High-Sensitivity C-Reactive Protein (hs-CRP): A Novel Inflammatory Marker in Type 2 Diabetes Mellitus.

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ABSTRACT

Introduction: Diabetes mellitus is a prevalent and chronic progressive metabolic disorder afflicting mankind. The disease progression of diabetes mellitus has been linked with chronic subclinical inflammation, which can be detected through the measurement of the level of high-sensitivity C-reactive protein (hs-CRP). However, there is conflicting and limited data available regarding this issue. The goal of the current study was to look into the relationship between type 2 diabetes mellitus (T2DM) and hs-CRP.

Methods: 200 people participated in a descriptive cross-sectional study, 100 of whom had type 2 diabetes mellitus (T2DM) and the other 100 appeared to be healthy subjects which served as controls. Half of the participants in the case and control groups were female. All the participants were age and sex matched and were only enrolled if they satisfied the diagnostic criteria of T2DM as per the American Diabetes Association. All the subjects underwent detailed history, clinical examination, and biochemical analysis. The biochemical analysis included Glycosylated hemoglobin (HbA1c), hs-CRP, fasting blood glucose (FBG) and postprandial blood glucose (PPBG).

Results: Compared to the control group with mean hs-CRP level of 0.97±0.26 mg/L, T2DM patients had a substantially higher mean hs-CRP level of 2.65± 3.65 mg/L. Additionally, a substantial correlation was discovered between the mean hs-CRP level and FBG, PPBG, HbA1c level, and duration of T2DM. The mean value of hs-CRP was substantially higher (4.76 ± 4.73 mg/L) in ≥ 10-year duration patients of T2DM than in < 10-year duration T2DM patients having mean value of 1.30 ± 1.76 mg/L (P value of < 0.0001).

Conclusion: Underlying low-grade subclinical inflammation can result in diabetes mellitus, which can be assessed by the level of hs-CRP

INTRODUCTION:

With changing lifestyles, dietary patterns, and rapid urbanization, the frequency of non-communicable metabolic illnesses such as diabetes, raised blood pressure and lipid disorders has rapidly increased during the last two decades in south Asia, especially India (1). Diabetes mellitus is a long lasting, multifaceted illness that results either due to a qualitative or quantitative decrease in the insulin molecule or insulin is not being appropriately utilized by the body cells. The hormone that largely controls blood glucose homeostasis is insulin. Over time, diabetes mellitus may result in serious damage to the heart, blood vessels, eyes, kidneys, and nerves and eventually may lead to early death (2). As per the information given by International Diabetes Federation (IDF) in 2021, 537 million people worldwide were having diabetes, which may rise at the current rate to 643 million by 2030 and 783 million by 2045 (3). Globally, diabetes has become the major cause of mortality and morbidity. It accounted for 6.7 million deaths in the year 2021which equals 1 death

every 5 seconds (3). In India, as per IDF estimates, its prevalence by 2045 will be 124.9 million people in the age group of 20–79 years , up from 74.9 million in 2021. Every seventh diabetic globally resides in India (3). Type 2 DM is the most common form of diabetes, and it accounts for more than 90% of all diabetic cases. Genetics and lifestyle factors remain the dominant cause of type 2 diabetes (4). Though such huge population is suffering, the specific underlying mechanism of insulin resistance or altered insulin production is not exactly clear. The idea that chronic subclinical inflammation plays a decisive factor in the manifestation of type 2 diabetes is supported by mounting data. Chronic low-grade inflammation reveals elevated inflammatory proteins along with CRP levels, which have been shown to be associated with the origin and development of T2DM (5-7). CRP is the classical inflammatory biomarker synthesized by liver, and its level is modulated by adipocyte-derived proinflammatory cytokines like interleukin-6 (IL-6), tumour necrosis factor α (TNF-α) (8). So, elevated CRP level has been linked to increased body weight. These biomarkers are intricately correlated with development of impaired insulin sensitivity and altered glucose metabolism. The lower detection limit of the conventional clinical tests for CRP is usually 3 to 8 mg/L. These assays are therefore ineffective for predicting vascular risk since they have low-normal sensitivity. Currently, an easily quantifiable and well researched biomarker of inflammation, high-sensitivity C-reactive protein (hs-CRP) is accessible in typical clinical labs. It can ascertain even low-grade inflammation by measuring CRP levels between 0.01 and 10 mg/L. Low systemic inflammatory status and a decreased risk of cardiovascular disease are indicated by hs-CRP levels < 1 mg/L, while moderate risk is associated with levels 1-3 mg/L and high risk with levels >3 mg/L (9). Previous research has shown that even after controlling for body mass index, smoking, family history of diabetes mellitus, and other variables, hs-CRP remained a significant predictor of diabetes risk (10). Since the role of hs-CRP levels in patient with diabetes is increasingly evaluated, the need to carry out further such studies has become obvious. As far as we are aware, no such study has been done in the tirhut region of Bihar in India. Our study was planned to investigate and analyse the association of hs-CRP levels among type 2 DM subjects.

Methods

The cross-sectional hospital-based study was conducted in the department of medicine and biochemistry at RDJM Medical College and Hospital, Muzaffarpur, Bihar, between July 2023 to June 2024. The study population included 100 type 2 diabetic patients who were managed in the medicine outpatient department, as well as 100 volunteers who appeared to be in good health and were matched for age and sex.

Both the case and control groups involved 50 male and 50 female participants. The T2DM patients were selected irrespective of glycaemic control and only if they fulfilled the diagnostic criteria as per the American Diabetes Association (FBG, PPBG, HbA1c) (11). The control group subjects were selected by simple random sampling technique from different strata of society who were apparently healthy. Type 1 and T2DM diabetes mellitus were differentiated on the basis of careful history-taking and scrutinizing previous laboratory results of various biochemical parameters. Onset of DM in childhood, history of ketoacidosis and insulin dependency for survival has traditionally been sufficient to diagnose type 1 diabetes mellitus. (12)

Inclusion criteria: Type 2 DM patients aged 35 years or more and who fulfilled the diagnostic criteria as per the American Diabetes Association (FBG, PPBG, HbA1c) (11).

Exclusion criteria: Type 1 DM patients, patients on medications known to affect the CRP levels like corticosteroids, amiodarone, tamoxifen, methotrexate were not included in the study. Additionally, the study did not include subjects with known heart disease, patients with malignancy, chronic alcoholics, and pregnant and lactating women.

Techniques of blood sample collection: After an overnight fasting, total 6 milliliters of venous blood was collected from the median cubital vein while maintaining all aseptic precautions. To estimate fasting and postprandial blood glucose, two milliliters of blood were used in a fluoride vial.

For estimating hs-CRP, two milliliters of blood were used in a clot activator or empty vial. To estimate HbA1c, two milliliters of blood were used in an EDTA vial.
Methods of evaluation: Estimation of postprandial blood glucose and fasting blood glucose were done in a biochemistry autoanalyzer by the hexokinase method. Estimation of HbA1c was done in the Innovastar Analyzer by particle-enhanced immunoturbidimetric test, and estimation of hs-CRP was done in Erba Mannheim by measurement of the antigen-antibody reaction by the end point method. Following biochemical procedure, the results were statistically analyzed and contrasted among the study groups.

Statistical analysis:
GraphPad InStat version 3.00 for Windows, developed by GraphPad Software in San Diego, California, USA, was used to statistically analyze the data results. For both cases and the control, the mean and standard deviation were computed. An independent t-test was utilized to compare the mean values of continuous variables. Statistical significance was established when the two-tailed p-value was less than 0.05.

Results and observations:
The present cross-sectional hospital-based study was conducted on 100 T2DM patients and 100 healthy controls aged 35 years or more at RDJM Medical College and Hospital between June 2023 and July 2024. All the participants were investigated for hs-CRP, HbA1c, FBG, and PPBG, and the results were analysed using an unpaired student's t-test. The blood glucose quantification provides an assessment of short-term glycaemic control, while HbA1c provides an assessment of average glycaemic control over the past 3 months (13).
Table 1: The mean age of T2DM patients and the healthy control were 52.57±11.38 years and 52.12 ± 11.19 years respectively.

TABLE 1: MEAN AGE IN YEARS AND SD OF STUDIED GROUPS

STUDY GROUP	NO OF SUBJECTS	MEAN AGE IN YEARS ± SD
CASES	100	52.57 ± 11.38
CONTROLS	100	52.12 ± 11.19

Table 2 and Tabe 3: The mean age of male participants was 52.92±11.88 years and 52.64 ± 11 years for cases and control, while for the female participants it was 52.22 ± 10.96 years and 51.6 ± 10.54 years respectively.

TABLE 2: MEAN AGE IN YEARS AND SD OF MALE SUBJECTS

MALE SUBJECTS	NO OF SUBJECTS	MEAN AGE IN YEARS ± SD
CASES	50	52.92 ± 11.88
CONTROLS	50	52.64 ± 11.88

TABLE 3: MEAN AGE IN YEARS AND SD OF FEMALE SUBJECTS

FEMALE SUBJECTS	NO OF SUBJECTS	MEAN AGE IN YEARS ± SD
CASES	50	52.22 ± 10.96
CONTROLS	50	51.6 ± 10.54

TABLE 4: COMPARISON OF GLYCEMIC PARAMETERS AND HS-CRP LEVEL BETWEEN T2DM PATIENTS AND HEALTHY CONTROLS.

VARIABLES	CASES (Mean ± SD)	CONTROL (Mean ± SD)	p Value
FBG (mg/dl)	191.70 ±101.08	100.19±12.22	0.0
PPBG (mg/dl)	268.00 ± 131.16	135.40 ± 10.81	0.0
HbA1c (%)	8.78± 2.24	5.21±0.43	0.0
hs-CRP (mg/l)	2.65± 3.65	0.97±0.26	0.0

Table 4 showed that the FBS level in T2DM patients was significantly higher with mean value of 191.70 ±101.08 mg/dl than the healthy controls having mean value of 100.19±12.22 mg/dl (p <0.05). The post prandial blood sugar level, Glycosylated hemoglobin (HbA1c) and hs-CRP level also showed significantly higher level among T2DM patients with mean value of 268.00 ± 131.16 mg/dl, 8.78± 2.24 %, 2.65± 3.65 mg/dl than the healthy controls with the mean value of 135.40 ± 10.81 mg/dl, 5.21±0.43 % and 0.97±0.26 mg/dl respectively with p value <0.05.

TABLE 5: ANALYSIS OF HS-CRP level ON THE BASIS OF DURATION OF DM

DURATION OF DM	MEAN (mg/dl)	SD	SEM	95% CI	p VALUE
<10Years	1.30	1.76	0.22	0.84 – 1.75	<0.0001
≥10 Years	4.76	4.73	0.75	3.22– 6.29	

Table 5 shows that the mean level of hs-CRP in ≥ 10 years duration T2DM patients was 4.76 ± 4.73 ng/dl, which was significantly higher than hs-CRP level in < 10-year duration T2DM patients with the mean value of 1.30 ± 1.76 mg/dl (P value of <0.0001).

TABLE 6: ANALYSIS OF HS-CRP LEVEL ON THE BASIS OF MEAN FASTING BLOOD GLUCOSE.

MEAN BLOOD GLUCOSE	MEAN (mg/dl	SD	SEM	95%CI	p VALUE
≤126mg/dl	1.62	2.30	0.38	0.84—2.39	<0.05
>126mg/dl	3.23	4.13	0.51	2.19—4.26	

Table 6 shows that the mean levels of hs-CRP in T2DM patients having mean FBG level ≤126 mg/dl was found to be 1.62 ± 2.30 mg/dl while those T2DM patients having FBG level >126 mg/dl it was 3.23 ± 4.13 mg/dl with the two-tailed P value of <0.05.

TABLE 7: ANALYSIS OF HS-CRP ON THE BASIS OF MEAN POST PRANDIAL BLOOD GLUCOSE

MEAN BLOOD GLUCOSE	MEAN (mg/dl)	SD	SEM	95%CI	p VALUE
≤200mg/dl	1.59	2.63	0.43	0.71—2.46	<0.05
>200mg/dl	3.27	4.03	0.50	2.25—4.28	

Table 7 shows that the mean level of hs-CRP in T2DM patients having mean PPBG level ≤200 mg/dl was found to be 1.59 ± 2.63 mg/dl, while in >200 mg/dl diabetic cases was 3.27 ± 4.03 mg/dl with the two-tailed P value of <0.05.

TABLE 8: ANALYSIS OF HS-CRP LEVEL ON THE BASIS OF HbA1c

HbA1c	MEAN (mg/dl)	SD	SEM	95% CI	p VALUE
≤7%	1.34	1.91	0.37	0.58—2.09	<0.05
>7%	3.11	4.00	0.46	2.18—4.03	

Table 8 shows that the mean levels of hs-CRP in T2DM with HbA1c>7% was found to be significantly higher with value of 3.11± 4.00 mg/dl than those with HbA1c≤7% having value of 1.34 ± 1.91 mg/dl (p <0.05).

Discussion:

To examine the role of hs-CRP in T2DM patients, the current study was planned which involved 100 T2DM patients and 100 healthy controls. With a standard deviation of 11.38 years, the average age of the cases was 52.57 years. The mean age of controls was 52.12 years with a standard deviation of 11.19 years. The mean fasting blood glucose level in cases was found to be 191.70 ± 101.08 mg/dl and in controls 100.19± 12.22 mg/dl with a significant two-tailed P value< 0.0001. The mean blood glucose level in postprandial plasma glucose in diabetic cases was found to be 268.00 ± 131.16 mg/dl, which was significantly higher than the controls with a mean value of 135.40±10.81 mg/dl. The present study revealed that the mean hs-CRP level in T2DM patients was 2.65 ± 3.65 mg/L, while in control it was 0.97 ± 0.26 mg/L with a significant two-tailed p value of< 0.0001, which supports the previous study by Wang X et al., which suggested that the population with elevated CRP levels is more prone to develop T2DM (6). Mahajan et al. also showed that hs-CRP is an independent predictor of developing T2DM (14). The average hs-CRP level was found to be 4.76 ± 4.73 mg/L for T2DM patients of ≥ 10 years duration, which was significantly higher than T2DM patients of < 10 years duration. This suggest that duration of diabetes mellitus has strong correlation with higher hs-CRP level which supports the study by Gupta R et al which revealed a positive linear association between hs-CRP and duration of diabetes (15). They also established a linear association between hs-CRP and Hb1AC level which supports the present study which revealed that hs-CRP level for cases with poor glycaemic control (Hb1AC >7%) was significantly higher compared to cases with good glycaemic control (Hb1AC ≤ 7%) (15). However, the present study is in contrary to a meta-analysis which described no such definite correlation between hs-CRP and Hb1AC level, the level of hs-CRP was not regarded as an independent risk factor for diabetes development (16,17). Ramesh SS et al. demonstrated a similar correlation between hs-CRP and HbA1c but not with fasting blood sugar or postprandial blood sugar. This suggests that FBG or PPBG may not be as effective as HbA1c in monitoring and prediction of the disease (18). The present study revealed that hs-CRP level was significantly higher in T2DM patients with FBG levels > 126 mg/dl than those cases with FBG levels ≤ 126 mg/dl (p-value < 0.05). A population-based study by Aronson D et al. demonstrated that there is an independent association of hs-CRP with fasting blood glucose (19). Furthermore, the present study also showed that hs-CRP level was also correlated with PPBG, which was significantly higher in cases with PPBG level > 200 mg/dl than cases with PPBG level ≤ 200 mg/dl. In a different investigation, the scientists discovered that hs-CRP was linked to metabolic syndrome, obesity, glucose intolerance, impaired fasting blood sugar, and insulin resistance on its own (20).

Limitations: The present study could not consider BMI (body mass index), waist circumference, lipid profile status, or blood pressure of the participants, which may be the confounding factors. Moreover, we could not evaluate the effect of drugs on diabetic individuals, which probably might influence the blood parameters, including hs-CRP.

Conclusion: This study suggests that hs-CRP is positively correlated with glycaemic parameters and duration of T2DM. Among several markers of inflammation, hs-CRP may be a defining marker in people prone to diabetes or may be useful in the prognosis of diabetes. The high level of hs-CRP in diabetic patients suggests the vital role of low grade systemic subclinical inflammation in diabetogenesis and the insulin resistance syndrome. Further research is needed to establish the causal relationship between inflammation and T2DM.

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