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Original article:

A CROSS-SECTIONAL STUDY ON HIGH-SENSITIVITY C-REACTIVE PROTEINS (hs-CRP) IN TYPE 2 DIABETIC PATIENTS OF HARYANA REGION

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ABSTRACT

Introduction: Chronic systemic low grade inflammation evidenced by elevated high-sensitivity C-reactive protein (hs-CRP) levels has been investigated widely in the pathogenesis of vascular disease in type 2 DM. In view of the gap of information from this region, the study aimed to investigate the association of hs-CRP with Type 2 DM ,in patients visiting the tertiary care centre in Haryana.

Material and Methods: In the present study inflammatory status was measured by determining the plasma hs-CRP levels in 50 type 2 diabetic patients without any complications and in 50 age and sex matched healthy control subjects. Type 2 diabetes was diagnosed according to ADA criteria. Patients were included after applying inclusion and exclusion criteria. Data was analysed and there seemed to be correlation between chronic inflammatory protein hs-CRP and HbA1Clevels.

Results: Mean hs-CRP levels in type 2 diabetic was 6.59±2.88 mg/l which was significantly higher compared to mean hs-CRP levels of normal healthy controls, 1.32±0.34 mg/l. Correlation coefficient (r values)were used to study the association of hs-CRP and HbA1C levels.

Conclusion: There was significant positive correlation observed between hs-CRP levels and HbA1C levels. Diabetic patients having poor control of glycemic status are in more active inflammatory state. Chronic inflammation may have a role in vascular toxicity resulting in endothelial damage in type 2 diabetic patients of this region of Haryana.

Keywords: hs-CRP, Type 2 diabetes mellitus, Inflammation, HbA1C

INTRODUCTION

Diabetes Mellitus is the most common non –communicable disease globally.[1] Based on the current trends, the International Diabetes Federation projects that 438 million individuals will have diabetes by the year 2030.[2] The risk of chronic complications increases as a function of the duration and degree of glycemia; they do not become apparent until the second decade of hyperglycemia. Since type 2 DM often have a long asymptomatic period of hyperglycemia, many individuals with type 2 DM have complications at the time of diagnosis.[1] Currently diabetes mellitus is considered as a disease with low grade inflammation. Liver produces acute phase proteins in response to

early inflammation. Activated chronic inflammation may lead to thromboembolic manifestations in the body. Different studies have shown that inflammatory markers in the blood like C-Reactive Protein (CRP), IL, PAI and fibrinogen are elevated significantly in diabetic population.[3] The inflammatory milieu promotes atherosclerosis and gives rise to other complications in diabetes. CRP is not only an associated factor with other established prognostic markers, but promising as an independent risk marker, especially for cardiovascular disease in diabetes.[4] High CRP levels may be a marker of oxidative stress on the endothelium in diabetic patients. In fact, high levels of serum CRP and other inflammatory markers in a normal population is an indicator of future development of diabetes.[5] The level of CRP in the blood correlate with severity of diabetes and degree of control.[6] Recent studies have suggested that low grade inflammation may be used to predict the onset of cardiovascular disease and type 2 DM.[7,8] To our knowledge inflammatory status in Type2 diabetics of this population has not been studied. Hence, the present cross sectional study was undertaken to: 1. To determine serum hs-CRP level in type 2 DM patients, 2. To determine hs-CRP levels in healthy controls, 3. Compare hs-CRP levels between type 2 DM patients and healthy control subjects and 4. To find correlation between HbA1c and Hs-CRP in type 2 diabetes mellitus patients.

MATERIAL AND METHODS

Subjects and study design:

This was a cross sectional study undertaken in the Department of Biochemistry in collaboration with Department of Medicine at Shaheed Hasan Khan Govt. Medical College, Nalhar, Nuh, Haryana, from August 2017 to August 2018. The study group included 50 diabetic subjects who were diagnosed type 2 DM according to ADA criteria, attending Out Patient Department of Medicine for follow up and 50 healthy control subjects (Table 1). The two groups were closely matched with respect to age.

The average duration of diabetes was 4 years. Glycemic control was achieved by diet alone, by diet plus sulfonylurea or diet plus daily insulin injection. The patient receiving insulin met the criteria for classification as non-insulin dependent diabetes since he/she had previously achieved adequate control of hyperglycemia with diet and sulfonylurea and never had diabetic ketoacidosis. All subjects underwent a screening that included medical history, physical examination. No subject had overt evidence of atherosclerosis as judged by the absence of symptoms of angina, claudication or cerebrovascular ischaemia and each had a normal vascular examination including normal pulses and absence of bruits. The protocol was approved by the Institutional Ethical committee and each volunteer gave written informed consent.

Patient's selection criteria

The study targeted type 2 diabetic patients, medically diagnosed by American Diabetes Association (ADA) criteria, above 30 years of age and upto 70 years age and were on routine medical review for their type 2 diabetes mellitus treatment in Medicine OPD. Randomly selected age and sex matched individuals, with no history of diabetes or any type of illness and not on statins and anti-inflammatory drugs were used as controls.

Patient's exclusion criteria:

- Smokers
- Alcoholic

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- Patients diagnosed with liver dysfunction and renal disease
- Pregnant females
- Patients with carcinoma
- Patients with inflammatory disease
- Patients on statin
- Patients on anti-inflammatory drugs
- Abdominal obesity

Samples and investigations

Blood samples were taken from diabetic patients and control patients. Investigations carried out were blood glucose fasting and 2 hours postprandial, glycated haemoglobin (HbA1c) and hs-CRP levels. Blood sugar was determined by glucose oxidase peroxidase method. Glycated haemoglobin by Immunoturbidimetry method and hs-CRP levels was estimated by quantitative immunoturbidimetric method .

All patients were first clinically examined. Then relevant tests were done in central laboratory of SHKMGMC Hospital. Other biochemical parameters tested included Blood Urea, Serum Creatinine, Serum Uric acid, Lipid Profile.

The data was first tabulated in Microsoft Excel worksheet. They were analysed using online software. Continuous variables were presented as mean±SD. Discrete variables were expressed as absolute number and percentages. Correlation was calculated using Pearson Correlation Coefficient. Student's t-test was used to find significance of difference of the mean. P value <0.05 was considered significant. Correlation Coefficient was calculated using Pearson Correlation Coefficient between hs-CRP and HbA1C.

RESULTS

We had a total of 100 patients in our study. The mean age of the study group was 51.06 ± 11.36 years and the control group 55.16±11.29 years (Table 1). The various biochemical study parameters of the study group and control group are shown in Table 2. Mean hs-CRP in the study group was 6.59±2.88 mg/l which was significantly higher than the control group (1.32±0.34 mg/l, p <0.001). There was a trend towards higher mean total cholesterol and mean LDL in diabetics compared to healthy individuals (Table 2).

FBS, HbA1c,hs-CRP levels are shown in Figure1, Figure 2, Figure 3 respectively. A positive correlation was observed between hs-CRP and Hb A1C in the study group(Figure 4).

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Table 1: Distribution of patients according to age in study (n=50) and control group (n=50)

Age range	Study group	Percentage	Control group	Percentage
30-40	11	22	8	16
41-50	14	28	10	20
51-60	15	30	12	24
61-70	10	20	20	40
Range	30-70		30-70	
Mean±SD	51.06±11.36		55.16±11.29	

Table 2:Various biochemical parameters in study group (n=50) and control group (n=50)

Parameters	Study group Mean±SD	Range	Control group Mean±SD	Range	t value	p value	Significance
Age	51.06±11.36	30-70	55.16±11.29	30-70	1.80	0.07	>0.05 NS
Sex (number) Male Female	 32 18	 -	 27 23				>0.05 NS
Urea (mg%)	40.32±32.76	22-114	14.06±4.47	7-20	5.61	0.000	<0.001 HS
Creatinine (mg%)	1.29±1.07	0.6-3.7	0.95±0.20	0.6-1.2	2.20	0.03	<0.05 Sig.
Uric acid (mg%)	4.77±1.28	2.6-6.2	5.22±0.70	3.4-6	2.14	0.03	<0.05 Sig.
Calcium (mg%)	9.67±0.75	8.2-10.5	9.32±0.49	8.5-10	2.71	0.007	<0.01 Sig.
Phosphate (mg%)	4.05±0.57	3.2-5.1	4.10±0.61	3.1-5.1	0.484	0.629	>0.05 NS
Sodium (meq/l)	139.04±1.53	137-142	139.12±3.0	135-144	0.167	0.867	>0.05 NS
Potassium (meq/l)	4.13±0.12	4-4.4	4.16±0.48	3.5-5	0.397	0.692	>0.05 NS
Bil. (Total) (mg%)	0.35±0.12	0.2-0.5	0.7±0.38	0.1-1.2	6.03	0.000	<0.001 HS
SGOT (IU/l)	30.52±10.35	20-52	30.36±10.46	20-52	0.07	0.938	>0.05 NS
SGPT (IU/l)	37.64±18.43	17-74	36.58±18.43	17-74	0.287	0.774	>0.05 NS
T. protein (gm%)	7.69±0.56	6.8-	7.22±0.77	6-8.2	3.44	0.000	<0.001 HS

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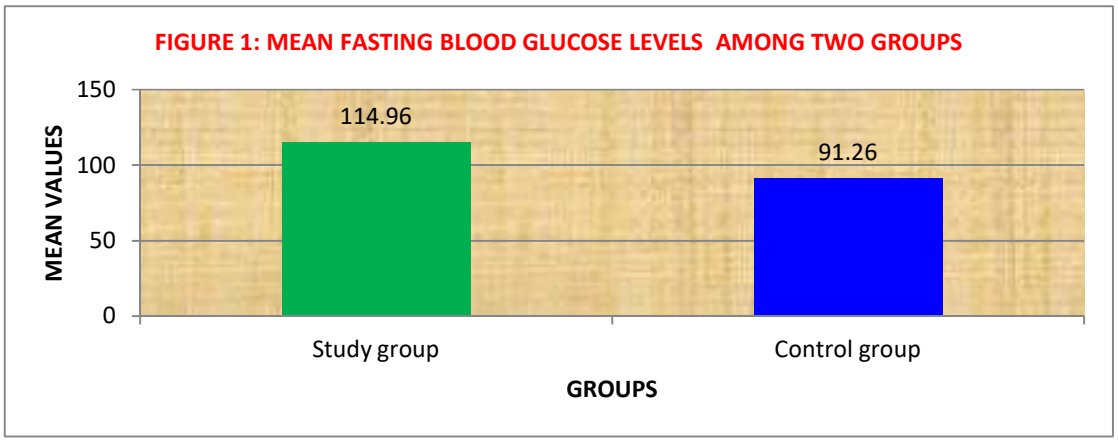
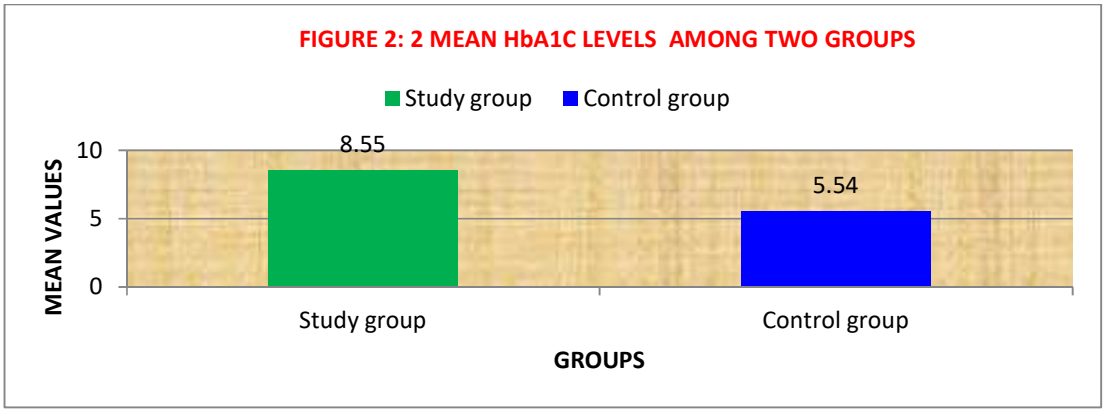
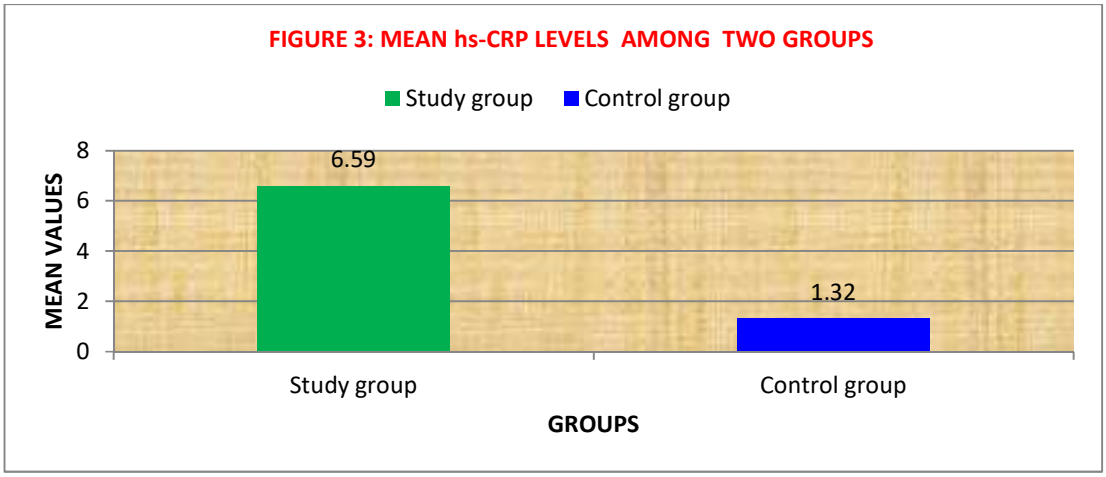
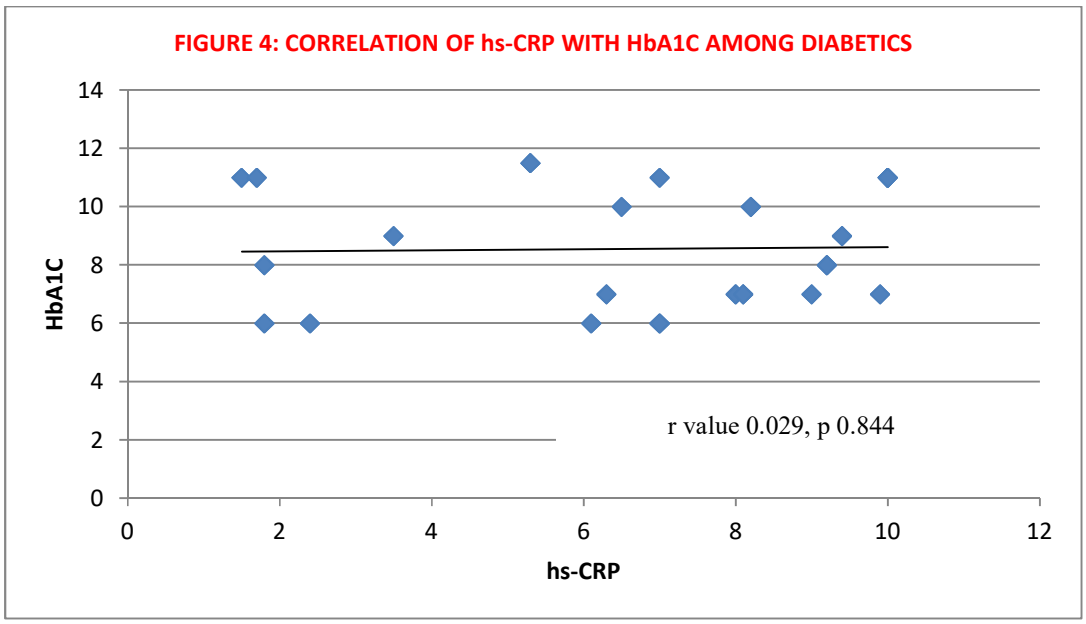
Albumin (gm%)	4.54±0.49	3.7-5	4.28±0.58	3.5-5.5	2.31	0.02	<0.05 Sig.
Cho. (mg%)	188.9±41.39	131-259	188.16±36.36	131-259	0.09	0.924	>0.05 NS
TG (mg%)	291.92±224.40	104-894	107.38±24.26	67-158	5.78	0.000	<0.001 HS
HDL (mg%)	29.72±11.74	15-53	43.88±4.85	36-52	7.87	0.000	<0.001 HS
Magnesium(mg/dl)	1.89±0.303	1.5-2.5	2.14±0.26	1.9-2.7	4.38	0.000	<0.001 HS
hs-CRP (mg/l)	6.59±2.88	1.5-10	1.32±0.343	0.7-2	12.83	0.000	<0.001 HS
HbA1c (%)	8.55±2.0	6-11.5	5.54±0.63	3.9-6.6	10.10	0.000	<0.001 HS
FBS (mg%)	114.96±4.87	110-125	91.26±6.73	78-100	20.14	0.000	<0.001 HS

NS = Not significant, HS = Highly significant, Sig. = Significant









DISCUSSION:

In our hospital based study we found high hs-CRP values in Diabetic subjects who did not have their blood sugar level in control. hs-CRP values showed significant correlation with HbA1C. Cardiovascular events are increased in Type 2 DM subjects due to a complex combination of various traditional and non-traditional risk factors that have an important role to play in the beginning and in the evolution of atherosclerosis from endothelial dysfunction to clinical events. DM2 might induce inflammation by increasing advanced glycation end products that may activate macrophages and increase oxidative stress and interleukin -6 synthesis, resulting in production of CRP.[9] Arterial inflammation induced due to toxic effects of inflammatory proteins has emerged to be central in initiation and progression of atherosclerosis. In people with diabetes, high hs-CRP levels (> 1 mg/l) were associated with increase

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in CV mortality after adjusting for age, sex and glucose tolerance tests.[10] It is reported that relative cardiovascular risk categories for serum hs-CRP levels are: low risk < 1.0 mg/L, average risk 1.0-3.0 mg/L, high risk 3.0-10.0 mg/L and unspecific elevation being >10mg/l and needs to be evaluated for acute inflammatory conditions.[10]

In the present study the hs-CRP values are significantly higher in diabetics who had no complications compared to controls ($p < 0.001$) Yasufumi et al[11], Simin et al[12] and Abbas et al[13] projected similar results in studies done from many parts of the world. The mechanisms by which chronic inflammation is evoked in type 2 DM to cause cardiovascular complications is not clear. In agreement with our study there are reports from India who have also reported significantly higher hs-CRP levels in diabetic patients with poor glycemic control.[14-16] Though we have several studies on hs-CRP and diabetes mellitus, we did not find any information about the inflammatory status from this population, so the study focused on the association of hs-CRP with glycemic control in type 2 Diabetic patients of this region of Haryana. In the present study, we found that the mean hs-CRP levels were significantly higher in both diabetic men and women as compared to their non diabetic counterparts. Higher hs-CRP was also positively correlated with higher HbA1C and fasting hyperglycemia.

CONCLUSION

Current evidence supports the usefulness of hs-CRP measurement for vascular risk assessment, in the diabetic patients, for preventing cardiovascular complications. Focus on decreasing hs-CRP levels by nutritional intervention and pharmaceutical means, from the early stage of diagnosis of type 2 DM, may have a important role.

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