with serum HDL-C (r=-0.223 w.o.c; r=-0.441 w.c).

Yuji *et al.,* (2005) in their study have made a comparison of log hsCRP with FBS, Total Cholesterol, Triglycerides and HDL-C. They found a positive correlation of hsCRP with FBS, T-Cholesterol, Triglycerides and negative correlation with HDL-C.

Pradhan *et al.,* (2001) proposed many possible mechanisms by which hsCRP enhance atherosclerosis-hsCRP activates the complement pathway and induces adhesion molecule expression by human endothelial cells. hsCRP is also known to play a role in monocyte recruitment into the arterial wall. hsCRP enhances the entry of LDL particles into macrophages and it has been found to induce plasminogen activator inhibitor-1 expression. Elevated CRP also stimulates endothelial production of E-Selectin, ICAM-1 and VCAM-1, important mediators of impaired vascular reactivity, reduced insulin delivery and increased peripheral insulin resistance, Thus, the positive association between CRP and type 2 diabetes may simply reflect underlying endothelial dysfunction and subclinical atherosclerosis

Since elevated levels of hs-CRP correlative positively with altered lipid profile, these changes seem to be related to the degree of severity of the complications and hence may be useful as markers of complications in type 2 diabetes.

Hence further studies on serum hs-CRP to prevent the complications of diabetes will be interesting and helpful.

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