Comparison of serum ADA (Adenosine Deaminase) levels in Diabetic and Normal individuals

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Abstract

Introduction: Type 2 Diabetes Mellitus (T2DM) which is characterized by hyperglycemia, impaired cell-mediated immune response, dyslipidemia and inflammation results in chronic complications like Coronary artery disease (CAD). Studies have revealed that Adenosine deaminase (ADA) play an essential role in the regulation of T-lymphocyte proliferation and gluconeogenesis. Our study was conducted to find out if ADA can be used as a prognostic marker for CAD in T2DM.

Aim and objective: To compare the levels of ADA in T2DM and controls and to determine whether ADA can be utilized as a prognostic marker for CAD in diabetic patients.

Material and methods: This is a cross-sectional study conducted in Amrita Institute of Medical Sciences and Research Centre, Kochi which included 104 patients with T2DM and 55 healthy individuals. Blood samples were collected and analyzed for ADA, HbA1C, Lipid profile and hsCRP.

Results: The mean glycemic indices, lipid profile and hsCRP showed statistically significant increase in the group with T2DM when compared to the group without T2DM. Serum ADA showed a statistically significant association with T2DM with a p value of < 0.001. A significant positive correlation (r=0.625) was observed between ADA and HbA1c in the group with diabetes. The ROC curve analysis showed that ADA, if used as a predictive marker for the prediction of CAD in T2DM, has 95.8% and 92.5% sensitivity and specificity respectively.

Conclusion: ADA can be used as a prognostic biomarker for predicting complications like CAD in T2DM.

Keywords: Type 2 Diabetes Mellitus, Adenosine deaminase, Coronary Artery Disease

Introduction

Type 2 Diabetes Mellitus (T2DM) is a metabolic disorder characterized by hyperglycemia caused due to abnormalities in insulin secretion and action. It has become a major health issue since the last several years and the incidence has been increasing due to insulin resistance, concurrent obesity, cell dysfunction and dysregulated immune function [1].

T2DM induced cell-mediated dysfunction can be assessed by an enzyme Adenosine Deaminase (ADA) which is a major enzyme involved in purine metabolism. ADA is distributed in various tissues such as cardiac muscles, adipose tissue and lymphoid tissue and in the latter it plays an important role in lymphocyte differentiation and maturation. Activation and production of cytokines is an important part of cell-mediated immunity and this is handled by CD26 and adenosine receptors (A1R, A2AR, A2BR, A3R). Any rise in the levels of ADA will lead to depletion of adenosine which thereby results in the down-regulation of the aforementioned receptors. This would consequently affect the cell-mediated immunity which can further lead to decrease in IL2 and its receptors[2,3,4,5,6]. All these events can have a significant impact in the pathogenesis of T2DM. Studies have shown that features of T2DM like insulin resistance can be modified by suppressing ADA [7, 8].

Adenosine is said to have a cardio protective role and thus high levels of ADA is strongly associated with Coronary Artery disease (CAD). Thus, increase of ADA in T2DM results in decreased adenosine levels that can give rise to CAD related complications [9]. The novelty of this study is that though there are literatures comparing the levels of ADA in T2DM and CAD

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separately, not many studies have been conducted to find the utility of ADA in predicting CAD in T2DM patients; especially in Indians.

In this study we aimed to measure the levels of serum ADA in T2DM patients and thus find out if it can be used as a marker that can predict future development of CAD in these patients.

Material and Methods

This cross-sectional study was conducted in patients visiting the Department of Endocrinology and General Medicine OPDs in Amrita Institute of Medical Sciences and Research Centre, Kochi for a period of 6months from January 2022 to July 2022. The study was conducted according to the approval and guidelines of the ethical committee of the institution (ECASM-AIMS-2022-088) and with the informed, written consent of the participants.

A total of 159 subjects of both sexes in the age group 35 - 65 years were taken for the study out of whom 104 were T2DM patients and 55 were in the non-diabetic control group. Non-diabetic controls were normal healthy subjects presenting for health check-ups in the Comprehensive Health Check-up Clinic of the hospital.

Inclusion Criteria: Male and female participants between the age group of 35 and 65 years diagnosed with T2DM for the past six months and those patients with T2DM who have been diagnosed with CAD for the past 6 months were included in the study.

Exclusion Criteria: Those patients taking Insulin/ Oral anti-hyperglycemic agents, steroids and those patients having gestational diabetes mellitus or hemoglobin variants or having any condition other than T2DM which can alter the levels of ADA like pneumonia, typhoid, tuberculosis, infectious mononucleosis, rheumatoid arthritis or any type of malignancies were excluded from the study.

The blood samples were collected under aseptic conditions. Samples for measuring serum ADA were collected in red color vacutainers without anticoagulant. Samples for HbA1C were collected in lavender EDTA coated vacutainers and those for FBS and PPBS in grey colored sodium fluoride coated vacutainers. Green colored heparinized vacutainers were used for collecting samples for estimating lipid profile and hsCRP. HbA1C, FBS and PPBS were analyzed immediately whereas the samples for analyzing other parameters were centrifuged at 3000 RPM for 10 minutes. The separated serum and plasma were stored at -20 degree Celsius till examination.

Serum ADA, Plasma FBS, PPBS and lipid profile were estimated using photometry and hsCRP by immunoturbidimetery in the Cobas 8000 analyzer. HbA1C was analyzed by HPLC method in the BioradD-100 analyzer.

Results

Table1: Comparison of age and gender between the study groups

	T2DM (n=104)	Control (n=55)	p-value
Age	53.07±9.710	36.35±10.827	<0.001
Gender			
Males	65	26	0.065
Females	39	29	

The mean age of T2DM patients were higher than the control group and was statistically significant (p<0.001). The number of males were higher than females in both the groups and was not statistically significant (p=0.065).

Table 2: Comparison of mean and median of Hba1c, lipid profile and hscrp between the study groups.

Parameters	Mean±SD / Median (Q1-Q3)		p-value
	T2DM	Control	
HbA1c	8.2683±1.83495	5.1418±0.26784	<0.001
ADA	15.92±4.29	5.88±1.14	<0.001
High density lipoprotein (HDL)	41.6135±10.89659	56.2164±6.93196	<0.001
Total Cholesterol	168.95 (135.65-212.47)	152 (133-170)	0.005
Low density lipoprotein (LDL)	99.8 (75.85- 142.27)	93 (76- 107)	0.008
Very low density lipoprotein (VLDL)	26.4 (19.1-33.4)	14.1(11.7- 17)	<0.001
Triglycerides (TG)	131.9 (95.82-166.8)	70 (58- 87.5)	<0.001
High sensitive C Reactive protein (hsCRP)	15.95(8.31- 35.24)	2.3(1.5- 2.7)	<0.001

The mean HbA1C levels were higher in T2DM patients than in controls. The mean HDL levels were lower in T2DM patients than in controls. The median Total cholesterol, LDL, VLDL, TG and hs CRP were higher in T2DM patients than in controls.

Table 3: Association of ADA with CAD among T2DM group

ADA	T2DM		p-value
	CAD (n-24)	Non-CAD (n-80)	
<18.5	1 (4.2%)	74 (92.5%)	<0.001
>18.5	23 (95.8%)	6 (7.5%)	

The cut off of ADA was found to be 18.5. ADA levels of >18.5 was observed in 95.8% of T2DM with CAD and 7.5% of T2DM without CAD. ADA levels of <18.5 was observed in 4.2% of T2DM with CAD and 92.5% of T2DM without CAD. These were statistically significant.

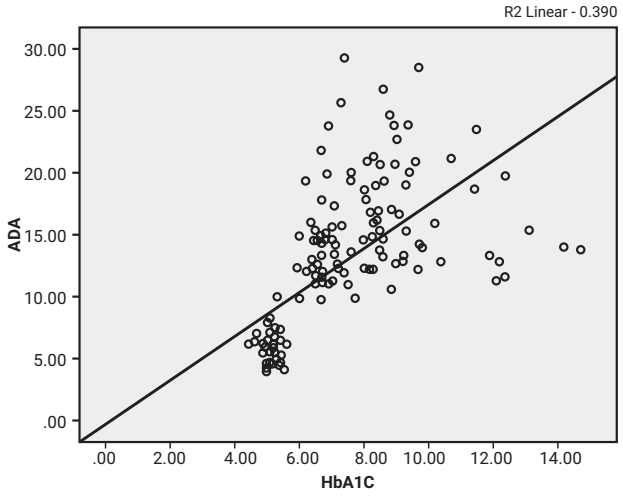

Figure 1: Pearson's Correlation of ADA with HbA1C in T2DM group

A positive intermediate correlation of ADA was observed with HbA1c; r–value of 0.625.

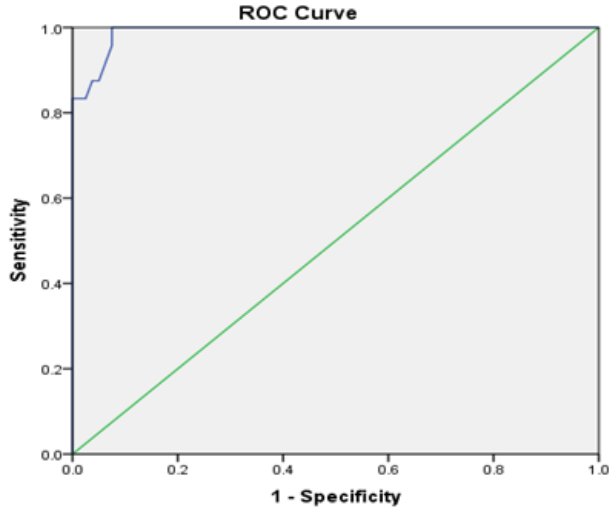

Figure 2: Receiver Operating Characteristic curves (ROC) for ADA with CAD

The area under curve (AUC) was 0.99 with p-value <0.001. The cut-off of ADA with CAD was 18.5 with a sensitivity of 95.8% and specificity of 92.5%.

Discussion

T2DM is a rapidly rising condition due to obesity, insulin resistance, relative insulin deficiency and dysfunction of β-cells which result in hyperglycemia along with chronic complications. The morbidity and mortality are expected to rise in the forthcoming years due to CAD as complication [1].

The major issue in T2DM is the diminished cell-mediated immune response. ADA plays a key role in assessing the inadequacy of cell function [2,10,11] and can be used an immunological marker in diabetes. Elevated ADA will activate the advanced glycation end products (AGE) which in turn stimulates the cytokines, Tumor necrosis factor (TNF) and macrophages resulting in the activation of T-lymphocyte to bind to the AGE receptors. ADA thus causes T-lymphocyte proliferation which results in release of inflammatory mediators by them leading to diabetic complications [12,13].

The current study establishes the importance of ADA in patients with T2DM and the utility of ADA as a prognostic marker in order to prevent the development of chronic complications like CAD in these patients.

There were 159 participants enrolled in our study out of which 104 patients were diagnosed with T2DM and 55 were controls. In our study we observed a statistically significant increase in the age of the patients with T2DM than in controls which shows the dependency of age in insulin secretion and development of insulin resistance. There were totally 65 males and 39 females in the T2DM group and 26 males and 29 females in the control group and no statistically significant difference was observed between gender and T2DM. (Table 1) We observed that the glycemic indices (FBS, PPBS and HbA1c), lipid profile and inflammatory marker hsCRP were significantly increased in T2DM than in controls. (Table 2)

T2DM has association with HbA1c, lipid profile and inflammation as dyslipidemia causes accumulation of free fatty acids and low HDL elevates inflammatory response in adipose tissues. Elevated hsCRP is due to inflammation and deposition of oxidized LDL particles in arterial walls causing atherosclerosis. Similar findings were observed in studies done by Khan HA et al, Omodanisi EI et al and Otter W et al [14,15,16]

The serum ADA was significantly elevated in T2DM compared to controls. (Table 2) Rise in ADA could be due to depletion of adenosine which thereby causes imbalance in surface protein expression and cell-mediated dysfunction. Studies done by Singh S et al and Niraula A et al also reported similar observations [17,18].

A positive correlation was observed between ADA and HbA1c in T2DM patients with a r-value of 0.625 which shows that HbA1c is directly proportional to the serum ADA levels [17]. Studies done by Singh S et al also show similar results. (Figure 1)

The Area under Curve (AUC) for ADA with CAD is 0.99 which is statistically significant with a p-value of <0.001. The specificity and sensitivity of ADA in diagnosis of CAD were 95.8% and 92.5% respectively with a cut-off value of 18.5. (Table 3) (Figure 2) Though a study done by Xuan C et al had observed that the levels of ADA are high in patients with T2DM with CAD [19], they had not checked the sensitivity and specificity of the same. A cut-off value for the same was also not established.

Thus, from our study we conclude that ADA can be considered as a prognostic marker for the development of CAD in T2DM patients.

Conclusions

We have observed in our study that ADA levels are elevated in patients within one year of detecting T2DM. In addition, we are able to conclude that ADA levels are significantly higher in diabetic patients who developed CAD during the time course of the disease. Thus our study proves that serum ADA is a promising biomarker in terms of sensitivity and specificity in evaluating the prognosis of CAD in T2DM. Further prospective studies in more number of populations involving categorization of ADA could help in identifying different stages of CAD.

Recommendations

ADA serves as a sensitive and specific marker for predicting development of CAD in patients with T2DM. Routine measurement of ADA along with other glycemic indices in T2DM patients could lead to development of new diagnostic and therapeutic strategies for the management of T2DM complications.

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