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Original Article

Internal Medicine Section

Adenosine Deaminase- Relation with Diabetes and its Complication

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ABSTRACT

Introduction: Adenosine Deaminase (ADA), a purine metabolic enzyme is suggested to contribute significantly to the modulation of bioactivity of insulin but its relation with diabetes is not yet fully understood.

Aim: To assess the relation of ADA with diabetes and its complications.

Materials and Methods: This study included a total of 100 diabetic patients and 100 well matched healthy non-diabetic as control subjects. Serum ADA levels of patients with Diabetes Mellitus (DM) as diagnosed by current American Diabetic Association (ADA) criteria were compared with well-matched healthy non-diabetic patients, further ADA levels were compared with micro and macrovascular complications of diabetes. The recorded data was exported to Statistical Package for the Social Sciences (SPSS) version 23.0 after being compiled in Microsoft excel.

Results: The mean age of diabetic patients was 57.7±9.49 and 54.9±11.29 years for controls. Significant difference in ADA in diabetic and non-diabetics with mean ADA of 38.7±16.02 and 28.9±6.39 IU/L respectively with a p-value of <0.001 was noted. In the study group, the duration of diabetes was ≥ 10 years in 51%. Microvascular complication like retinopathy was seen in 65%, nephropathy in 31% and neuropathy in 52% cases. Macrovascular complications like Peripheral Vascular Disease (PVD) in 5%, Coronary Artery Disease (CAD) in 11% and Cerebrovascular Disease (CVD) in 9% cases. HbA1c <7% was seen in 8%, 7-10 in 44% and >10 in 48% cases. Significant correlation was observed between serum ADA and microvascular complication and duration of diabetes.

Conclusion: Serum ADA can be an addition marker of diabetes and has significant correlation with its various complications.

Keywords: Diabetes mellitus, Macrovascular complications, Microvascular complication

INTRODUCTION

Globally, an estimated 9.3% (463 million) people were living with diabetes in 2019 and this is estimated to rise to 10.2% (578 million) people by 2030. Additionally prevalence was found to be higher in urban areas (10.8%) than rural (7.2%) areas [1]. Type 2 diabetes makes up about 85-90% of all cases [2]. Overall, increases in the diabetes prevalence rates are mainly due to surge in risk factors for type 2 DM, especially increased longevity and obesity [3]. DM is characterised by several immunological disturbances. These immunological disturbances are usually associated with cell-mediated immune responses and abnormal T-cell lymphocyte function which is further linked to insulin defect. ADA has been suggested to be a marker of altered immunity and marker of hyperglycaemia in diabetes [4].

ADA is a major catalyst in purine metabolism. It acts by irreversibly catalysing hydrolytic deamination of adenosine to inosine and 2-deoxyadenosine to 2-deoxyinosine, respectively. These two products are further converted to hypoxanthine, xanthine and uric acid [5]. Although widely distributed in body tissues, highest ADA activity is seen in T-lymphocytes where it plays a major role both in their differentiation and proliferation and is regarded as an excellent marker of cell mediated immune response. Elevated lymphocytic ADA activity is well documented in various pathological processes which are accompanied with altered cell mediated immune response [6]. Significant reduction in elevated ADA levels has been documented by insulin administration [7]. The half-life of serum ADA is about 30 minutes. In multiple tissues, ADA has been seen modulating. It decreases free fatty acid levels by its potent anti-lipolytic property and increases insulin sensitivity in adipose tissue. Adenosine is found to have an anti-inflammatory effect, thus it is suggested that ADA may regulate the inflammatory response [8]. Severity of inflammation has been proven to have a direct relation with expression and activity of ADA [9].

To study the ADA levels in diabetic and compare it with ADA in non-diabetic population and to find any correlation of ADA with diabetic complications.

MATERIALS AND METHODS

This was a hospital-based case control study which was conducted over a period of two years from December 2017 to November 2019, at Sher-I-Kashmir Institute of Medical Sciences, Soura, Jammu and Kashmir, India. This study included a total of 200 participants. One hundred diabetic patients were enrolled as case cohort and 100 non-diabetic were enrolled as controls. The acceptable sample size with confidence interval of =95% and margin of error 10% was 96. The study protocol was reviewed and cleared by the Institutional Ethics Committee (IEC) and an informed consent was obtained from patients/relatives for utilisation of data for research purposes (Ref no. IEC/SKIMSMC protocol104/2017).

Inclusion Criteria

For cases

The patients diagnosed with type 2 diabetes with HbA1c ≥6.5, Fasting blood sugar ≥126 mg/dL or random blood sugar ≥200mg/dl as per ADA were taken up for study [10]. Type 2 DM patients with or without any complications (macrovascular or microvascular complication) were included.

For control group

The age and sex matched control subjects were taken from general population (Hospital staff and consenting Attendants from other specialties) with no history of DM and normal blood sugars as documented by normal fasting/random blood sugar levels and normal HbA1c.

Exclusion Criteria

For case and control group

Participants with Tuberculosis, Hepatitis, Chronic liver disease, Rheumatoid arthritis, Sarcoidosis, Gout, Chronic kidney disease, Malignancy and patients taking supplements like Antioxidants, Vitamins and Minerals were excluded from the study as these condition are known to be associated with increase in ADA levels.

The clinical evaluation and investigations were done in the all patients. This included, detailed history and clinical examination, baseline investigations including Complete Blood Count (CBC), Renal Function Test (RFT), Liver Function Test (LFT), Blood sugar levels (fasting and random), Blood HbA1c levels assessment were performed in all patients at one particular laboratory, so as to standardise the test value and normal range. The method used was High Performance liquid chromatography (using BIORAD machine), Lipid profile and Estimation of serum ADA levels.

Method of Estimation of Serum ADA

The method used for quantitative determination of ADA concentration was Colourimetric method. This is based on the principle that ADA hydrolyses adenosine to ammonia and inosine. In alkaline medium with sodium nitropruside acting as a catalyst, ammonia reacts with phenol and hypocholorite to form blue indophenol complex. The amount of ADA present is directly related to the intensity of blue coloured indophenol complex which is quantified with the help of colourimeter.

Reference values [11]:

Normal < 30 U/L

Suspect 30 U/L to 40 U/L

Positive (High) > 40 U/L to 60 U/L

Strong Positive > 60 U/L

STATISTICAL ANALYSIS

The recorded data was exported to SPSS version 23.0 after being compiled in Microsoft excel. Continuous variables were expressed as Mean±SD and categorical variables were expressed as frequencies and percentages. Student's independent t-test was employed for comparing continuous variables (ADA). To determine the correlation of serum ADA levels with age, BMI, duration of diabetes and HbA1c, Karl Pearson's correlation coefficient was applied. A p-value of <0.05 was considered statistically significant and all p-values were two tailed.

RESULTS

The case and control population were well matched in age, sex, and residence with no significant statistical difference between the two groups. Significant statistical difference in Body Mass Index (BMI) was observed between the two groups as depicted in [Table/Fig-1]. There was a statistically significant difference in serum ADA levels in case and control population group as shown in [Table/Fig-2]. The mean ADA±SD in males was 40.3±15.46 and in females was 37.2±16.54 in the case cohort. There was no significant correlation of gender with serum ADA levels (p-value=0.332). The clinical parameters of the diabetic cohort of the study have been given in the [Table/Fig-3]. About half of the patient population had diabetes for more than 10 years. Hypertension was the most common associated comorbidity and significant number of patients had associated microvascular complication.

Distribution of HbA1c in the diabetic cohort is shown in [Table/Fig-4]. Nearly half of patients had severely uncontrolled diabetes with HbA1c above 10%. Only 8% of study population had levels below 7%. Fundus examination finding are also shown in [Table/Fig-4].

There was a positive co-relation between microvascular complication of diabetes and serum ADA level. No significant correlation was noted between macrovascular complications and Serum ADA levels except for Cerebrovascular Disease (CVD) as shown in [Table/Fig-5].

		Case (total 100)	Control (total 100)	
		n	n	p-value
Sex	Male	52	63	0.116
	Female	48	37	
Age (years)	<40	2	5	0.059
	40-49	17	33	
	50-59	30	34	
	60-69	36	16	
	≥70	15	12	
	Mean±SD	57.7±9.49	54.9±11.29	
Residence	Rural	59	64	0.467
	Urban	41	36	
BMI (kg/m²)	<18.4	0	0	0.009*
	18.4-24.9	32	40	
	25-29.9	56	59	
	30-34.9	12	1	
	Mean±SD	26.5±2.79	25.4±1.69	

[Table/Fig-1]: Demographic distribution of case and control population.
*Statistically Significant Difference (p-value<0.05)

		Case (total 100)	Control (total 100)	p-value
		n	n	
Serum ADA (IU/L)	<40	35	95	<0.001*
	≥40	65	5	
	Mean±SD	38.7±16.02	28.9±6.39	

[Table/Fig-2]: Serum ADA levels in case and control population.
*Statistically significant difference (p-value<0.05)

Parameters			n
Duration of diabetes (years)		<1	2
		1-5	20
		5-10	27
		≥ 10	51
		Mean±SD=9.5±6.38	
Comorbidities/other risk factors		Smoking	58
		Hypothyroidism	49
		Hypertension	61
		COPD	8
Complications	Microvascular	Retinopathy	65
		Nephropathy	31
		Neuropathy	52
	Macrovascular	PVD	5
		CAD	11
		CVD	9
		Multiple	7
	Others	Gastroparesis	3
		Hearing Loss	1
		Multiple	6
Drugs		Metformin	1
		Insulin	48
		OHA	36
		Both Insulin & OHA	15
24 hr urinary protein		Normal	75
		Subnephrotic proteinuria	21
		Nephrotic proteinuria	4

[Table/Fig-3]: Clinical parameters of the total 100 patients of case cohort.
COPD: Chronic obstructive pulmonary disease; PVD: Peripheral vascular disease; CAD: Coronary artery diseases; CVD: Cerebrovascular disease; OHA-Oral hypoglycaemic agents; Subnephrotic proteinuria: 300 to 3500 mg/day; Nephrotic proteinuria: >3500 mg/day

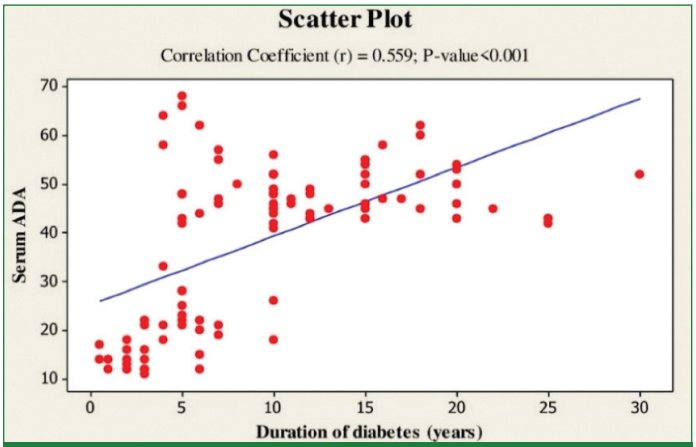
		n
HbA1c (%)	<7	8
	7-10	44
	>10	48
	Mean±SD=10.38±2.62	
Fundus examination	Normal	35
	NPDR	59
	PDR	6

[Table/Fig-4]: Distribution of HbA1c and retinopathy in the total 100 patients of case cohort.
NDPR: Non proliferative diabetic retinopathy; PDR: Proliferative diabetic retinopathy

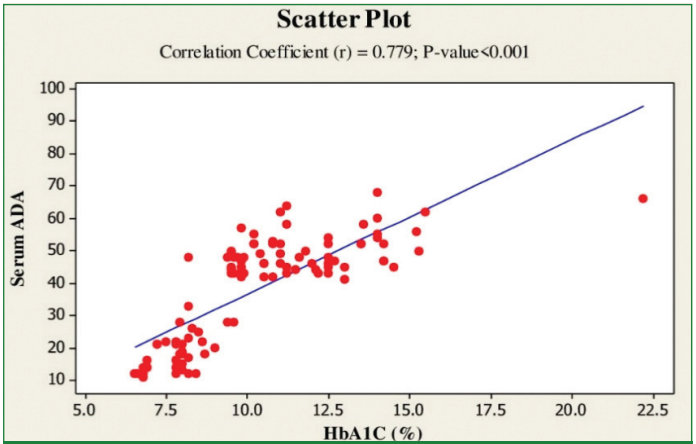
Microvascular complication		ADA normal (Total 35) (n%)	ADA high (Total 65) (n%)	p-value*
Retinopathy	Present	1 (2.86)	64 (98.46)	<0.001
Nephropathy	Present	2 (5.72)	29 (44.62)	<0.001
Neuropathy	Present	0 (0)	52 (80)	<0.001
Macrovascular complications				
PVD	Present	0 (0)	5 (7.69)	0.159
CAD	Present	1 (2.86)	10 (15.38)	0.091
CVD	Present	0 (0)	9 (13.84)	0.025

[Table/Fig-5]: Correlation between Micro- and Macro-vascular complications of T2DM with ADA.
Statistically significant difference (p-value<0.05); PVD: Peripheral vascular disease; CAD: Coronary artery diseases; CVD: Cerebrovascular disease

This study also showed that there was a positive correlation between the serum ADA levels and age of cases using Karl Pearsons coefficient (r=0.461) (p<0.001), between serum ADA levels and BMI of cases (r=0.263) (p=0.008). Significant co-relation was found between serum ADA and duration of diabetes (scatterplot, [Table/Fig-6]). Significant co-relation was also found between serum ADA and HbA1c level (scatter plot, [Table/Fig-7]).



[Table/Fig-6]: Scatter plot showing positive correlation between the serum ADA levels and the duration of diabetes in cases.



[Table/Fig-7]: Scatter plot showing positive correlation between the serum ADA levels and the HbA1c % of the cases.

DISCUSSION

The present study was a hospital based prospective case-control study conducted at the centre over a period of two years. Hundred Type 2 DM patients diagnosed as per ADA criteria were enrolled. Hundred healthy, well matched in age and sex control subjects were taken from the general population. There was a statistically significant difference in the BMI of cases versus controls (p=0.009). Most of the patients (51%) in this study had more than 10 years duration of illness and the mean duration of illness was 9.5±6.38 years. Hypertension was the most common co-morbidity present in 61% of the cases. In this study, it was observed that the mean±SD serum ADA levels in the diabetic cases 38.7±16.02, which was higher than that in the control group, 28.9±6.39 and it was statistically significant (p<0.001).

Similar results were obtained in other studies [12-20]. All these studies showed higher levels of ADA in the case cohort as compared to the control group. Additionally, most of these studies have comparably similar duration of diabetes in the study cohort. This study was consistent with the above studies, strengthening the fact that serum ADA levels can also be used to predict the glycaemic control in diabetes. The high ADA activity in the current study could be due to altered T-lymphocyte responses or proliferation; which may suggest a mechanism of releasing ADA into circulation. Insulin plays a classical role in the T-cell intermediary metabolism. It is believed that insulin enhances lymphocyte function, differentiation and proliferation and maintains the activated state of the T-lymphocytes by increasing the energy expenditure and augment the protein synthesis necessary for proper lymphocyte functioning [12]. Altered insulin action may lead to delayed responsiveness of T lymphocytes to antigens, thus high ADA activity in type 2 DM might be due to altered insulin related T-lymphocyte function. Cellular studies have shown that severe hyperglycaemia reduces the function of immune cells and increases inflammation and also insulin may modulate its action on glucose metabolism in the tissues by changing the activity of ADA in type 2 diabetic patients [21]. This study found that there was no significant correlation of gender with ADA levels (p=0.332). This is in alliance with the study conducted by Ramani NS et al., and Vasuda KC et al., [4,22].

In this study, out of 100 diabetic subjects, 65 (65%) had retinopathy and out of those 65, 64 (98.5%) had higher serum ADA levels as compared to controls. It was found that there was positive correlation (p<0.001) of diabetic retinopathy with serum ADA levels. Most of cases in the current study had higher ADA levels and poor glycaemic control as reflected by HbA1c% that explains higher prevalence of diabetic retinopathy. Serum ADA was high in 44.6% of the diabetic nephropathy patients as compared to the normal subjects and there was a positive correlation (p<0.001) of nephropathy with serum ADA levels. This study was possibly the first to evaluate the relation of micro and microvascular complications with serum ADA levels. In this study, among the cases that had high serum ADA levels, 80% had diabetic neuropathy while as among the cases with normal serum ADA levels, none of the subjects had neuropathy. This study found that there was a statistically significant correlation of neuropathy with serum ADA levels (p<0.001). Despite chronic hyperglycaemia appearing to be the main factor for development of neuropathy, multiple other disturbances were found in the microvasculature of nerves of patients with diabetes [23]. These contributing factors included insulin resistance, diabetic dyslipidemia and hypertension. In this study, most of the cases had poor glycaemic control (mean±SD HbA1c% =10.38±2.62), diabetic dyslipidemia and associated hypertension (61% of the cases) which can partly explain higher prevalence of neuropathy in this study.

Among macrovascular complications, the statistically significant correlation (p=0.025) was found between CVD and high ADA levels. Among the diabetic cases who had high serum ADA levels, 13.8% had CVD while as in cases with normal serum ADA levels; none had CVD. There was no significant correlation between serum

ADA levels and other macrovascular complications like Peripheral Vascular Disease (PVD) and CAD. It was found in study patients that there was a significant correlation of serum ADA levels with age (r=0.461; p<0.001), with BMI (r=0.263; p=0.008), and with duration of diabetes (r=0.559; p<0.001). This was in contrary to Sapkota LB et al., who observed that there was no significant correlation of serum ADA levels in study patients with age (r=0.148; p=0.189), with BMI (r=0.164; p=0.145) and with duration of diabetes (r=0.060; p=0.499) [24], these differences may be attributed to smaller study population, different ethnic population and an overall lower BMI in the later study. In the case cohort, the mean±SD of HbA1c % of the cases was equal to 10.38±2.62. There was a strong positive correlation (r=0.779, p<0.001) between the serum ADA levels and the HbA1c % of the cases. Analysis was done by Pearson's correlation test and p-value was <0.05. Similar results were reported in the study conducted by Pinnelli VB et al., implying a role of ADA in diabetic control [25]. Additionally, Ramani NS et al., found a positive correlation (r=0.290, p<0.05) between serum ADA (mean±SD = 32.06±17.09) and HbA1c (mean±SD = 8.38±4.00) [4]. Furthermore, lowering or normalisation of HbA1c level was associated with decreased ADA activity. This is in consistent with the previous studies of Kurtul N et al., and Erbagci AB et al., [13,26].

Limitation(s)

The main limitation of this study was that it was a single centre study. A multicentre study may be required to better validate the results.

CONCLUSION(S)

The study showed that there was a significant positive correlation between increased serum ADA in diabetes patients compared to individuals with normal blood sugars. It is clear from the results of the present study that increased ADA levels are associated with poor glycaemic control in diabetic patients and also associated with complications of type 2 DM like retinopathy, nephropathy, neuropathy, CVD. The major concern put forth by this study is whether to estimate serum ADA routinely in all Type 2 diabetes patients and whether to set a cut-off value of serum ADA for good glycaemic control. Although strongly favouring these finding there is need of further research in this field.

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