Original Research Paper

Volume-8 | Issue-11 | November-2018 | PRINT ISSN No 2249-555X

Biochemistry

"ESTIMATION OF SERUM ADENOSINE DEAMINASE IN TYPE 2 DIABETIC PATIENTS AND ITS CORRELATION WITH GLYCEMIC INDEX: A TEACHING HOSPITAL BASED STUDY"

Dr Prafulla Kumar Mishra Professor & Head, Department of Biochemistry Hi-Tech Medical College & Hospital, Rourkela, Odisha, India

Dr Tapan Kumar Mohapatra* Professor & Head, Department of Biochemistry, Santosh Medical College and Hospital, Ghaziabad *Corresponding Author

ABSTRACT

Background: Diabetes mellitus is a group of metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Diabetes mellitus is a common disorder of glucose homeostasis which grows epidemically.

Methods: Randomly selected, 45 patients with type-2 diabetic patients with an age ranged from 30 to 65 years along with 45 healthy controls were recruited from the General Medicine Department.

Results: A significant positive correlation was observed between serum ADA with HbA1c, FBS, PPBS, TC and TG in cases compared to controls with P value <0.001 and r value 0.65, 0.20, 0.24, 0.29 and 0.31 respectively.

Conclusion: Significantly higher values of ADA in cases compared to controls suggest that ADA plays a role in the pathophysiology of type 2 DM. A positive significantly correlation between serum ADA levels and glycemic parameters.

KEYWORDS : Type 2 DM, Hyperglycemia and Adenosine deaminase(ADA)

INTRODUCTION:

Diabetes mellitus is a common disorder of glucose homeostasis which grows epidemically. The incidence and the prevalence of type 2 DM is globally increasing and becoming a major public health problem for health care providers. It is estimated that by 2030 this would have risen to 552million.[1] Thus, understanding the pathogenesis and preventing and/or ameliorating the long term complications have been major goals of research in diabetes mellitus. In India, currently there are 62 million people with diabetes.[2] By 2030, this number is estimated to rise to 80 million.[3]

Immunological disturbances in type 2 diabetic individuals have an association with cell mediated responses[4]; and inappropriate T-lymphocyte function, which is vital in this pathogenic condition, has a link with insulin defect.[5] Adenosine deaminase, an enzyme distributed in the human tissues[6], was considered as good marker of cell mediated immunity[7]. It plays a crucial role in lymphocyte proliferation and differentiation[8], and shows its highest activity in T-lymphocytes. Previously, adenosine deaminase has been reported to be a marker for insulin function.[9,10] But its connection with the immune system was not yet established in diabetic subjects. Even though there are some reports available on ADA levels in diabetic subjects, these are all inconclusive and controversial.[11] Since a relationship exists between adenosine deaminase and cell mediated immunity. In the present study, we measured serum ADA activity in T2DM patients to evaluate the relationship between serum ADA activity with glycemic status and various metabolic parameters in T2DM patients.

MATERIAL AND METHODS:

This study was conducted in the Department of Biochemistry in association with Department of Medicine, Hi-Tech Medical College & Hospital Rourkela, Odisha, India, during the period from September 2016 to August 2017. Randomly selected, 45 patients with type-2 diabetic patients with an age ranged from 30 to 65 years along with 45 healthy controls were recruited from the General Medicine Department of HMCH, Rourkela. 5 mL of venous blood sample was collected after 12 hours of fasting for estimation of fasting plasma glucose, HbA1c and lipid profile and 2 ml venous blood sample 2 hours after breakfast for postprandial plasma glucose were studied for following parameters.

1. Blood Glucose by GOD-POD methods.[12]
2. Glycated Haemoglobin (GHb) by cation exchange resin methods.[13]
3. Total Cholesterol (TC) by enzymatic end point CHODPOD methods.[14]
4. Triglyceride (TG) by enzymatic glycerol phosphate oxidase/peroxidase methods.[15]
5. HDL-Cholesterol by direct enzymatic end point method.[16,17]
6. LDL-Cholesterol by Friedewald's formula.[18]
7. VLDL-Cholesterol by Friedewald's eqution. LDL-c = Tc-HDL-c(TG/5)
8. Sr. ADA was estimated by Colorimetric method described by Guiseppe Guisti.[19]

STATISTICAL ANALYSIS:

Statistical analysis of data was performed using the SPSS (Version 16.0). For comparison of parameters between the two groups, students t test was used. Statistical significance was considered at a 'p' value of < 0.05. For correlation, Pearson's correlation coefficient (r) was used.

RESULTS AND DISCUSSION:

The base line characteristics of the patient and control group are shown in Table I. The mean age of both the controls and subjects were 36.07+8.51 and 43.16+10.05 respectively. There was a statistically significant increase in mean value of fasting blood sugar 224.56 & 83.69 mg/dl with p<0.0001 and postprandial blood sugar 287.24 & 112.26 mg/dl with p<0.0001 in cases as compared to controls. There was a statistically significant increase in mean value of HbA1c (8.05 & 4.34) % with p<0.0001 in cases compared to controls and also a statistically significant increase (p<0.0001) in mean value of ADA 46.01 & 18.04 U/L with p<0.0001 in cases compared to controls. There was a statistically significant increase in mean value of serum total cholesterol p=0.014, and triglycerides p<0.001 in cases as compared to controls, and there was also positive correlation between total cholesterol, triglycerides and serum ADA.

TABLE 1: Comparision between Glycemic status, lipid profile and ADA of type-2 diabetes and controls:

Variables	Type 2 DM (mean+SD) (n=45)	Controls (mean+SD) (n=45)	P- value
Age in years	43.16+10.05	36.07+8.51	0.16
HbA1c (%)	8.05+2.21	4.34+0.76	0.0001
FBS	224.56+131.05	83.69 +10.41	0.0001
PPBS	287.24+141.04	112.26+20.23	0.0001
TC (mg/dl)	207.32+58.04	180.26+31.21	0.014
TG (mg/dl)	223.04+ 64.41	140.25+48.62	0.0001
HDL-C (mg/dl)	30.02+3.07	36.03+4.8	0.16
LDL-C (mg/dl)	132.5±30.42	126.03±28.07	0.46
ADA (U/L)	46.01+4.9	18.04+4.02	0.0001

TABLE 2: Pearson correlation between serum ADA and HbA1c, FPG, PPG, TC and TG:

Variables	r-value	P-value
Serum ADA v/s HbA1c	0.65	0.001
Serum ADA v/s FPG	0.20	0.001
Serum ADA v/s PPG	0.24	0.001
Serum ADA v/s TC	0.29	0.001
Serum ADA v/s TG	0.31	0.001

A significant positive correlation was observed between serum ADA with HbA1c, FBS, PPBS, TC and TG in cases compared to controls with P value <0.001 and r value 0.65, 0.20, 0.24, 0.29 and 0.31 respectively. Immunological disturbances of cell-mediated origin are believed to initiate from T-lymphocyte dysfunction. Recent in vitro studies implicated that in type 2 diabetes mellitus, inappropriate immune responses may result from the defects in the action of insulin that is required for the function of T-lymphocytes.[4] Adenosine deaminase plays a crucial role in lymphocyte proliferation and differentiation[7] and shows its highest activity in T- lymphocytes.[8] In the present study we observed a significant elevation in the adenosine deaminase levels in diabetic subjects when compared to controls. ADA is an enzyme that converts adenosine into inosine through an irreversible deamination reaction.[20] It is hypothesized that adenosine has got insulin like activity on glucose and lipid metabolism particularly in adipose tissue and skeletal muscles. ADA is found as a producer of reactive oxygen species (ROS), stimulator of lipid peroxidation and marker of both T-cell activation and glycemic status in diabetes mellitus (DM).[21,22,23] An increase in ADA activity in T2DM patients has been reported, while the mechanism that increases serum and tissue ADA activity is not well known, with higher ADA activity in insulinsensitive tissues, the level of adenosine, which increases glucose uptake into cells, will be reduced.[24,25] The high plasma adenosine deaminase activity might be due to abnormal T-lymphocyte responses or proliferation; may point towards a mechanism that involves its release into circulation.[7] Therefore, we report that increased adenosine deaminase activity in diabetic individuals could be due to altered insulin related T-lymphocyte function. Previously, Chang and Shaio, have demonstrated that impaired cell mediated immunity was associated with abnormal lymphocyte proliferation1. We report that, as adenosine deaminase is associated with T- lymphocyte activity[8], its altered blood levels may help in predicting immunological dysfunction in diabetic individuals and might be one of the important biomarkers in predicting diabetes mellitus. We hypothesise that these observations may furnish better insights on the role of cell-mediated immunity in the pathophysiology of type 2 diabetes. It is also thought that in diabetic individuals, deranged immunity may also originate from antibody dependant cellular cytotoxic responses, which are believed to target insulin[26] that has control over T- lymphocyte function.[4] Such non-specific cellular immune responses, described as one of the four possible mechanisms responsible for the production of lymphocytotoxins, were observed earlier in autoimmune diseases like systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA),[27] where elevated adenosine deaminase levels have also been detected.[28,29] The role of adenosine deaminase in the cellular immunity was first identified in patients with severe combined immuno deficiency (SCID).[30,31] The high activity of this enzyme was considered to be a reflection of immunological disturbances observed in tuberculosis,[28] infectious mononucleosis[32], jaundice[33], leukaemia[34], and other conditions[35-37]. Our study on adenosine deaminase activity in type 2 diabetic individuals is the first of its kind of description in the immunological context. The use of adenosine deaminase is a cost-effective process and the efficient exploitation of this strategy may help in better establishing this enzyme as a good marker for assessing CMI in diabetic individuals. Therefore, we conclude that elevated adenosine deaminase activity may be an important indicator in the immuno-pathogenesis of type 2 diabetes mellitus. However, this study has a few limitations. A concomitant lymphocytic/plasma adenosine deaminase and its activity on insulin or vice versa, and a correlation with oral glucose tolerance test (OGTT) are to be carried out to strengthen this concept. Further studies on ADA activity in lymphocytes is required to consider ADA as an effective prognostic and pathological marker in type 2 diabetes mellitus.

CONCLUSION:

In conclusion, significantly higher values of ADA in cases compared to controls suggest that ADA plays a role in the pathophysiology of type 2 DM. A positive significantly correlation between serum ADA levels and glycemic parameters. Though it is evident that there is an elevation of serum ADA values in individuals with T2DM, the exact mechanism behind the elevation and the implication of altered expression need to be further elucidated. Therefore, estimation of serum ADA might serve as a glycemic marker for assessing the glycemic status of a diabetic patient.

REFERENCES

1. Olokoba AB, Obateru OA, Olokoba LB. Type 2 diabetes mellitus: a review of current trends. Oman Medical Journal 2012;27:269-273.
2. "Diabetes can be controlled in 80 percent of Cases in India". IANS. news. biharprabha.com. Retrieved 6 February 2014.
3. Mehta SR, Kashyap CA, Das CS. Diabetes mellitus in India: The Modern Scourge. MJAFI 2009;65:50-54.
4. Frankie B, Abbas E. Activated T-lymphocytes in type2 diabetes: Implications from in vitro studies. Curr Drug Targets 2003; 4: 493-503.
5. Vader Weyden MB, Kelly WN. Human adenosine deaminase: distribution and properties. J Biol Chem 1976; 251: 5448-56.
6. Baghanha MF, Pego A, Lima MA et al. Serum and pleural adenosine deaminase correlation with lymphocyte populations. Chest 1990; 87: 605-10.
7. Hovi T, Smyth JF, Allison AC, Williams SC. Role of adenosine deaminase in lymphocyte proliferation. Clin Exp Immunol 1976; 23: 395-403.
8. Sullivan JL, Oxborne WRA, Wedgewood RJ. Adenosine deaminase activity in lymphocytes. Br J Haematol 1977; 37: 157-8.
9. Kurtul N, Pence S, Akarsu E et al. Adenosine deaminase activity in the serum of type 2 diabetic patients. Acta Medica (Hradec Kralove) 2004; 47 (1): 33-5.
10. Hoshino T, Yamada K, Masuoka K et al. Elevated adenosine deaminase activity in the serum of patients with diabetes mellitus. Diabetes Res Clin Pract 1994; 25: 97-102.
11. Kurtul N, Pence S, Akarsu E et al. Adenosine deaminase activity in the serum of type 2 diabetic patients. Acta Medica (Hradec Kralove) 2004; 47 (1): 33-5.
12. Bergmayer H.V. – "Methods of Enzymatic Analysis",A.P., N.Y. 1974, Page 1196.
13. Gabby, K H et al. – J. Clin. End. Met. 44:859,1977.
14. Richmond W. – Preparation and properties of cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of total cholesterol in serum. Clin Chem. 19: 1350-1356, 1973.
15. Foosati P. and Prencipe L. – Serum triglyceride determined colorimetrically with an enzyme that produce hydrogen peroxide. Clin Chem. 28: 2077-2080, 1982.
16. Rifai N. and Warnick G.R., Ed.- Laboratory measurements of lipids, lipoproteins and apolipoproteins. AACC press, Washington, DC, USA 1994.
17. Burtis, C.A. and Ashwood, E.R. Ed. – Tietz Textbook of clinical chemistry, 2nd Ed, Saunders, Philadelphia, 1994.
18. Friedewald W.T., Levy R.I., Fredrickson D.S., clin Chem. 18:499, 1972.
19. Giusti G. Adenosine deaminase. In :H.U. Bergmeyer (ed), Methods of enzymatic analysis, Verlag chemie, Weinheim and Academic Press, 2nd edition. New York. 1974:1092-9.
20. Spencer N, Hopkinson DA, Harris H. Adenosine deaminase polymorphism in man. Annals of Human Genetics. 1968;32(1):9-14.
21. Shiva Prakash M, Chennaiah S, Murthy YSR. Altered Adenosine Deaminase activity in type 2 Diabetes Mellitus. JICAM. 2006;7:114-7.
22. Gitangali G, Neerja. The effect of Hyperglycemia on some Biochemical parameters in Diabetes Mellitus. JCDR. 2010;4:3181-6.
23. Erkilic K, Evereklioglu C, Cekmen M. Adenosine deaminase enzyme activity is increased and negatively correlates with catalase, SOD, and GSH in patients Behcet's Disease. Original contributions/ clinical and laboratory investigations. Mediators Infamm. 2003;12:107-16.
24. Lee JG, Kang DG, Yu JR, Kim Y, Kim J, Koh G, et al. Changes in adenosine deaminase activity in patients with type 2 diabetes mellitus and effect of DPP-4 inhibitor treatment on ADA activity. Diabetes and Metabolism Journal. 2011;35(2):149-58.
25. Warrier AC, Rao NY, Mishra TK, Kulpati DS, Mishra KT, Kabi BC. Evaluation of Adenosine Deaminase activity and lipid peroxidation levels in Diabetes Mellitus. Indian Journal of Clinical Biochemistry. 1995;10(1):9-13.
26. Fovenyi J, Totpal K, Thaisz E, Garam T. Non-specific cellular immunity in type I and type II diabetes. Exp Clin Endocrinol 1984; 83: 203-6.
27. Paul IT, Vilma D Mottironi, Eugene V Barnett. Cytotoxins in disease: Autocytotoxins in lupus. N Eng J Med 1970; 283: 724-8.
28. Pettersson T, Ojala K, Weber TH. Adenosine deaminase in the diagnosis of pleural effusions. Act Med Scand 1984; 215: 299-304.
29. Ungerer JPG, Oustuizen HM, Bissbort SH, Vermaak WJH. Serum adenosine deaminase: isoenzyme and diagnostic applications. Clin Chem 1992; 38: 1322-6.
30. Giblett ER, Anderson JE, Cohen F et al. Adenosine deaminase deficiency in two patients with impaired cellular immunity. Lancet 1972; 2: 1067-9.
31. Meuwissen HJ, Pollara B, Pickering RJ. Combined Immunodeficiency disease associate with adenosine deaminase deficiency. J Pediatr 1975; 86: 169-81.
32. Koehler LH, Benz EJ. Serum adenosine deaminase: methodology and applications. Clin Chem 1962; 8: 133-40.
33. Goldberg DM. Serum adenosine deaminase in the differential diagnosis of jaundice. Br Med J 1965; 1: 353-5.
34. Grever MR, Coleman MS, Balcerzak SP. Adenosine deaminase and terminal deoxynucleotidyl transferase: biochemical markers in the management of chronic myelogenous leukemia. Cancer Res 1983; 43:1442-5.
35. Mishra OP, Garg R, Ali Z , Usha. Adenosine deaminase activity in nephrotic syndrome. J Trop Pediatr 1997; 43: 33-7.
36. Kavitha K, Yashoda Devi P, Shiva Prakash M. Adenosine deaminase in cord blood as an immunoenzyme marker in low birth weight neonates. Ind J Med Sci 2000; 54: 92-4.
37. Surekha Rani H, Dayasagar Rao V, Shiva Prakash M. Serum adenosine deaminase activity and C-reactive protein levels in unstable angina. Indian J Hum Genet 2003; 9: 17-20.