Diagnostic value of Adenosine deaminase and its isoenzymaticactivity in Nepalese patients with type 2 Diabetes Mellitus

Sapkota LB[1], Pokharel DR[2], Shukla PS[3], Thapa S[4]

Correspondence to:

sdrloken@gmail.com

[1]**Dr. Lokendra Bahadur Sapkota,** MD, Assistant Professor, Department of Biochemistry, Chitwan Medical College, Bharatpur, Chitwan, Nepal.

[2]**Dr. Daya Ram Pokharel,** Ph.D., Associate Professor, Department of Biochemistry, Manipal College of Medical Sciences, Pokhara, Nepal.

[3]**Dr. Pramod Shankar Shukla,** Ph.D., Professor & HOD, Department of Biochemistry, Manipal College of Medical Sciences, Pokhara, Nepal.

[4]**Dr. Sangita Thapa**, MD, Department of Clinical Microbiology and Immunology, Chitwan Medical College, Bharatpur, Chitwan, Nepal.

Abstract

Background

Adenosine deaminase, an enzyme of purine metabolism is suggested to play an important role in cell mediated immunity and modulation of insulin activity, but its clinical and diagnostic significance in Nepalese type 2 diabetes mellitus is not yet characterized.

Methods

This is a hospital based case control study including 80 type 2 DM patients and same number of age and sex matched healthy controls aged between 30-70 years attending Manipal Teaching Hospital (MTH), Pokhara, Nepal. All the demographic and anthropometric data were collected using a preformed set of questionnaire and biochemical data were obtained from the laboratory analysis of the patients' blood samples. Statistical analysis was done with SPSS version 20.

Results

A significantly higher (p<0.001) mean values of BMI, FBS, PPBS, HbA1c and lipid profiles were found in type 2 diabetic cases compared to the controls. Similarly, the cases were also found to have significantly higher (p<0.001) serum ADA (35.55 $\pm$ 7.53U/L), ADA1 (14.16 $\pm$ 4.74 U/L) and ADA2 (21.48 $\pm$ 4.60 U/L) activities compared to controls (ADA- 20.46 $\pm$ 3.27 U/L, ADA1-8.3 $\pm$ 2.21 U/Land ADA2-12.2 $\pm$ 2.17 U/L). Highest diagnostic accuracy and agreement with blood glucose test was observed with ADA2 followed by total ADA and ADA1.

Conclusion

Based on their higher serum activities, diagnostic sensitivity, specificity and substantial agreement with plasma glucose estimation, serum total ADA and its isoenzymes could be utilized as biomarkers for identifying the immunological origin of the uncomplicated type 2 DM.

Key words

Adenosine deaminase, Isoenzymes, MTH, Pokhara, Nepal, Type 2 Diabetes Mellitus
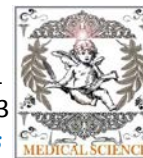
Background

Adenosine deaminase (ADA) is an enzyme involved in the irreversible deamination of adenosine to inosine and deoxyadenosine to deoxyinosine respectively [1]. Many studies have reported increased activity of ADA in diabetic patients compared to normal individuals [2, 3]. ADA has two major isoenzymes ADA1 and ADA2. ADA activity is reported to be highest in T-lymphocytes and thereby considered a novel biomarker of cell mediated immunity [4, 5]. Adenosine on the other hand has been proven to modulate insulin action in various tissues [6].

Type 2 Diabetes Mellitus (DM) is a chronic metabolic disorder characterized by immunologic disturbances and inappropriate T-lymphocyte function [7, 8]. So there exist a clear relationship between the alteration of ADA activity and immunopathogenesis of type 2 DM. Increased ADA activity in type 2 DM is well documented [2, 9, 10]. Determination of ADA and it's isoenzymes could therefore be used as an important biomarkers to predict immunological dysfunction in type 2 DM. To our knowledge, there is no report regarding the activity of ADA and its isoenzymes ADA1 and ADA2 in Nepalese diabetic subjects. So, this present study aims to determine the activity of serum total ADA and its isoenzymes ADA1 and ADA2 in Nepalese type 2 diabetic patients and explore its diagnostic significance.

Material and Methods

Study Period

This is a hospital based case-control study conducted in the Department of Biochemistry, Manipal Teaching Hospital (MTH), Pokhara during March to September 2016.

Inclusion criteria

Males and females aged between 30-70 years without having any acute or chronic complications of diabetes were included in this study. An equal number of age and sex matched healthy individuals were used as controls. World Health Organization (WHO) criteria were used for the diagnosis of subjects with type 2 DM [11].

Exclusion criteria

Patients with chronic complications of DM, pregnancy, addictive habits and other systemic illnesses were excluded from the study.

Data collection

Five 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 vial, ethylenediaminetetraacetic acid (EDTA) vacutaner 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 [12]. Glycated hemoglobin (HbA1c) was estimated by Nycocard Reader [13]. Blood collected in plain test tube was allowed for clotting at room temperature and the serum was carefully separated. Serum lipids (TG- triglyceride, TC- total cholesterol, and HDL-C- high density lipoprotein cholesterol) were directly measured and the value of LDL-C (low density lipoprotein cholesterol)was calculated using the Friedewald's formula [14]. All these parameters were analyzed in a semi automated chemistry analyzer (HumaLyzer-3500) using ready-to-use reagent kits following 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 (1972) [15]. The absorbance (OD) of blue colored complex formed at the end of reaction was measured using semi-automated chemistry analyzer at 620 nm. The ADA1 isoenzyme inhibitorerythro-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 approval of this study was done by the institutional ethical committee. Informed consent was taken from all the subjects included in this study.

Data analysis:

Demographic, clinical, and biochemical data of the patients were collected from personal interviews using a preformed set of questionnaires. The obtained data were analyzed using SPSS version 20. Comparison of mean values between controls and cases were done using student's t test. p value<0.05 was considered statistically significant.

Results

Table 1 - Baseline characteristics of patients			
Parameters	Controls	Cases	p value
Age	52.3±9.7	52.3±9.2	0.914[x]
BMI (kg/m²)	23.9±2.1	25.9±3.3	<0.001[†]
WHR	0.91±0.05	0.92±0.07	0.348[x]
SBP (mmHg)	127.6±8.2	135.5±7.6	<0.001[†]
DBP (mmHg)	83.2±5.4	87.3±6.0	<0.001[†]
FBS (mg/dl)	97.6±9.1	140.4±39.9	<0.001[†]
PPBS(mg/dl)	118.5±17.4	219.4±59.6	<0.001[†]
HbA1c (%)	5.6± 0.5	7.3±1.1	<0.001[†]
TC (mg/dl)	177.7±28.2	206.0±26.0	<0.001[†]
TG (mg/dl)	135.7±35.4	181.9±45.0	<0.001[†]
HDL-C (mg/dl)	50.6±8.7	46.1±5.8	<0.001[†]
LDL-C (mg/dl)	99.6±23.8	123.3±24.7	<0.001[†]
VLDL-C (mg/dl)	27.5±7.3	36.5± 9.0	<0.001[†]

[x]P>0.05 statistically not significant
[†]P<0.01 statistically significant

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BMI - body mass index, WHR-waist hip ratio, SBP - systolic blood pressure, DBP - diastolic blood pressure, FBS - fasting blood sugar, PPBS - postprandial blood sugar, HbA1c - glycated hemoglobin A1c, TC - total cholesterol, TG - triglyceride, HDL-C - high density lipoprotein cholesterol, LDL-C - low density lipoprotein cholesterol, VLDL-C - very low density lipoprotein cholesterol.

Mean age of the control and cases were 52.3+9.7 and 52.3+9.2 years respectively. BMI, WHR, SBP, DBP, FBS, PPBS, HbA1c, TC, TG, LDL and VLDL were significantly higher (p<0.001) in cases compared to controls while HDL-C was significantly lower (p<0.001) in cases compared to controls (Table 1).

Table – 2 Comparison of total ADA and its isoenzymes ADA1and ADA2 in controls and case groups			
Parameters	Controls	Cases	p value
ADA1	8.3 + 2.21	14.16 + 4.74	0.000[†]
ADA2	12.2 + 2.17	21.48 + 4.60	0.000[†]
Total ADA	20.46 + 3.27	35.55 + 7.53	0.000[†]

[†]P<0.01 statistically significant

The mean value of serum total ADA, ADA1 and ADA2 were found to be significantly higher in cases compared to controls (Table 2).

Table – 3 Comparison of serum ADA parameters: total ADA, ADA1 and ADA2 among male and female study subjects					
ADA parameters	Controls			Cases		
	Male	Female	p value	Male	Female	p value
ADA1	8.0 + 2.24	8.62 + 2.15	0.211[x]	14.19+4.89	14.12+4.63	0.953[x]
ADA2	12.29+2.23	12.11+ 2.13	0.71[x]	21.9+4.42	20.95+ 4.79	0.336[x]
total ADA	20.2+3.0	20.68+ 3.51	0.573[x]	36.3+7.37	34.63+7.7	0.306[x]

[x]P>0.05 statistically not significant

Mean values of ADA parameters were compared between male and female study subjects in controls and cases. The obtained p value showed no significant differences in serum ADA1, ADA2 and total ADA between male and female study subjects in both cases and controls (Table 3).

Table – 4 Distribution pattern of Serum ADA activity in the study subjects				
Parameters	Controls (N=80)		Cases (N=80)	
	Normal (True negative)	High (False positive)	Normal (False negative)	High (True positive)
ADA1	62 (77.5%)	18 (22.5%)	13 (16.3%)	67 (83.8%)
ADA2	71 (88.8%)	9 (11.3%)	5 (6.3%)	75 (93.8%)
Total ADA	70 (87.5%)	10 (12.5%)	8 (10%)	72 (90 %)

When we observed the serum distribution pattern of ADA activity, 90% of cases have increased serum ADA level compared to controls in which only 12.5 % have increased serum ADA level. Similarly, 83.8% of cases and 22.5% of control groups were found to have elevated serum ADA1 level. For ADA2, serum elevation was observed with 93.8% of cases and 11.3% of control groups (Table 4).

Table-5 Diagnostic accuracy of Serum ADA measurement					
ADA parameters	Sensitivity (%)	Specificity (%)	PPV	NPV	Kappa value
ADA1	83.8	77.5	78.8	82.6	0.66
ADA2	93.8	88.8	89.2	93.4	0.76
total ADA	90	87.5	87.8	87.9	0.7

PPV- positive predictive value, NPV=negative predictive value

This shows that the highest diagnostic accuracy was seen with ADA2 having sensitivity of 93.8%, specificity-88.8%, positive predictive value (PPV)- 89.2%, negative predictive value (NPV)-93.4%and Kappa value (χ)-0.76 followed by total ADA with sensitivity of 90%, specificity-87.5%, PPV-87.8, NPV-87.9 and Kappa value (χ)-0.7. The least diagnostic accuracy was observed with ADA1 with sensitivity of 83.8%, specificity-77.5%, PPV-78.8, NPV-82.6 and Kappa value(χ)-0.66 (Table 5). Kappa statistics showed that there is highest level of concordance between FBS and ADA2 measurement, followed by total ADA and ADA1 respectively.

Discussion

This study is first of its kind among Nepalese diabetic individuals that aimed to explore the diagnostic potential of ADA and its isoenzymes in type 2 DM.

Relationship between ADA and type 2 DM
It is a well-known fact that the abnormal function of T-lymphocytes results in immunological disturbances of cell-mediated origin. Many in vitro studies have shown that the insulin resistance developed in the type 2 diabetic patients triggers the inflammatory conditions leading to abnormal proliferation of T-lymphocytes [16-17].

Metabolic changes that occur in the course of diabetes are reflected by elevated adenosine concentrations in some tissues [18, 19]. The rapid proliferation of T cells and their sustained activity require the clearance of adenosine which may be toxic at high concentration with the help of ADA. So the conditions in which tissue adenosine concentration increases, ADA activity also increases. The significant elevation of serum ADA and its isoenzyme activity in type 2 DM individuals in this study might be both due to elevated adenosine concentration and abnormal T-lymphocyte responses or proliferation resulting its release into the circulation.

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Significance of ADA activity in type 2 DM:

Hoshino *et al*. also reported increased level of serum ADA1 and ADA2 in both type 1 and type 2 DM compared to healthy donor [20]. Among the Diabetic groups also, the ADA1 and ADA2 activity was shown to be higher in type 2 DM. Shivaprakash *et al*. (2006) observed significantly increased ADA activity in type 2 DM and hypothesized that the increased ADA activity is related to the alteration of immune function in type 2 diabetic individuals [2]. Gitanjali *et al*. (2010) had also reported elevation of serum ADA activity in type 2 diabetic patients and correlated it with markers of lipid peroxidation [21]. Our study had also shown significantly higher total ADA and it's isoenzymes ADA1 and ADA2 activities in type 2 DM. Furthermore, we established ADA and it's isoenzymes have high sensitivity, specificity, PPV, NPV and Kappa (χ) value in predicting type 2 DM. In this study we found highest diagnostic accuracy with ADA2 followed by total ADA and ADA1.

Possible explanation for raised ADA activity in type 2 DM

The ADA1 isoenzyme being intracellular in location, its extracellular concentration can rise during extensive cellular necrosis or increased turnover of lymphoid cells [22]. ADA2 found only in monocytes and macrophages is released extracellularly during infections or chronic inflammation as observed in type 2 DM [23]. ADA2-dependent T cell proliferation and monocyte-macrophage differentiation /proliferation may explain predominantly increased serum ADA2 activity compared to ADA1 in this study. Not only for TB, these findings in Nepalese type 2 DM patients are suggestive of the diagnostic potential of serum ADA and its isoenzymatic activity in the differential diagnosis of the disease.

Conclusion

Serum ADA and its isoenzymatic activity aids in the differential diagnosis of type 2 DM and it is a potential marker of immunopathology of type 2 DM. It could be used as a diagnostic biomarker in the differential diagnosis of type 2 DM because as a diagnostic marker it possesses high diagnostic accuracy.

Limitations & future scope of the study

The main drawback of this study was the sample size, which was less and it is recommended to perform a multicenter study in future, with a larger population. Long term follows up and inclusion of more investigations even at molecular basis also needed to get accurate result.

Abbreviations

Adenosine deaminase (ADA), Body mass index (BMI), diastolic blood pressure (DBP), Diabetes Mellitus (DM), ethylenediaminetetraacetic acid (EDTA), erythro-9-(2-Hydroxy-3-nonyl) adenine(EHNA), Fasting blood sugar (FBS), glucose oxidase peroxidase (GOD-POD), Glycated haemoglobin (HbA1c), High density lipoprotein cholesterol (HDL-C), Low density lipoprotein cholesterol (LDL-C), negative predictive value (NPV), optical density (OD), postprandial blood sugar (PPBS), positive predictive value (PPV), systolic blood pressure (SBP), Statistical Package for the Social Sciences (SPSS), total cholesterol (TC), triglyceride (TG), Very low density lipoprotein cholesterol (VLDL-C), World Health Organization (WHO), waist hip ratio (WHR)

Competing interests

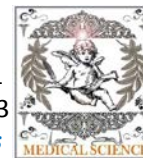The authors declare that they have no competing interests.

Authors' contribution

DRP, PSS and LBS conceived and designed the study. LBS prepared the questionnaire. LBS collected the samples and performed biochemical tests.LBS, ST and DRP analyzed the results. LBS and ST prepared and refined the manuscript.

Acknowledgments

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