Adenosine Deaminase Activity and its Relation with Glycated Hemoglobin and Uric Acid in Type 2 Diabetic Patients

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Received: 5 September 2013
Accepted: 20 November 2013
Published in 28 December 2013

Abstract

Objective: It has been reported that adenosine deaminase (ADA) is a good marker for insulin function but its clinical significance in type 2 diabetes mellitus (T2DM) is not yet characterized. This study aims to assess the association of ADA with glycated hemoglobin (HbA1c) and uric acid (UA) in T2DM patients.

Materials and Methods: The study population consisted of 120 subjects divided into 3 groups: Group A: non diabetic controls (n=40), Group B: diabetic subjects with HbA1c<7% (n=40), and Group C: diabetic subjects with HbA1c>7% (n=40). This study was carried out in the Nepalgunj medical college and Hospital, Nepal, between April 2012 and April 2013.

Results: In our study, fasting plasma glucose (FPG), HbA1c and ADA levels were found to be increased in T2DM patients as compared to controls. ADA activity is found to be higher in Group s B and C as compared to group A. The correlation between ADA and $HbA1_C$ was positive in both Group B (r=0.03) and C (r=0.28). There was negative correlation between UA levels and HbA1c (r=-0.07).

Conclusion: There was an increase in serum ADA levels with increase in HbA1c levels, which may play an important role in determining the glycemic status in diabetes. It was found that the UA levels increased with moderately increasing levels of HbA1c (<7%) and then decreased with further increasing levels of HbA1c (>7%). Serum ADA and UA levels reflect closely related components of T2DM.

Keywords: Type 2 Diabetes mellitus, ADA, Glycated hemoglobin, Uric acid, Nepal.

Introduction

Diabetes mellitus is the major health problem affecting both developed and developing countries. It is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid, and protein metabolism. Defects in carbohydrate metabolizing machinery and consistent efforts of the physiological system to correct the imbalance in carbohydrate metabolism place an over exertion on the endocrine system. Continuing deterioration of endocrine control exacerbates the metabolic disturbances and leads primarily to hyperglycemia (1-3). According to WHO, diabetes affects more than

170 million people worldwide (4), and affects more than 436,000 people in Nepal, and this number will rise to 1,328,000 by 2030 (5). The percentage of diabetic patients has increased from 19.04% in 2002 to 25.9% in 2009 in Nepal (6). Adenosine deaminase (ADA), an enzyme presents in red cells and the vessel wall catalyses the irreversible hydrolytic deamination of adenosine to inosine and 2'-deoxyadenosine to 2'-deoxyinosine. Both inosine and 2'-deoxyinosine are converted to hypoxanthine, xanthine and finally to uric acid (UA) (7). The enzyme exists in two isoenzyme forms: (ADA1 and ADA2) which are coded by separate genes (8). ADA is considered as a good marker of cell mediated immunity (9). High lymphocyte ADA activities were found to be elevated in diseases in which there is a cell mediated immune response (10).Chronic hyperglycemia leads to increased oxidative stress by forming enediol radicals and superoxide ions by NADPH oxidase system and increases ADA levels, both leading to insulin resistance. GLUT4 receptors are downregulated in the absence of adenosine. This is one of the reasons for insulin resistance. (11)

In a study, Prakashet al. (10) reported elevated serum ADA activity in patients with type 2 diabetes mellitus (T2DM). Kramer et al. (12) reported the association of high UA levels with T2DM but Tuomilehto et al. (13) demonstrated low UA levels in diabetic patients contradicting the earlier report. Thus, reports are available on serum ADA level and serum UA level in patients with T2DM, but no conclusive correlation could be established till now.

Given to lack of such a study in Nepalese population, this study was conducted to evaluate the serum ADA activity and serum UA, and to find the correlation, if any, with glycated hemoglobin (HbA1c) in Nepalese patients with T2DM.

Materials and Methods

Included were 80 patients with T2DM in the age group of 35-65 years of either sex, on oral hypoglycemic drugs, attending the outpatient department of Nepalgunj medical college and teaching hospital, Nepal. A group of 40 age- and sex-matched normal healthy individuals from the same population served as controls (group A). Eighty T2DM patients were further divided into group B (HbA1c<7%, n=40) and group C (HbA1c>7%, n=40) on the basis of the HbA1c levels.

This study was carried out in the central laboratory of biochemistry of the Nepalgunj medical college and teaching Hospital, Banke, Nepal between April 1, 2012 and April 30, 2013. Blood samples from subjects and controls were taken for measuring fasting plasma glucose (FPG), HbA1c, UA and ADA. The criteria for the diagnosis of T2DM were on the basis of new American Diabetes Association guidelines (14). Exclusion criteria consisted of type 1 diabetic patient, acute complications of diabetes, GDM, history of other acute illness or infections , tuberculosis, gout, rheumatoid arthritis, skeletal muscle injury and patients on insulin therapy. Serum glucose measurement was done by glucose oxidase and peroxidase methods (15). HbA1c was measured by Nycocard Reader (16). The total activity of serum ADA was assayed with a commercially supplied kit (Tulip Diagnostic (P) Ltd, Verna Goa, India); instructions followed according to the manufacturer. The assay was based on the colorimetric method described by Guisti and Galanti (17). One unit of ADA was defined as the amount of enzyme required to release three micromoles of ammonia per minute from adenosine in one hour at 37°C. The total ADA activity was expressed as U/L. Serum UA was determined by uricase/PAP method (Fossati & Prencipe, 1980) (18).

The results obtained from the above investigations were analyzed and expressed as mean±SD by using Excel 2007. The comparison was done by student t-test on variables of each parameter by using SPSS software, version 16, (SPSS Inc, Chicago (IL). The Pearson's correlation coefficient was used for examining the relationships between serum

ADA, UA and HbA1c. Ethical approval for the study was taken from the institutional research ethical committee.

Results

Table 1 represents the sex and age distribution of the study groups. Mean FPG levels were 89.65±8.22 mg/dl for group A, 131.63±13.79 mg/dl for group B, and 162.92±21 mg/dl for group C. There was a significant difference of FPG level between groups A and B (P<0.0001) as well as groups B and C (P<0.0001)(Table2).

The mean HbA1c levels are shown in Table 3. There was a significant difference (p<0.0001) ofHbA1c level between any paired of the groups.

Mean serum ADA levels in the groups are presented in table 4. Statistical analysis showed significant difference of serum ADA between the groups (P<0.0001).Mean serum UA levels was also statistically different between any paired of the groups except for the groups A vs. B (P>0.05)(Table5).

The Pearson correlation coefficient for the relationships between serum ADA, HbA1c and UA levels in Group B showed positive correlation between HbA1c and ADA (r=0.03). Similarly, comparison of serum UA and HbA1c levels revealed positive correlation (r=0.25). There was also significant relationships between serum ADA and HbA1c levels, as well as between serum UA levels and HbA1c in Group C(r=0.28, r=-0.07 respectively) (Table 6).

DISCUSSION

Diabetes mellitus, a common endocrine metabolic disorder, is a leading cause of death worldwide (19).It is characterized by hyperglycemia resulting from a variable interaction of hereditary and environmental factors and is due to the combination of insulin resistance (impairment in insulin-mediated glucose disposal) and defective secretion of insulin by pancreatic β cells (20).

In the present study, the mean serum ADA levels of group C were significantly higher than group B (P<0.001). Also, the levels of ADA were significantly higher in both groups B and C than Group A (P<0.001).Similar results were reported by Hoshino et al.(7), Kurtal et al. (21) and Kaur et al. (22).

Immunological disturbances of cell-mediated origin are believed to initiate from T-lymphocyte dysfunction. Recent in vitro studies implicated that in T2DM, inappropriate immune responses may result from the defects in the action of insulin required for the function of T-lymphocytes (23).ADA plays a crucial role in lymphocyte proliferation and differentiation (24) and shows its highest activity in T-lymphocytes (25). In the present study, a significant elevation in the ADA levels was observed in diabetic subjects compared to the controls. High plasma ADA activity might be due to abnormal T-lymphocyte responses or proliferation and may point to a mechanism that involves its release into circulation (24). Therefore, as presented in Figure 1, increased ADA activity in diabetic individuals could be due to altered insulin-related T-lymphocyte function (25-33).

Table 1- Sex and number distribution of subjects in group A, B and C

Group	Male	Female	Mean age(years)
Group A(n=40)	20	20	48.42±12.12
Group B(n=40)	25	15	47.82±13.12
Group C(n=40)	23	17	48.10±10.12

Table 2- Showing FPG level in all groups

Group	FPG(mg/dl)		
	Mean SD	Comparison	*P* value
A	89.65±8.22	Group A vs. B	<0.0001
B	131.63±13.79	Group A vs. C	<0.0001
C	162.92 ± 21	Group B vs. C	<0.0001

Table 3- Showing HbA1c level in all groups

Group	HbA1c (%)		
	Mean SD	Comparison	*P* value
A	5.14 ±0.49	Group A vs. B	<0.0001
B	6.03±0.50	Group A vs. C	<0.0001
C	9 ±. 81	Group B vs. C	<0.0001

Table 4- Comparison of Serum Adenosine Deaminase (ADA) levels in three groups

Group	Number	Mean ±SD	Comparison	*P* value
A	40	18.15±4.21	Group A vs. B	<0.0001
B	40	33.88±7.89	Group A vs. C	<0.0001
C	40	42.93 ±14.67	Group B vs. C	≈0.0009

Table 5- Comparison of Serum Uric acid levels in three groups

Group	Number	Mean ±SD	Comparison	*P* value
A	40	6.07 ± .96	Group A vs. B	>0.05NS
B	40	6.64 ± 1.54	Group A vs. C	≈0.0002
C	40	5.17 ±1.13	Group B vs. C	<0.0001

Table- 6 Comparison of Serum ADA, Uric acid and HbA1c in Group B and Group C

Parameter		Group B		Group C	
		HbA1c	ADA	HbA1c	ADA
ADA	**r value**	+ 0.03		+0.28	
	***p* value**	<0.0001		<0.0001	
Uric acid	**r value**	+ 0.25	0.2	- 0.07	0.000
	***p* value**	<0.0001	<0.0001	<0.0001	<0.0001

Mokhtari et al (2009) documented that ADA activity is significantly higher in gestational diabetes mellitus and pregnant individuals than normal group (34).

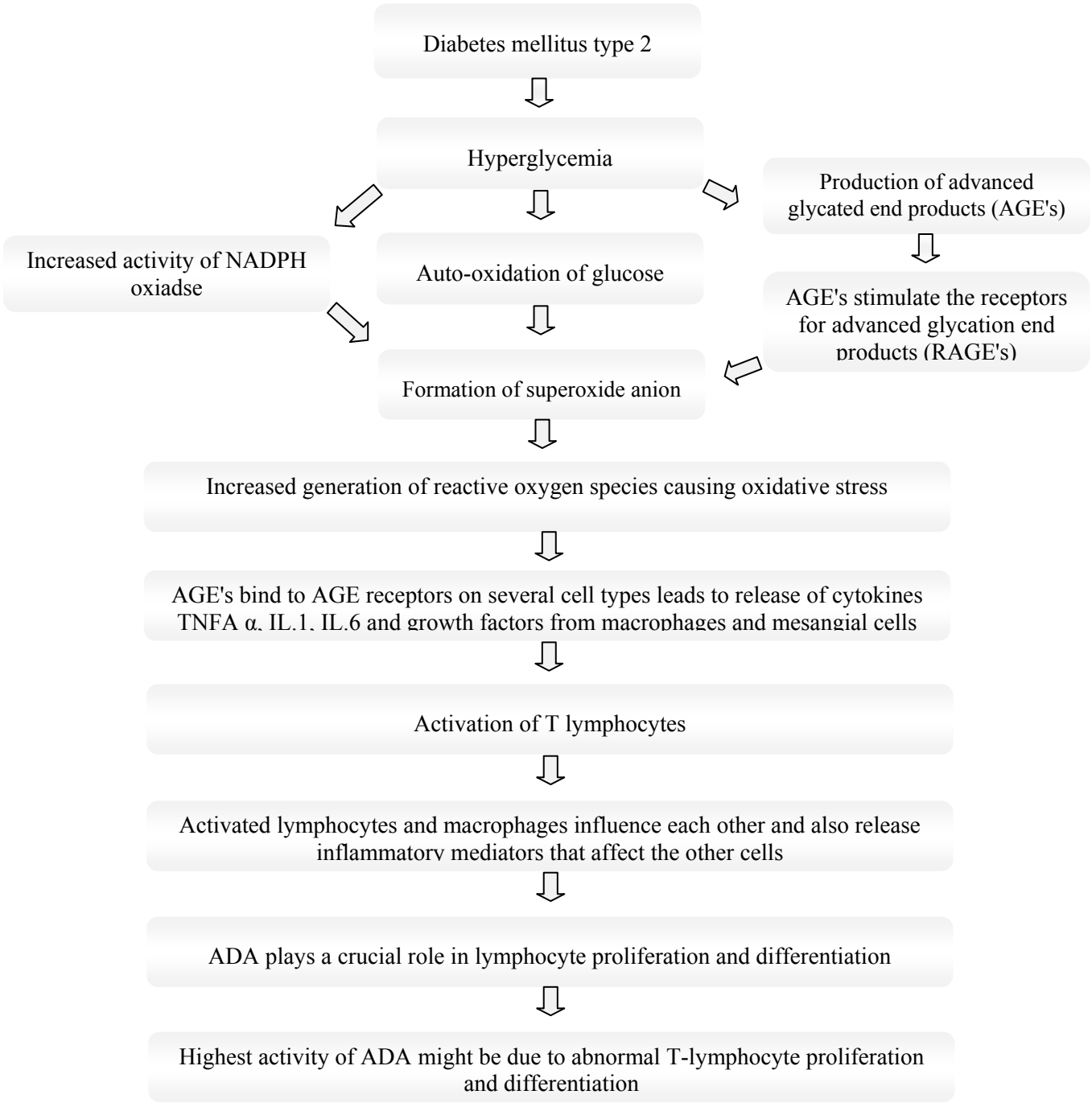

Figure 1- Possible Mechanism of high activity of ADA in type 2 diabetic patients

Results of this study showed positive correlation between HbA1c and ADA and between HbA1c and UA in group B (r=0.03, 0.25 respectively). The correspondent comparisons were also significant in group C (r=0.28, -0.07 respectively).These finding were in accordance with Choi et al. (35) and Tuomilehto et al. studies (13).

Conclusion

In our study, there was an increase in serum ADA levels with increase in HbA1c levels, which may play an important role in determining the glycemic status in diabetes. It was found that the serum UA levels increased with moderately increasing levels of HbA1c (<7%) and then decreased with further increasing levels of HbA1c (>7%). Serum ADA and serum UA levels reflect closely related components of T2DM.Further studies are required in Nepal to support these findings. We believe that our study results serve as baseline data to plan such studies in future in Nepal.

Acknowledgements

It is our proud privilege to express profound sense of gratitude and sincere thanks to the all the participants and specially Managing Director of Nepalgunj Medical College & Teaching Hospital, Banke, Nepal, for their support to make this study successful which has been completed with logical and fruitful conclusion.

References

1. Tiwari AK, Madhusudanarao J. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. Current science 2002;83:30-38, 2002.
2. Saravanan G, Pari L. Effect of an herbal drug, cogent db on plasma and tissue glycoproteins in alloxan-induced diabetic rats. Res J Med Plant 2007;1:83-91.
3. Bobb A, Gale D, Manmohan S, Mohammed A, Seetahal F, Small P, et al. The impact of the chronic disease assistance plan (CDAP) on the control of type 2 diabetes in Trinidad .Diabetes Research and clinical practice 2008;80(3):360-4.
4. Wokoma FS. Diabetes and hypertension in Africa, an overview. Diabetes Int 2002;12(12):36-40.
5. Wild S, Roglic G, Sicree R, Green A, King H. Global burden of diabetes mellitus in the year 2000. Global Burden of Disease 2000.
6. Dulal RK, Karki S. Disease management programme for Diabetes mellitus in Nepal. Journal of Nepal Medical Association 2009;48(176):281-6
7. Hoshino T, Yamada K, Masuoka K, Tsuboi I, Itoh K, Nonaka K, et al. Elevatedadenosine deaminase activity in the serum of patients with diabetes mellitus. Diabetes Research and clinical practice 1994;25(2):97-102.
8. Gakis C. Adenosine deaminase (ADA) isoenzymes ADA1 and ADA2: diagnostic and biological role. European Respiratory Journal 1996;9(4):632-3.
9. Sullivan JL, Oxborne WRA, Wedgewood RJ. Adenosine deaminase activity in lymphocytes. Br J Haematol 1977;37:157-8.
10. Prakash MS, Chennaiah S, Murthy YSR, Anjaiah E, Rao SA, Suresh C. Altered adenosine deaminase activity in type 2 diabetes mellitus. Age 2006;43(6.2):44-6.
11. Havilah P., PanditVinodh B, Durga Prasad K. Adenosine Deaminase Activity in Type-2 Diabetes Mellitus – An Independent Marker of Glycemic Status and Stimulator of Lipid Peroxidation. Int. J. Chem. and Life Sciences.2013;2(6):1175-8.
12. Kramer CK, Muhlen DV, Jassal SK, Connor EB. Serum uric acid levels improve prediction of incident Type 2 Diabetes in individuals with impaired fasting glucose. Diabetes Care 2009;32:1272-3.
13. Tuomilehto J, Zimmet P, Wolf E, Richard T, Ram P, King H. Plasma uric acid level and its association with Diabetes Mellitus and some biologic parameters in a biracial population of Fiji. American Journal of Epidemiology 1987;127(2):321-36.
14. American Diabetes Association Clinical Practice Recommendations: Executive Summary: Standards of Medical Care in Diabetes-2010 Diabetes Care 2010;33:4-5.
15. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann ClinBiochem 1969;6:24-7.
16. Jeppson JO. Approved IFCC Reference Method for the measurement of HbA1c in human blood. ClinChem Lab Method 2002;40(1):78-89.
17. Giusti G. Adenosine deaminase. Methods of enzymatic analysis. In: Bergmeyer HU editor. New York: Academic press Inc 1974;2:1092-9.
18. Fossati P, Prencipe L, Berti G. Use of 3, 5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric

acid in serum and urine. ClinChem 1980;26:227–31.
19. Faghilimnai, S, Hashemipour M. and Kelishadi, B. (2006). Lipid profile of children with type 1 diabetes compared to controls: ARYA .J. 2(1):36-8.
20. World Health Organization.Study Group on Diabetes Mellitus. Diabetes Mellitus: Report of a WHO Study Group on Diabetes Mellitus. WHO; 1985.
21. Kurtul N, Pence S, Akarsu E, Kocoglu H, Aksoy Y, Aksoy H. Adenosine deaminase activity in the serum oftype 2 diabetic patients. ActaMedica-Hradec Kralove- 2004;47(1):33-6.
22. Kaur A. Serum Adenosine Deaminase Activity and Its Correlation with GlycatedHaemoglobin Levels in Patients of Type 2 Diabetes Mellitus AmandeepKaur, SahibaKukreja, NareshMalhotra, Neha. Journal of Clinical and Diagnostic Research 2012;6(2):252-6.
23. Frankie B, Abbas E. Activated T-lymphocytes in type2diabetes: Implications from in vitro studies. Curr Drug Targets 2003;4:493-503.
24. Hovi T, Smyth JF, Allison AC, Williams SC. Role of adenosine deaminase in lymphocyte proliferation. ClinExpImmunol .1976;23:395-403.
25. Sullivan JL, Oxborne WRA, Wedgewood RJ. Adenosine deaminase activity in lymphocytes. Br J Haematol 1977;37:157-8
26. Ankush RD, Suryakar AN, Ankush, NR. Hypomagnesaemia in Type-2 Diabetes Mellitus patients: A Study on the status of oxidative and nitrosative stress. Indian Journal of Clinical Biochemistry 2009;24(2):184-89.
27. Gitanjali G, Sudeep G, Neerja, Mili G, Deepak A, Priyanka S. The Effect OfHyperglycaemia On Some Biochemical Parameters In Diabetes Mellitus. 2010.
28. Singh PP, Mahadi F, Roy A, Sharma P. Reactive oxygen species, reactive nitrogen species and antioxidants in etiopathogenesis of diabetes mellitus type -2. Indian Journal of Clinical Biochemistry 2009;24(4):324-42.
29. Kumar V, Abbas AK, Fausto N. The endocrine system. Robbins and Cotran Pathologic basis of disease 7th ed 2008;1198.
30. Ahmed N. Advanced glycation end products role in pathology of diabetic complications. Diab Res ClinPract 2005;67:3-21.
31. Goldsby RA, Kindt TJ, Osborne BA. Cytokines. Kuby immunology. 4th ed. New York: W.H. Freeman and Company 2000;320.
32. Desrosiers MD, Cembrola KM, Fakir MJ, Stephens LA, Jama FM, Shameli A, et al. Adenosine deamination sustains dendritic cell activation in inflammation. The Journal of Immunology 2007;179:1884-92.
33. Hovi T, Smyth JF, Allison AC, Williams SC. Role of adenosine deaminase in lymphocyte proliferation. ClinExpImmunol 1976;23:395-403.
34. Mokhtari M, Hashemi M, Yaghmaei M, Molashahi F, Shikhzadeh A, Niazi A, et al. Serum adenosine deaminase activity in gestational diabetes mellitus and normal pregnancy. Archives of gynecology and obstetrics 2010;281(4):623-6.
35. Choi HK, Ford ES. Haemoglobin A1c, fasting glucose, serum C-peptide and insulin resistance in relation to serum uric acid level- the Third National Health and Nutrition Examination Survey. Rheumatology 2008;47(5):713-7.