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General Medicine

ASSOCIATION BETWEEN C-REACTIVE PROTEIN AND GLYCOSYLATED HEMOGLOBIN IN TYPE-2 DIABETES MELLITUS: AN INDIAN PERSPECTIVE

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ABSTRACT

Background: Diabetes mellitus (DM) is a complex metabolic disorder that alters the glucose status of the human body. There has been a steady increase in the prevalence of DM in the past few decades leading to macro-and microvascular complications. There has been mounting evidence suggesting that inflammation plays a role in the pathophysiology of DM.

Methods: A single centre prospective study was conducted on 50 consecutive patients with de novo or prior Type-2 DM (T2DM) history between JANUARY 2021 to OCTOBER 2021. CRP and HbA1c samples were taken at the start of the study and were retaken after 12 weeks of pharmacotherapy and lifestyle modification. The relationship between CRP and HbA1c was analysed.

Results: The mean HbA1c and CRP for the initial 50 participants were 9.6500 ± 1.8816 and 1.1520 ± 0.9984, respectively. At the 12th-week follow up, the mean HbA1c fell to 7.3952 ± 1.3155 ($P<0.05$), and the mean CRP was reduced to 0.2857 ± 0.5237 ($P<0.05$). Furthermore, the rise in HbA1c was correlated with a statistically significant rise in CRP.

Conclusion: This study establishes a positive correlation between serum CRP and HbA1c. Improving glycemic control can help reduce the risk of adverse events associated with sustained inflammation.

KEYWORDS : C-reactive protein; HbA1c; Type-2 Diabetes Mellitus; Inflammation; Diabetes; Glycosylated Hemoglobin.

INTRODUCTION

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both.[1,2] DM is a growing global burden. The latest International Diabetes Federation (IDF) figures reveal that 537 million worldwide live with DM - a 16% rise from the 2019 estimates. By 2045, one in eight adults (783 million) might be living with DM.[3] Type-2 DM (T2DM) is the most common form of diabetes (90%). It is characterised by insulin action and secretion disorders, either of which may be the predominant feature.[2,3]

The risk of developing T2DM increases with age, obesity, physical inactivity, family history, hypertension, dyslipidaemia and women with a history of gestational diabetes. The frequency of T2DM varies considerably among different racial or ethnic subgroups. Persons of Native American, Polynesian or Micronesian, Asian-Indian, Hispanic, or African-American descent are at higher risk than persons of European origin.[4]

Impaired glucose tolerance or hyperglycemia establishes the diagnosis.[5]

Persistent hyperglycemia is associated with macro-and microvascular complications.[6]

The clinical relationship between DM and atherosclerosis is well established, with the risk for coronary heart disease, peripheral arterial disease and cerebrovascular disease (CVD) being significantly elevated in patients with diabetes.[7] Several studies have implicated that T2DM is associated with increased concentrations of the acute-phase reactants and pro-inflammatory cytokines (IL-1, IL-6, TNF-a), which are promoters of atherosclerosis.[8,9]

C-reactive protein (CRP) is an acute-phase reactant pentameric protein synthesised in the liver. Its production is primarily induced by the IL-6 action on the gene responsible for CRP transcription during the acute stage of the inflammatory process. CRP is a direct marker for inflammation. The CRP levels rapidly rise and fall with the onset and elimination of the inflammatory stimulus, respectively.[10]

The glycosylated hemoglobin (HbA1c) test shows an average blood sugar level over the past 90 days and represents a percentage. The test can also establish the diagnosis of diabetes.[11] Hemoglobin becomes glycated or coated with glucose from the bloodstream in proportion to the blood glucose levels. Higher average blood sugar levels will reflect on the surface of the hemoglobin protein as higher HbA1c levels.[12,13]

MATERIALS AND METHODS

We prospectively studied the association of CRP (an acute phase reactant) and HbA1c (an indicator of glycemic control). The study comprised 50 consecutive patients with de novo or prior history of T2DM visiting the medicine out-patient department between JANUARY 2021 to OCTOBER 2021. The purpose of the study was explained to the patients and their attenders, and informed consent was obtained. Out of the 50 initial participants, only 37 patients presented for their follow up.

CRP and HbA1c samples were drawn simultaneously. The patients were advised to follow up after 12 weeks of optimised medical therapy (insulin/oral antidiabetic medicines) and lifestyle changes (exercise, weight loss and diet counselling).

Inclusion Criteria

- Fasting venous blood glucose value equal to or more than 126mg/dl
- Postprandial venous blood glucose >200mg/dl
- Age >18

Exclusion Criteria

- Patients on statins, thiazolidinediones (TZDs), and other anti-inflammatory drugs
- Type-1 DM
- Known heart failure
- Acute febrile illnesses
- Renal impairment
- Hepatic impairment
- Arthritis or other chronic illnesses
- Malignancies
- Asymptomatic infections
- Smokers

At their 12th-week follow-up, CRP and HbA1c samples were drawn again. Antidiabetic treatment's efficacy, adherence to lifestyle changes, and the relation between CRP and HbA1c were analysed. The Institutional Ethics committee approved the study.

CRP Testing

Diagnostic Kit

A diagnostic reagent kit for the in vitro detection of CRP in human serum by qualitative and semi-quantitative rapid latex slide test.

Procedure

A series of dilutions of the test serum in normal saline, as 1:2, 1:4, 1:8 etc., was prepared, and one drop of each of these dilutions was tested with one drop of Latex CRP Reagent. Agglutination occurring within 2 minutes was observed on the glass slide provided in the kit. The highest dilution which showed agglutination was taken as CRP titre of the test serum.

Calculation

The concentration of CRP in serum can be calculated as:

CRP concentration = Sensitivity x Highest dilution of serum showing agglutination.

The sensitivity and detection limit of this test is 0.6 mg/dl.

HbA1c Testing

Testing kit: Teco Diagnostics Glycohemoglobin Reagent Set.

Procedure

Step A- Hemolysate preparation:

1. Dispense 500 ml Lysing reagent into tube labelled as standard, control and sample.
2. Place 100 microlitre well-mixed blood sample, standard or control, into an appropriately labelled tube and mix well.
3. Allow the tube to stand for 5 minutes.

Step B- Glyco haemoglobin preparation:

1. Dispense 3 ml of glycol haemoglobin cation exchange resin into 13 x 100mm glass tube labelled as standard, control, sample.
2. Mix resin by inverting at least ten times. Swirl the bottle after adding it to each tube.
3. Add 100microlitre of hemolysate from step A. Position the filter separately from the tube so that the rubber sleeve is approximately 1cm above the liquid level.
4. Place the tube on the rotator or rocker and mix for 5 minutes. Remove the tube from the rotator.
5. Push the filter separator into the tube to firmly pack the resin. The supernatant can be poured into another tube or directly into a cubic or absorbent's measurement.
6. Adjust the instrument to 0' absorbent at 415 nanometres with deionised water as blank (wavelength range 390-420 nanometre).
7. Read and record the absorbent value for standard, control, sample, etc. These readings are for Glycohaemoglobin.

Step C -Total haemoglobin fraction:

1. Dispense 5ml deionised water into tube labelled as standard, control, and sample.
2. Place 20 microliters of the haemolysed from step A.3 into an appropriately labelled tube and mix. Adjust the instrument to 0' absorbent at 415 nanometres with deionised water as blank.
3. Read and record the absorbent values for standard, control, sample etc. These readings are for total haemoglobin.

CALCULATION

Results of the unknown should be determined in the following manner. Percentage glycolhaemoglobin (unknown)= [R(unknown)/R (standard)] x standard concentration

R unknown = ratio unknown = absorbent of glycol unknown/absorbent of total Hb unknown.

R standard= Absorbent of glycol standard / Absorbent of total Hb standard

Absorbent = value that we get in median

The standard containing 8% glycol Hb had absorbent = 0.480 for glycol Hb and absorbent 0.578 for total Hb.

An unknown sample had glycol Hb absorbent = 0.962 and total Hb absorbent = 0.764 glyco Hb.

Statistical analysis was done using the SPSS package and MS Excel. Student's 't' test and $\chi 2$ test were used. Pearson correlation and P values were calculated. P values < 0.05 were considered to be significant.

RESULTS

The following observations were made in the study population:

For the initial 50 patients (**Table 1**),

1. The mean HbA1c was 9.6500 ± 1.8816 (**Figure 1**)
2. The mean CRP was 1.1520 ± 0.9984 (**Figure 2**)

For the initial 50 patients, the rise in HbA1c was associated with a statistically significant rise in CRP (**Table 2, Figure 3**).

For the 37 follow up patients (**Table 1**),

1. The mean HbA1c reduced to 7.3952 ± 1.3155 (P<0.05) (**Figure 1**)
2. The mean CRP was reduced to 0.2857 ± 0.5237 (P<0.05) (**Figure 2**)

There was a significant decrease of HbA1c after treatment, and this was associated with a significant corresponding reduction of CRP levels.

DISCUSSION

CRP is an independent predictor of cardiovascular diseases and is linked to an increased risk of thrombotic events, including myocardial infarction and stroke. CRP has also been linked to an increased risk of later development of diabetes. Prior research has also established that CRP levels are higher in people with diabetes and is associated with higher HbA1c in people without diabetes.[14,15,16,17] Furthermore, CRP levels are higher in people with diabetes when compared with non-diabetics. Less is known about whether CRP in people with diabetes is related to the level of glycemic control.

Activation of the immune system is highly related to the incidence and progression of T2DM. Both innate and adaptive immunity are involved in adipose tissue inflammation. The phenotype switching of macrophages from M2-type (predominantly anti-inflammatory) to increased proportions of M1-type macrophages (pro-inflammatory) plays an essential role in initiating and amplifying islet cell inflammation.[18] The evidence also shows that the recruitment of T and B cells precedes adipose tissue infiltration by macrophage infiltration of the adipose tissue.[19]

Moreover, several other organs have been implicated in the metabolic homeostasis and inflammatory state in T2DM, such as the nervous system, liver and possibly skeletal muscles.[20,21,22,23,24] However, more research is needed to support this evidence.[5]

In a cross-sectional study by Yudkin et al., CRP levels were related to insulin resistance, obesity and endothelial dysfunction.[25] Data derived from the Women's Health Study showed that elevated levels of CRP predicted the development of T2DM.[26] This was further confirmed by data from the Nurses' Health Study.[27] CRP levels have also been associated with future development of hypertension and are known to play a direct role in the pathogenesis of atherosclerosis and thrombosis.[28,29]

In a cross-sectional study in urban North Indians of 2520 urban subjects with 1410 T2DM patients and 1110 non-diabetic subjects (controls), carried out with the assessment of 18 metabolic traits, it was found that in T2DM patients, there was a statistically significant positive correlation between CRP and all the indices of obesity, hyperglycaemia, insulin resistance and dyslipidaemia. The strongest correlation of CRP was observed with BMI, WHR, triglycerides, and HbA1c. The strongest correlation of CRP in controls was observed with WC, followed by BMI and HbA1c.[30]

Several studies demonstrated that low-grade systemic inflammation is associated with an increased risk of cardiovascular disease.[31] elevation of serum C-reactive protein is an indicator of systemic inflammation. The associations of C-reactive protein with blood insulin and glucose may thus help to elucidate the roles of inflammation in insulin resistance and the development of cardiovascular disease.[32]

Elevated CRP levels are also linked to the increased risk of the development of diabetes.[26] Furthermore, CRP levels are higher in people with diabetes than those without diabetes. In the study by Wu et al., investigations on the association between serum CRP and fasting blood levels of insulin, glucose and HbA1c have revealed that elevated CRP was associated with high HbA1c among men and women.[15]

King et al. found that in unadjusted analyses, respondents with diabetes who had elevated HbA1c levels (>9.0%) had a significantly higher per cent of elevated CRP than people with low (<7%) HbA1c

levels (P< 0.001). Furthermore, HbA1c was significantly associated with an increased likelihood of elevated CRP for HbA1c >9.0% (OR 2.15, 95% CI 1.07–4.32) and for HbA1c >11.0% (4.40, 1.87–10.38). Higher HbA1c also predicted an elevated CRP in the regression model when HbA1c was analysed as a continuous variable (1.20, 1.07–1.34).[33]

In this study, the mean CRP when correlated with HbA1c at different levels < 7, 7-9, 9-10, >10, were 0.40, 0.51,1.41 and 2.15 respectively. This showed that a rise in HbA1c correlates significantly with increasing CRP values (P < 0.05). Therefore, like other studies, this study shows that higher glycaemic levels are associated with higher CRP values.

Having excluded all other causes of raised CRP, the only implication is that increased CRP at the molecular level could be involved in the pathogenesis of T2 DM.

The mean HbA1c of 50 patients who came initially was 9.6500, and the mean HbA1c of the 37 patients who came for follow-up was 7.3752. This showed a significant decrease in HbA1c in patients on follow-up (P< 0.01). The mean CRP of the 50 patients initially was 1.1520, and the mean CRP of 37 patients when came for follow-up was 0.2857. This also showed a significant decrease in CRP when patients came for follow-up (P<0.05).

A further subanalysis was done on 37 patients out of the 50 who came initially and for follow-up. The mean HbA1c of the 37 patients who came initially and for follow-up was 9.285 and 7.385, respectively. This showed a significant decrease in HbA1c when patients came for follow-up. This reduction was found to be significant (P < 0.05). The mean CRP of 37 patients who came initially and for follow-up was 0.78 and 0.53, respectively. These values also showed a significant decrease in CRP when patients came for follow-up (P<0.05), a finding not found in other studies so far. This is likely related to compliance with pharmacotherapy and lifestyle changes.

In this study, we go a step further with the finding that with successively higher levels of HbA1c, the number of patients with CRP positive is significantly higher. The main implication of these findings is that inflammation may be implicated in the development of diabetes and in ongoing levels of hyperglycemia once diabetes is established.

This study has found a positive correlation between CRP and Glycemic control.

There were several limitations to this study. Firstly, the CRP values were measured using a Semi-quantitative method.

Secondly, the onslaught of the second wave of the Coronavirus disease 2019 pandemic made the routine follow up difficult and likely reduced the number of follow up patients.

TABLES

Table 1: HbA1c and CRP of 50 initial and 37 follow up cases.

	HbA1c (Initial 50)	HbA1c (Follow up 37)	CRP 1 (Initial 50)	CRP 2 (Follow up 37)
Mean	9.6500	7.3952	1.1520	0.2857
Standard deviation	1.8816	1.3155	0.9984	0.5237
P value		0.0000		0.000359

Table 2: Relation between HbA1c and CRP (P<005).

Hba1c (%)	Number of patients	Mean CRP
<7	9	0.4
7 - 9	14	0.51
9.1 - 10	11	1.41
>10	16	2.15

FIGURES

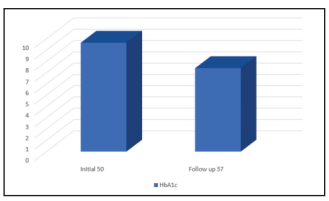

Figure 1: Comparsion of HbA1c between the initial 50 patients and the follow up 37 (P<0.05).

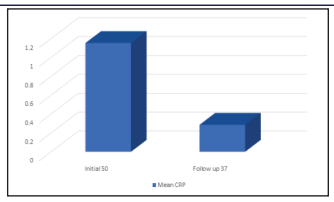

Figure 2: Comparsion of CRP between the initial 50 patients and the follow up 37 (P<0.05).

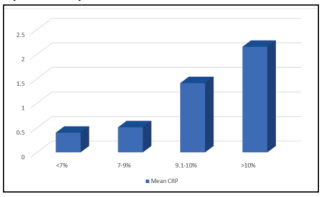

Figure 3: Relation of CRP with increasing levels of HbA1c (%).

CONCLUSION

This study established a positive correlation between serum CRP and HbA1c (glycemic level). Sustained elevation of CRP is associated with detrimental health outcomes such as coronary and other vascular disorders. Improving glycemic control in people with diabetes can help reduce the risk of such adverse events.

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