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Correlation study of adenosine deaminase and its isoenzymes in type 2 diabetes mellitus

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ABSTRACT

Objective: Adenosine deaminase (ADA) plays an important role in cell-mediated immunity and modulation of insulin activity. Its clinical and diagnostic significance in Nepalese type 2 diabetes is not yet characterized. So, this study's objective was to determine the isoenzymatic activities of ADA (ADA1, ADA2, and total ADA) and show its correlation with demographic, anthropometric, and biochemical characteristics of type 2 Nepalese subjects with diabetes.

Research design and methods: This is a hospital-based cross-sectional study including 80 type 2 diabetes mellitus (DM) patients and same number of age-matched and sex-matched healthy controls. Data were collected using preformed set of questionnaires and biochemical data were obtained from the laboratory analysis of the patient's blood samples. Statistical analysis was performed with SPSS V.20.

Results: A significantly higher ($p<0.001$) mean values of body mass index (BMI), fasting blood sugar (FBS), postprandial blood sugar (PPBS), glycated hemoglobin (HbA1c), and lipid profiles except high-density lipoprotein cholesterol (HDL-C) were found in type 2 diabetic cases compared with controls. Serum ADA activities were significantly higher in cases compared with controls ($p<0.001$) showing significant positive correlation ($p<0.05$) with FBS, PPBS, HbA1c, and alcoholism; while no correlation was found with age, sex, ethnicity, BMI, waist–hip ratio, dietary habits, smoking, and duration of diabetes.

Conclusions: Serum ADA activities were significantly higher in type 2 diabetic patients compared with controls having significant positive correlation with glycemic parameters. Serum ADA and its isoenzymes could be used as biomarkers for assessing glycemic status in patients with type 2 DM.

Significance of this study

What is already known about this subject?

- Type 2 diabetes mellitus (DM) is characterized by immunological disturbances and inappropriate T-lymphocyte function. Adenosine deaminase (ADA), an enzyme of purine metabolism is considered as a good marker of cell-mediated immune response.
- ADA and its isoenzymatic activities are increased in many conditions which are associated with alteration of cell-mediated immune responses including type 2 DM.

What are the new findings?

- ADA and its isoenzymes are elevated in sera of type 2 diabetic patients and show strong positive correlation with glycemic parameters (fasting blood sugar (FBS), postprandial blood sugar (PPBS), glycated hemoglobin (HbA1c)). The correlation was highest with ADA2 followed by total ADA and ADA1.
- This study highlights the diagnostic potential of ADA2 isoenzyme over ADA1 and total ADA measurement in assessing glycemic status of type 2 diabetes.

How might these results change the focus of research or clinical practice?

- This study demonstrates that serum ADA and its isoenzymatic activities are predominantly elevated in type 2 DM.
- Serum ADA and its isoenzymes could be used as biomarkers for assessing glycemic status and immunological origin of type 2 DM.

INTRODUCTION

Type 2 diabetes mellitus (DM) is a chronic metabolic disorder with its prevalence steadily increasing worldwide. Hyperglycemia in type 2 DM occurs due to peripheral insulin resistance, declining β-cell function which eventually leads to β-cell failure.[1 2] It is a heterogenous group of metabolic disorders characterized by several immunological disturbances. These immunological disturbances have an association with cell-mediated immune responses;[3] and abnormal T-lymphocyte function, which is further linked with insulin defect.[4] Distinct genetic and metabolic imperfections in insulin action and/or secretion give rise to the basic phenotype of hyperglycemia in type 2 DM.[5] Hyperglycemia defines the disease and is the cause of its most characteristic symptoms and long-term complications. Assessment and monitoring of glycemia is an important aspect of diabetic care.[6] The quest for new markers in diabetes is

increasing day by day but not a single marker has surpassed the use of glycated hemoglobin (HbA1c) in assessing glycemic control until this date.

Adenosine deaminase (ADA) is an enzyme of purine metabolism catalyzing irreversible deamination of adenosine to inosine, and deoxyadenosine to deoxyinosine, respectively.[7] ADA has two major isoenzymes ADA1 and ADA2. ADA is widely distributed in human tissues with its highest activity in T-lymphocytes. It is considered as a good marker of cell-mediated immune response.[8] High lymphocytic ADA activities were reported in many diseases which are associated with alteration in cell-mediated immune responses.[9] Adenosine on the other hand has been proven to modulate insulin action in various tissues.[10] It reduces free fatty acid level by its potent antilipolytic property and improves insulin sensitivity in adipose tissue.

As ADA is associated with T-lymphocyte activity, its altered blood levels may help in predicting immunological dysfunction associated with type 2 DM. Many studies have reported increased activity of ADA in type 2 diabetic patients compared with healthy controls.[9–11] To the best of our knowledge, there is no report regarding the activity of ADA and its isoenzymes ADA1 and ADA2 in Nepalese subjects with diabetes. The present study aims to determine the activity of serum total ADA and its isoenzymes ADA1 and ADA2; and correlate these parameters with demographic, anthropometric, and biochemical characteristics of Nepalese type 2 diabetic individuals.

RESEARCH DESIGN AND METHODS

This is a hospital-based cross-sectional study conducted in the Department of Biochemistry, Manipal Teaching Hospital (MTH), Pokhara, during April to September 2015.

Study population

A total number of 80 patients with type 2 DM aged between 35 and 70 years attending MTH for their routine medical checkup were included in this study. Same number of age and sex-matched healthy individuals with no history of DM was used as controls. Staffs working in various departments of MTH and those patients who visited specimen collection center of MTH from different regions of Pokhara valley for screening DM were recruited as controls. WHO criteria were used for the diagnosis of subjects with type 2 DM.[12] Patients with chronic complications of diabetes, prolonged use of medicines other than those used for the treatment of DM, addictive habits, pregnancy, and other systemic illnesses like chronic liver disease, chronic kidney disease, tuberculosis, rheumatoid arthritis, systemic lupus erythematosus, infectious mononucleosis, Behcets disease, and any malignancies were excluded from the study. All data were collected from personal interviews using a preformed set of questionnaires.

Sample collection

In total, 5 mL of the venous blood in fasting state was collected with the help of a sterile 5 mL syringe from the antecubital vein of each of the consenting subjects and kept in fluoridated phial, EDTA vacutainer, and plain test tube as per the need of the tests.

Biochemical analysis

Fasting blood sugar (FBS) was measured in blood collected in fluoride-oxalate vials by glucose oxidase-peroxidase (GOD-POD) method.[13] HbA1c was estimated by Nycocard Reader.[14] Blood collected in plain test tube was allowed to clot at room temperature and the serum was carefully separated. Serum lipids (triglyceride (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C)) were directly measured and the value of low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald's formula.[15] The value of very low-density lipoprotein cholesterol VLDL-C was calculated as one-fifth of the concentration of TG. All these parameters were analyzed using a semiautomated chemistry analyzer (HumaLyzer-3500) and ready-to-use reagent kits according to the manufacturer's instructions (Human Diagnostics, Germany). Activity of serum total and isoenzyme forms of ADA was measured using adenosine substrate based on the colorimetric method described by Giusti and Galanti.[16] The absorbance (OD) of blue-colored complex formed at the end of reaction was measured using semiautomated chemistry analyzer at 620 nm. The ADA1 isoenzyme inhibitor erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) was used for the measurement of ADA2 while the difference between total ADA and ADA2 gave ADA1 activity.

Ethical issues

The study was approved by the institutional ethical committee and informed consent was obtained from all the patients. Social Research Association (SRA) 2003 ethical guidelines were followed during this study.

Data analysis

The obtained data were analyzed using SPSS V.20. Comparison of mean values between controls and cases were performed using student's t-test. p Value <0.05 was considered to be statistically significant.

RESULTS

Table 1 shows the baseline characteristics of the study subjects. We found no significant difference between the mean age (p=0.914) and waist–hip ratio (WHR) (p=0.348) between the type 2 diabetic cases and controls. In contrast significant difference was seen between the body mass index (BMI), systolic blood pressure (SBP), and diastolic blood pressure (DBP) between controls and cases with p value <0.001.

Table 2 shows that all the biochemical parameters (FBS, postprandial blood sugar (PPBS), HbA1c, TG, TC,

Table 1 Baseline characteristics of the study subjects

Parameters	Controls			Cases			p Value
	Male	Female	Total	Male	Female	Total	
Age	51.9±9.8	52.7±9.7	52.3±9.7	51.8±9.0	52.55±9.6	52.3±9.2	0.914
BMI (kg/m^2)	24.2±1.2	23.5±2.7	23.9±2.1	25.9±3.0	25.87±3.7	25.9±3.3	<0.001
WHR	0.91±0.04	0.90±0.05	0.91±0.05	0.92±0.07	0.92±0.06	0.92±0.07	0.348
SBP (mm Hg)	129.2±6.7	125.9±9.3	127.6±8.2	135.0±8.5	136.1±6.4	135.5±7.6	<0.001
DBP (mm Hg)	83.6±4.8	82.7±6.07	83.2±5.4	86.0±6.6	88.79±4.8	87.3±6.0	<0.001

p<0.05 is significant.
BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; WHR, waist-hip ratio.

LDL-C, VLDL-C, ADA1, ADA2, and total ADA) except HDL-C were significantly higher in diabetic cases compared with their healthy counterparts (p<0.001). Some biochemical parameters like TC, TG, and LDL even exhibit significant sex-specific variations within controls and cases (p<0.05).

Table 3 shows the comparison of serum ADA activities between non-obese and obese type 2 subjects with diabetes. We classified subjects with type 2 diabetes into obese and non-obese based on BMI. Individuals with BMI<25 kg/m^2 were classified as non-obese and those with BMI≥25 kg/m^2 were classified as being obese. Serum ADA levels were slightly higher in obese compared with non-obese subjects with diabetes but the difference was not statistically significant.

Tables 4 and 5 show the correlation analysis of ADA activities with the baseline characteristics and the biochemical parameters of the study subjects, respectively. Pearson's correlation analysis was performed because the variables were normally distributed with no outliers. Serum ADA activity did not show any significant correlation with age, BMI, WHR, ethnicity, residence, occupation, dietary habits, smoking, and duration of DM. In contrast, significant positive correlation was seen between the serum ADA and its isoenzymatic levels with glycemic parameters.

DISCUSSION

In the present study, we observed significantly increased serum ADA and its isoenzymatic activities in type 2 diabetic patients compared with their healthy counterparts. These findings in our study are supported by many other studies performed before. Kurtul *et al*[11] had shown increased level of serum ADA in patients with type 2 DM and suggested the role of ADA in modulating the bioactivity of insulin. Hoshino *et al*[17] also reported significantly elevated ADA1 and ADA2 levels in type 1 and type 2 diabetic patients compared with their controls. Gitanjali *et al*[18] reported elevated level of serum ADA activity in type 2 diabetic patients and correlated it with markers of lipid peroxidation. Shivaprakash *et al*[9] observed significantly increased ADA activity in subjects with diabetes and hypothesized that increased ADA activity may be due to altered immunity.

Very few studies had been conducted so far which have estimated isoenzymes of ADA (ADA1 and ADA2) in type 2 DM and shown their relationship with the

Table 2 Biochemical characteristics of study subjects

Parameters	Controls			Cases			p Value
	Male	Female	Total	Male	Female	Total	
FBS (mg/dL)	99.2±7.9	95.9±10.1	97.6±9.1	141.7±43.2	138.8±36.4	140.4±39.9	<0.001
PPBS(mg/dL)	117.5±14.7	119.5±19.9	118.5±17.4	219.4±57.2	219.2±63.0	219.4±59.6	<0.001
HbA1c (%)	5.6±0.4	5.4±0.5	5.6±0.5	7.37±1.2	7.3±1.0	7.3±1.1	<0.001
TC (mg/dL)	181.3±29.3	173.9±26.8	177.7±28.2	214.1±28.5	197.0±19.7	206.0±26.0*	<0.001
TG (mg/dL)	142.8±40.8	127.2±26.7	135.7±35.4*	188.9±53.6	174.1±31.9	181.9±45.0	<0.001
HDL-C (mg/dL)	48.9±6.5	52.5±10.3	50.6±8.7	46.57±6.6	45.63±4.8	46.1±5.8	<0.001
LDL-C (mg/dL)	102.4±23.4	96.6±24.1	99.6±23.8	129.4±27.7	116.6±19.1	123.3±24.7*	<0.001
VLDL-C (mg/dl)	28.6±8.0	26.3±6.2	27.5±7.3	38.0±10.7	34.73±6.3	36.5±9.0	<0.001
ADA1 (U/L)	8.0 ±2.24	8.62±2.15	8.3±2.21	14.19±4.89	14.12±4.63	14.16 ±4.74	<0.001
ADA2 (U/L)	12.29±2.23	12.11±2.13	12.2 ±2.17	21.9±4.42	20.95±4.79	21.48 ±4.60	<0.001
Total ADA	20.2±3.0	20.68±3.51	20.46 ±3.27	36.3±7.37	34.63±7.7	35.55 ±7.53	<0.001

p<0.05 is significant.
*Significant difference between male and female within controls and case groups, at the level of 0.05.
ADA, adenosine deaminase; FBS, fasting blood sugar; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PPBS, postprandial blood sugar; TC, total cholesterol; TG, triglyceride; VLDL-C, very low-density lipoprotein cholesterol.

Table 3 Comparison of ADA activities among obese and non-obese subjects with diabetes

	Non-obese type 2 DM (n=47)	Obese type 2 DM (n=33)	p Value
Serum ADA-1 level (U/L)	13.68±4.09	14.66±5.12	0.197
Serum ADA-2 level (U/L)	20.97±4.79	21.98±4.44	0.236
Serum total ADA level (U/L)	34.65±7.58	36.64±7.42	0.174

p<0.05 is significant.
ADA, adenosine deaminase; DM, diabetes mellitus.

glycemic status. Raised serum ADA1 isoenzyme can be attributed either to extensive cellular necrosis or to increased turnover of lymphoid cells because it is intracellular in location.[19] ADA2 is found only in monocytes and macrophages. ADA2 stimulates proliferation of CD4 cells, induces differentiation of monocytes into macrophages and is released during infections and chronic inflammation.[19 20] Expression and activity of ADA both have been directly correlated with the severity of inflammation.[21] Type 2 DM is characterized by chronic hyperglycemia and low-grade systemic inflammation. These factors might be responsible for predominantly increased serum ADA2 activity compared with ADA1 in type 2 diabetic individuals observed in this study.

Various demographic, anthropometric, and biochemical parameters were obtained to establish their correlation with serum ADA and its isoenzymes. Comparison of baseline characteristics between controls and case groups showed significant difference with respect to BMI and blood pressure (SBP & DBP) (p<0.001). A significant difference in the glycemic status (FBS, PPBS, HbA1c) and lipid profile of the cases and control groups was also seen in this study (p<0.001). We also substratified diabetic cases into obese and non-obese subjects and compared ADA and its isoenzymatic activities. We found no significant difference in ADA activities between obese and non-obese subjects with diabetes though ADA levels were slightly higher in obese subjects with diabetes. Our findings are concomitant with Khemka *et al*[22] who had shown raised serum ADA, TG, FBS in non-obese type 2 DM patients.

No significant correlation was seen between the activity of ADA and its isoenzymes with respect to demographic and anthropometric characteristics of controls and case groups except for alcoholism. These observations suggest that ADA and its isoenzyme activities are not subject to change with respect to age, sex, and other anthropometric characteristics of the study subjects. Significant correlation between alcoholism and serum ADA activities seen in diabetic groups might be related to alcohol induced inflammation of liver.[23]

A strong and positive correlation was also seen between serum ADA activities with FBS, PPBS, and HbA1c concentration in type 2 DM patients. These findings are in agreement with Nisha *et al*[24] who also found significant and positive correlation between ADA activity and FBS, PPBS, and HbA1c. Warrier *et al*[10] had shown

Table 4 Correlation of serum ADA activity with baseline characteristics

		Total ADA activity		ADA1 activity		ADA2 activity	
Correlation between		Controls	Cases	Controls	Cases	Controls	Cases
Age	r Value	0.198	0.148	−0.164	0.130	−0.129	0.156
	p Value	0.312	0.189	0.147	0.252	0.253	0.168
BMI	r Value	0.125	0.164	0.172	0.202	0.007	0.079
	p Value	0.271	0.145	0.127	0.072	0.953	0.489
WHR	r Value	0.008	−0.159	0.072	−0.088	−0.079	−0.033
	p Value	0.997	0.160	0.525	0.436	0.489	0.773
Ethnicity	r Value	0.119	0.055	0.159	0.014	0.038	0.003
	p Value	0.294	0.629	0.162	0.901	0.736	0.981
Residence	r Value	0.019	−0.017	0.112	0.037	−0.096	0.099
	p Value	0.870	0.879	0.323	0.743	0.399	0.396
Occupation	r Value	−0.003	0.066	−0.028	−0.108	0.023	0.110
	p Value	0.977	0.563	0.806	0.340	0.838	0.330
Dietary habits	r Value	0.125	0.044	0.255	0.005	0.074	0.053
	p Value	0.271	0.695	0.023	0.862	0.512	0.637
Smoking	r Value	0.027	0.219	0.140	0.119	−0.099	0.232
	p Value	0.813	0.104	0.216	0.292	0.384	0.039
Alcohol	r Value	0.068	0.227*	0.135	0.243*	−0.154	0.223*
	p Value	0.551	0.047	0.030	0.232	0.173	0.046
Duration of DM	r Value	NA	0.060	NA	0.091	NA	0.105
	p Value		0.499		0.421		0.352

*Correlation is significant at the 0.05 level (two tailed).
ADA, adenosine deaminase; BMI, body mass index; DM, diabetes mellitus; WHR, waist-hip ratio.

Table 5 Correlation of serum total ADA, ADA1, and ADA2 activity with various biochemical parameters

Correlation between		Total ADA activity		ADA1 activity		ADA2 activity	
		Controls	Cases	Controls	Cases	Controls	Cases
FBS	r Value	0.598*	0.689*	0.413*	0.453*	0.416*	0.671*
	p Value	0.007	0.000	0.03	0.006	0.009	0.000
PPBS	r Value	0.460†	0.595*	0.458*	0.476*	0.521†	0.583*
	p Value	0.02	0.000	0.042	0.001	0.049	0.000
HbA1c	r Value	0.343†	0.431*	0.319†	0.365†	0.465*	0.491*
	p Value	0.003	0.000	0.01	0.018	0.003	0.000
TC	r Value	0.044	0.022	0.061	0.023	0.012	0.065
	p Value	0.697	0.849	0.592	0.842	0.916	0.565
TG	r Value	0.128	0.087	0.038	0.079	0.159	0.004
	p Value	0.257	0.442	0.738	0.485	0.158	0.971
HDL-C	r Value	−0.012	−0.183	0.101	−0.036	−0.12	−0.179
	p Value	0.913	0.104	0.374	0.751	0.281	0.112
LDL-C	r Value	0.024	0.047	0.005	0.067	0.038	0.073
	p Value	0.829	0.682	0.967	0.558	0.740	0.521
VLDL-C	r Value	0.148	0.102	0.076	0.095	0.150	0.008
	p Value	0.189	0.367	0.503	0.403	0.185	0.943

*Correlation is significant at the 0.01 level (two tailed).
†Correlation is significant at the 0.05 level (two tailed).
ADA, adenosine deaminase; FBS, fasting blood sugar; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PPBS, postprandial blood sugar; TC, total cholesterol; TG, triglyceride; VLDL-C, very low-density lipoprotein cholesterol.

increased ADA activity and its positive correlation with hyperglycemia (HbA1c) and lipid peroxidation in DM patients. Gohe MG *et al*[25] showed sensitivity and specificity of serum ADA measurement in type 2 DM to be 86% and 96%, respectively, and positively correlated with FBS, PPBS, and HbA1c concentration in diabetic individuals.

All these studies performed in the past show elevation of serum ADA levels and their correlation with glycemic parameters. In addition to the above findings, our study highlights the importance of isoenzymatic assessment of ADA in type 2 DM. Our study confirms high serum ADA in type 2 DM is mainly due to elevation of ADA2 fraction and is most probably released from monocytes/macrophages. ADA2 measurement alone is more efficient marker of glycemic status compared with total ADA measurement. This is a hospital-based cross-sectional study with limited representation of general diabetic population. Large extended prospective study will therefore be required to establish the diagnostic significance of ADA and its isoenzymes in type 2 DM.

CONCLUSION

In our study, we observed significantly elevated serum ADA and its isoenzymatic levels in type 2 diabetic cases compared with controls. Serum ADA and its isoenzymatic levels were positively correlated with FBS, PPBS, and HbA1c. Age, BMI, WHR, duration of DM, and demographic parameters do not alter ADA activity. All these features are suggestive of the diagnostic potential of serum ADA measurement in type 2 DM. ADA2 isoenzyme possess significant properties to be used as a biomarker in assessing immunopathogenesis of type 2 DM.

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