Original Article

A Study of Inflammatory Markers in Type 2 Diabetes Mellitus Patients

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Abstract

Background: Type 2 diabetes mellitus (DM) is associated with low-grade inflammation. Inflammatory markers such as C-reactive protein (CRP) and adenosine deaminase (ADA) activity have been related to the development of insulin resistance in Type 2 DM. Both these inflammatory markers are found to be elevated in the case of Type 2 DM. Normally, serum CRP level is found to be 0-10 mg/l and serum ADA activity is found to be 0-30 U/l.

Objectives: The aim was to determine the serum levels of inflammatory markers (serum CRP and serum activity of ADA) in Type 2 DM and to compare it with that of normal healthy controls.

Materials and Methods: A total of 30 Type 2 DM patients and 30 age and sex-matched healthy controls were included for the study. A fasting serum sample was collected from each diabetic and healthy individual and was tested quantitatively by glucose oxidase-peroxidase method for estimation of fasting blood glucose, turbidimetric immunoassay method for estimation of serum CRP level and kinetic assay method for the estimation of serum ADA activity. The values for glycated hemoglobin were measured by Boronate affinity chromatography (NycoCard assay).

Results: Significantly higher amounts of serum CRP and serum ADA activity were found to be present in the Type 2 diabetes patients (15.77 ± 13.54 mg/l and 48.34 ± 21.05 U/l respectively) when compared to healthy controls (6.90 ± 3.30 mg/l and 25.02 ± 5.78 U/l respectively). On the comparison of these inflammatory markers (serum CRP and serum ADA activity), the values of serum ADA activity were significantly higher in the diabetic patients compared with non-diabetic individuals.

Conclusion: The present study demonstrated that inflammatory markers CRP and ADA have been related to the severity in Type 2 DM. Further, it has also been found that in Type 2 DM, ADA is a better inflammatory marker in comparison to serum CRP.

Keywords: Adenosine deaminase, C-reactive protein, Inflammation

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia with derangement of carbohydrate, fat, and protein metabolism due to absolute or relative deficiency of insulin secretion and action, or both. In Type 2 DM, functioning of the immune system is seen to be altered, which results in adverse changes occurring in circulating leukocytes. These immunological changes lead to altered level of cytokines and change in number and activation of various leukocytes and increased apoptosis. Therefore, these changes suggest that inflammation participates in the pathogenesis of Type 2 DM.[1,2]

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C-reactive protein (CRP) is a prototypical and most commonly used acute phase reactant marker of

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inflammation in the body. CRP is synthesized by the liver in response to inflammatory cytokines such as interleukin-6 and tumor necrosis factor-α (TNF-α). Inflammatory cytokines have been seen to mediate insulin resistance in liver, skeletal muscles and adipose tissues, which finally lead to the Type 2 DM. Elevated CRP levels have also been linked to an increased risk of later development of diabetes. Due to its stability in plasma or serum and the ease of measurement, CRP may be a useful predictor of DM.

Adenosine deaminase (ADA) is a purine metabolic enzyme that catalyzes the deamination of adenosine to inosine contributing to the regulation of intracellular and extracellular concentration of adenosine.[3] ADA may play a role in insulin effect and glycemic control as adenosine acts directly to stimulate insulin activity via several processes such as glucose transport, lipid synthesis, pyruvate dehydrogenase activity, leucine oxidation and cyclic nucleotide phosphodiesterase activity.[4] Therefore, activity of ADA in Type 2 DM might be a marker for prognosis in Type 2 DM.

The elevated level of CRP and ADA predicts the development of Type 2 DM supporting a possible role for inflammation in diabetogenesis.[5] These facts need further investigation to correlate the relationship between inflammatory markers (CRP and ADA) and Type 2 DM. Therefore, this study is being carried out to correlate the association between serum levels of inflammatory markers with the glycemic status in Type 2 DM.

MATERIALS AND METHODS

In the present study, 30 patients aged 40-70 years who were diagnosed as diabetics and confirmed by the estimation of fasting serum glucose (>126 mg/dl) on two occasions were selected from the Medicine OPD and IPD of Teerthanker Mahaveer Medical College and Research Centre, Moradabad. 30 normal healthy subjects, age and sex-matched with the diabetic patients, were selected as controls.

Those individuals who were suffering from other inflammatory conditions like tuberculosis, leprosy, pregnancy, cancer, skin diseases, gout, liver and kidney diseases were excluded to rule out any increase in inflammatory markers due to causes other than DM.

Fasting serum sample was taken from each patient and control and was analyzed for plasma glucose, glycated hemoglobin (HbA1c), serum CRP and serum ADA activity, by glucose oxidase-peroxidase method,[6] boronate affinity chromatography (NycoCard),[7] turbidimetric immunoassay method[8] and kinetic assay method[9] respectively.

Statistical Analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) 16 version. Mean ± standard deviation were calculated for all the parameters analyzed and were compared by Student's *t*-test and the parameters were correlated by determining the coefficient of correlation (*r* value) using SPSS program. *P* values considered significant were as follows:

1. $P < 0.05$: As significant
2. $P < 0.001$: As highly significant.

RESULTS

The mean level of serum CRP and ADA activity of diabetic patients was significantly higher when compared to normal subjects (Tables 1 and 2). Further, a significant positive correlation was found between ADA and HbA1c ($r = 0.38$). On the other hand, a non-significant positive correlation was seen between CRP and HbA1c ($r = 0.24$) (Table 3 and Figures 1-4).

DISCUSSION

Type 2 DM is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased glucose production. Type 2 DM is frequently associated with an acute-phase reaction, suggesting a low-grade

Table 1: Comparison of FPG, HbA1c, serum CRP and serum ADA level between study groups

Parameters	Mean±SD	
	Diabetic	Non diabetic
FPG (mg/dl)	217.96±53.98	90.16±12.23
HbA1c (%)	9.85±1.83	5.26±0.68
CRP (mg/L)	15.77±13.54	6.90±3.30
ADA (U/L)	48.34±21.05	25.02±5.78

ADA: Adenosine deaminase, CRP: C-reactive protein, HbA1c: Glycated hemoglobin, FPG: Fasting plasma glucose, SD: Standard deviation

Table 2: Comparison of serum CRP and ADA between diabetics and non-diabetics

Parameters	*t* value	*P* value
CRP (mg/L)	3.486	0.001
ADA (U/L)	5.835	<0.001

ADA: Adenosine deaminase, CRP: C-reactive protein

Table 3: Correlation of ADA and CRP with HbA1c

Correlation between	*r* value	*P* value
CRP and HbA1c	0.24	>0.05
ADA and HbA1c	0.38	<0.05

ADA: Adenosine deaminase, CRP: C-reactive protein, HbA1c: Glycated hemoglobin

inflammatory status.[1,10] In fact, markers of acute-phase response, including serum CRP and serum ADA, the main mediators of the response, have been shown to be elevated in patients with Type 2 DM and with metabolic syndrome.[10]

This study shows the increased level of serum CRP and serum ADA activity in the patients with Type 2 DM as compared to that of the non-diabetic individuals. On comparing the mean values using the Student's *t*-test, the mean value of the serum CRP (mg/l) in the diabetic patients was found to be 15.77 ± 13.54, which was significantly higher than that of the controls which was found to be 6.90 ± 3.30 ($P < 0.05$). Similarly, the mean value of the serum ADA (U/L) activity in the diabetic patients was found to be 48.34 ± 21.05, which was significantly higher as compared to that of the controls which was found to be 25.02 ± 5.78 ($P < 0.05$).

To evaluate the relationship of the inflammatory markers, CRP and ADA with the glycemic status of an individual, Karl Pearson's correlation coefficient (r value) was calculated. It showed a positive and significant correlation between the serum ADA activity and HbA1c level with a correlation coefficient of $r = 0.385$ ($P < 0.05$). Likewise, the serum CRP values showed a positive correlation with a correlation coefficient of $r = 0.24$ ($P > 0.05$), which was mildly correlated with HbA1c level of the diabetic patients.

In this study, out of 30 diabetic patients, 14 patients i.e., about 46% had increased level of serum CRP from the reference range (up to 10 mg/l). On the other hand, out of 30 diabetic patients, 24 patients i.e., about 80% had increased level of serum ADA from the reference range (up to 30 IU/L).

Role of CRP in Type 2 DM

CRP is a marker of low-grade inflammation and may have an indirect influence on insulin resistance and insulin secretion through altered innate immune response due to heightened systemic inflammation. The production of CRP is regulated by inflammatory cytokines such as TNF-α and interleukin-6. Nevertheless, several mechanisms both direct and indirect, may causally link acute phase mediators to diabetes pathogenesis. TNF-α level are elevated in animal models with insulin resistance,[11] and may have a direct effect on the insulin receptor to down-regulate tyrosine kinase activity. While, neutralization of TNF-α in rodent models of insulin resistance, results in a dramatic improvement in insulin sensitivity.

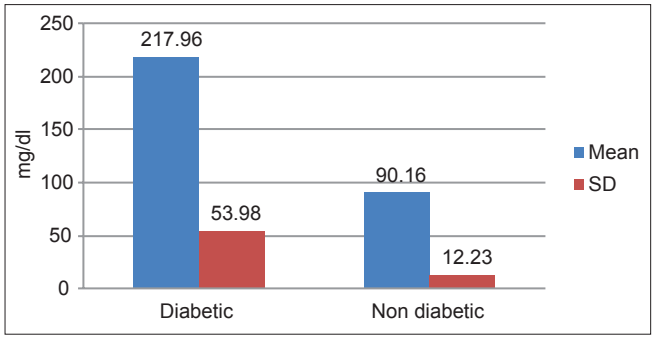

Figure 1: Comparison of fasting plasma glucose level between study groups

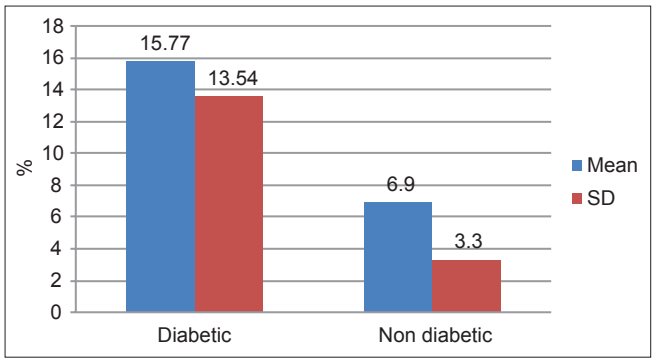

Figure 2: Comparison of glycated hemoglobin level between study groups

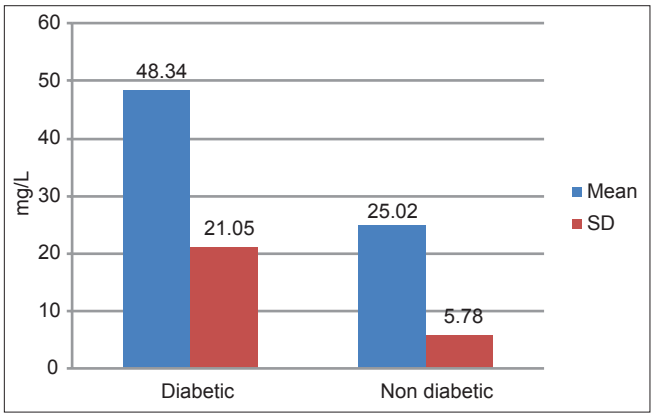

Figure 3: Comparison of serum C-reactive protein level between study groups

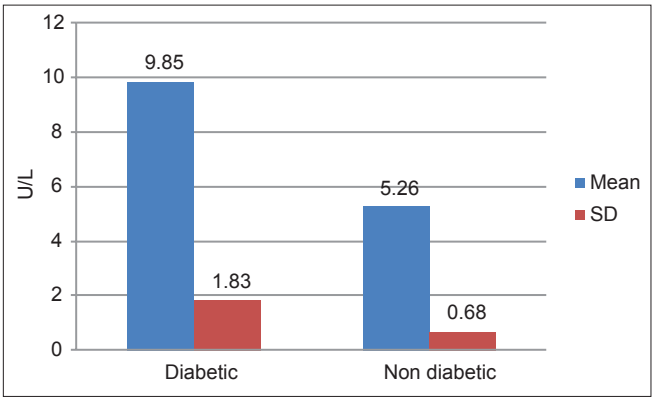

Figure 4: Comparison of serum adenosine deaminase activity between study groups

Role of ADA in Type 2 DM

ADA plays a crucial role in lymphocyte proliferation and differentiation[12] and shows its highest activity in T-lymphocytes.[13] The high plasma ADA activity might be due to abnormal T-lymphocyte responses or proliferation; may point towards the mechanism that involves its release into the circulation.[12] Therefore, it has been reported that increased ADA activity in diabetic individuals could be due to altered insulin related T-lymphocyte function. The elevation of serum ADA activity in Type 2 DM has also been explained through extracellular cyclic adenosine monophosphate (AMP) adenosine pathway.

Adenosine exerts its protective effects by inhibiting lipolysis through A1 receptors. ADA inactivates adenosine and hence activates lipolysis and markedly potentiates the increase in cyclic AMP accumulation due to nor epinephrine.[14,15] Thus, dysregulated fat metabolism and consequent elevation of free fatty acids leads to the subsequent development of Type 2 DM.[16,17]

ADA as a Better Inflammatory Marker in Type 2 DM

In our study, the levels of both the inflammatory markers studied, *viz.*; ADA activity and serum CRP were found to be elevated in the diabetic patients. To evaluate the correlation of these inflammatory markers with the glycemic status of a diabetic patient, Pearson's correlation coefficient (*r* value) was calculated. It revealed that both of these inflammatory markers, ADA and CRP were positively correlated with the HbA1c level of the diabetic patients with $r = +0.24$ and $r = +0.38$ respectively.

Among ADA and CRP as inflammatory markers, although CRP was positively correlated with the glycemic status, a significant correlation could not be established ($P > 0.05$). On the other hand, ADA activity was positively and significantly correlated with HbA1c concentration in DM patients ($P < 0.05$). About 80% had increased level of serum ADA from the reference range while only about 46% of the patients had increased level of serum CRP from the reference range. It can thus be suggested from this study that ADA is a better inflammatory marker as compared to CRP. ADA may play a role in the pathophysiology of Type 2 DM and its complications through biochemical mechanisms mentioned above. The correlation of serum activity of ADA was specifically strong in diabetes patients with poor glycemic control (HbA1c> 7.5%).

CONCLUSION

The present study demonstrated that inflammatory markers such as CRP and ADA have been related to the development of the severity in Type 2 DM. This study also demonstrated that ADA is a better inflammatory marker when compared to CRP, since a higher HbA1c level is significantly associated with a greater likelihood of higher ADA levels among diabetic patients. These observations have provided considerable insight which when further elaborated, may yield better measures to predict, prevent and treat Type 2 DM.

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