Journal of Cardiovascular Disease Research

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 11, 2024

STUDY OF SERUM ADENOSINE DEAMINASE ACTIVITY IN TYPE 2 DIABETES MELLITUS IN VIDARBHA REGION.

Dr Yogita Raj Dubey[1*], Sangeeta Dubey Bhargava[2]

[1]Assistant Professor, Department of Biochemistry , Netaji Subhash Chandra Bose Medical College Jabalpur (M.P).
[2]Assistant Professor, Department of Obstetrics & gynaecology R.D. Gardi Medical College, Ujjain (M.P).

Corresponding Author
Dr Yogita Raj Dubey
dr.yogita.raj@gmail.com

Abstract

Background: Diabetes mellitus (DM) is one of the most important cause of mortality in India characterized by hyperglycaemia due to deficiency of insulin and insulin resistance . Adenosine deaminase (ADA) is a purine metabolizing enzyme that catalyses the deamination of adenosine to inosine and modulates the insulin activity.
Aim: To determine the activity of adenosine deaminase in type 2 diabetic patients.
Material & Method: We measured the serum level of Adenosine Deaminase (ADA), and Fasting Plasma Glucose (FPG) in 50 patients with type 2 diabetes and 50 healthy controls. Subjects included in study were known diabetics.
Results: The levels of Serum Adenosine Deaminase were highly significant ($p<0.001$) in study group in comparison to control group.
Conclusion: Our study concludes that serum Adenosine Deaminase activity significantly increased in type 2 diabetes mellitus. High ADA activity reduces the glucose uptake into cells; therefore, insulin resistance is related to ADA activity.

Keywords: Adenosine deaminase, Type 2 Diabetes Mellitus, Hyperglycaemia, Insulin resistance

INTRODUCTION

Diabetes mellitus is one of the important metabolic disordercharacterised by hyperglycaemia, increasing worldwide, causing 4.8 million deaths and morbidity in 371 million people every year[1]. India has the second largest number of people with diabetes in the world (62.4 million) with 3.8% in rural and 11.8% in urban adults, and this number is expected to reach 100 million by the year 2030.[2,3,4]. Long term hyperglycaemia leads to glucotoxicity, which further leads to poor glycaemic control and predisposes to long term micro and macro vascular complications.

Insulin resistance and impaired insulin secretion are the main physiological abnormalities associated with T2DM [5].Immunological disturbances involving the cell mediated immune system and improper T-lymphocyte function also contributetothepathophysiologyofT2DM[6]. Adenosine deaminase (ADA) converts adenosine to inosine through an irreversible deamination reaction [7]. Highest ADA activity has been reported in lymphoid and fatty tissues ,liver, skeletal muscle, and heart[8]. Adenosine is responsible for increasing glucose uptake into cells [9]. Thus, higher ADA activity in insulin sensitive tissue will decrease adenosine levels which in turn decrease glucose uptake into cells. Adenosine has also been shown to increase gluconeogenesis and glycogenolysis via increasing cyclic AMP (CAMP) by stimulation of hepatic adenylate cyclase through adenosine A2 a receptor binding in liver. Both or either of these actions causes the increase of local insulin resistance and glucose output from the liver[10]. ADA also plays a crucial role in lymphocyte proliferation and differentiation and is highly active in T-lymphocytes [11]. Thus, a suppression of ADA activity may help improve insulin sensitivity and inflammation, cell proliferation, and T-lymphocyte activity, all of which are associated with the pathophysiology of T2DM.

Many studies have reported the increased activity of ADA in type 2 diabetic patients compared with healthy controls.[10,11,12] But in our knowledge, there is no study regarding the activity of ADA in Vidarbha region in diabetes. The present study aims to determine the activity of serum total ADA in type 2 diabetic patients of Vidarbha region.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 11, 2024

MATERIAL AND METHOD

This prospective case control study was carried in Department of Biochemistry, JNMC, Sawangi(Meghe), Wardha. Patients were taken from outpatient department as well from indoor admitted in Achrya Vinobha Bhave Rural Hospital .Consent was taken from all the participants.

Study group comprises of 100 subjects which further divided into two groups –

(1)Cases-[50]- These were the patients of diabetes mellitus between age group of 30-60 years of both male and female. Demographic data i.e. age, weight ,height, duration of disease, duration of treatment ,any past medical history, blood pressure was also recorded.

(2) Controls –[50]- These are normal healthy persons ,both male and female with age group of 30-60 years from general population.

Diagnosis of diabetes was done as per WHO criteria

Criteria for the Diagnosis of Diabetes Mellitus

- Symptoms of diabetes plus random blood glucose concentration 200 mg/dL or
- Fasting plasma glucose 126 mg/dL or
- Two-hour plasma glucose 200 mg/dl during an oral glucose tolerance test:

Exclusion Criteria

Patients with Type1 diabetes mellitus ,gestational diabetes, uricosuric drugs, chronic arthritis,gout, immunological disorder which alter ADAlevel hypertension, dyslipidaemia, smokers were excluded. Also patients on antioxidant vitamin supplements were also excluded. 3ml of serum sample will be collected from each patient.

Biochemical analysis

About 3 ml of fasting blood was collected for the determination of different biochemical parameters. The blood was drawn by venepuncture and collected into clean test tubes with ethylenediaminetetraacetic acid (EDTA). The blood samples were subjected to centrifugation at 3,000 rpm for 10 min for separation of plasma. The plasma thus obtained was analysed for biochemical parameters such as glucose, cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides by automated clinical analyzers (model Daytona, Randox).

The ADA levels were estimated using a commercially available kit . This procedure is based on the method reported by Giusti and Galanti[13]. Adenosine deaminase hydrolyses adenosine to ammonia and inosine. The ammonia formed further reacts with a phenol and hypochlorite in an alkaline medium to form a blue coloured indophenol complex, with sodium nitroprusside acting as a catalyst. The intensity of blue coloured indophenol complex formed is directly proportional to the amount of ADA present in the sample. The absorbance was read against water at 635 nm using a spectrophotometer

Statistical analysis

Statistical analysis was done by using descriptive and inferential statistics using chi square test, student's unpaired t test and Pearson's correlation coefficient. All variables were expressed as mean ± SD(standard deviation).

Software used in the analysis were SPSS 22.0 version and GraphPad Prism 6.0 version.

RESULT

In our study we have 62% male and 38% female in cases and 56% male and 44% female in controls. Maximum subjects belong to age group 41 – 60 years. Both the groups are age matched.

Table 1: Comparison of fasting and post prandial plasma glucose and serum ADA in two groups

Clinical Parameters	Cases		Controls		t-value	p-value
	Mean	SD	Mean	SD		
FPG (mg/dl)	191.16	55.44	94.06	15.25	11.93	0.0001,
PPPG (mg/dl)	328.5	92.01	136.74	21.27	14.35	0.0001,
ADA (U/L)	39.83	5.81	17.86	2.59	24.37	0.0001

p value <0.05 : significant, 0.0001: highly significant

Values of Fasting plasma glucose(FPG) and Postmeal plasma glucose(PPG) are compared in both the groups. FPG values are found to be more in cases (191.16 ±55.44) as compared to control (94.06±15.25) which is statistically highly significant (p=0.0001).

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 11, 2024

PPG values are also found higher (328.5±92.01) in cases, compared to control (136.74±21.27) which is also highly significant (p=0.0001).

Serum ADA is increased in cases (39.83±5.81) as compared to controls (17.86±2.59) which is highly significant (p=0.0001)

Table 2: Comparison of lipid profile in two groups

Clinical Parameters	Cases		Controls		t-value	p-value
	Mean	SD	Mean	SD		
Total cholesterol	199.98	48.13	169.34	31.65	3.76	0.0001
Triglyceride	206.9	65.73	91.62	40.81	10.53	0.0001
HDL Cholesterol	33.56	6.88	40.24	12.01	3.41	0.001
LDL Cholesterol	120.54	37.83	111.1	26.62	1.44	0.152
VLDL cholesterol	40.96	13.17	17.7	7.79	10.74	0.0001

p value <0.05 : significant, 0.0001: highly significant

In diabetic patients values of total cholesterol (199.98±48.13}, triglyceride (206.9±65.73) and VLDL Cholesterol (40.96±13.17) are increased as compared to controls (169.34±31.65), (91.62±40.81), (17.7±7.79) which is highly significant (p=0.0001). Value of LDL Cholesterol is also increased in cases (120.54±37.83) as compared to controls (111.1±26.62) but it is not significant (p=0.152) .HDL Cholesterol is decreased in diabetics significantly which is highly significant.

DISCUSSION

The relationship between the serum adenosine deaminase with the glycemic parameters (FBS, PPBS) and parameters of lipid profile (TG, TC, LDL cholesterol, HDL cholesterol) in diabetes mellitus type 2 was evaluated. There was a significant ($p < 0.001$) increase in serum ADA in individuals with diabetes mellitus type 2 in comparison with control group. Findings in our study also supported by many other studies done previously.. Similar study done by Bhavita Patel et al[14] ,Vineet Kumar Khemka,et al[15], Shaikh Sahema M et al[16] , Mohammed A. Al-Duais et al[17] with similar results. ADA is an enzyme that converts adenosine into inosine through an irreversible deamination reaction[18]. 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)[19,20,21]. 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 insulin sensitive tissues, the level of adenosine, which increases glucose uptake into cells, will be reduced[22,10]

Bhavita Patel et al[14] explained the pathogenesis of increased ADA levels in Type 2 D.M is by extra cellular CAMP – adenosine pathway. ADA inactivates adenosine and enhances lipolysis.However, it is difficult to conclude whether changes in ADA activity are the cause or result of actual insulin resistance. Adenosine potentiates insulin and contraction stimulated glucose transport in skeletal muscles by enhancing the increase in GLUT-4 at the cell surface and raised the possibility of decreased adenosine production or action by increased level of adenosine deaminase could play a causative role in insulin resistance[23].

In the present study, serum ADA and Insulin levels were markedly increased in type 2 diabetic patients ($p<0.0001$) in comparison to healthy subjects which is highly significant.Our results were in agreement with results obtained in previous studies that concluded the increased in adenosine content make similar effect to insulin on glucose and lipid metabolism.

Conclusion

In conclusion ADA has been viewed as a parameter of interest in type 2 diabetes due to its role in oxidative stress, as a marker of cell mediated immunity along with its effects on insulin by altering levels of adenosine. It is important to detect complications early and a single marker is required to identify. Therefore, ADA can be used as an important parameter as a marker of glycaemic status in the patients of type 2 diabetes mellitus.

Limitations

There are some limitations in our study. Estimation of serum insulin levels which are known to be related to ADA should be included. Prediabetic subjects were also not considered in this study as screening of serum ADA may be an alarming factor in the pathogenesis of T2DM. Despite these limitations, our study shows higher

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 11, 2024

serum ADA,TG, and FPG levels in T2DM patients which suggests an association between ADA and T2DM subjects. A larger cross-sectionalstudyneedstobedonetoconcludethe fact.

Acknowledgment
The authors would like to acknowledge the support and help of department of medicine and central clinical laboratory of AVBRH Sawangi (M) Wardha.

REFERENCES

1.IDF. Diabetes atlas. Brussels, Belgium: International Diabetes Federation. 2013;4.
2. Reddy KS, Shah B, Varghese C, Ramadoss A. Chronic diseases
3. Lancet 2005;366:1746-51. 3. Unwin N, Whiting D, Guariguata L, Ghyoot G, Gan D, editors. 5th ed. Brussels: International Diabetes Federation. International Diabetes Federation, Diabetes Atlas. 2011:11-74.
4. Anjana RM, Pradeepa R, Deepa M, Datta M, Sudha V, Unnikrishnan R, et al. Prevalence of diabetes and prediabetes (impaired fasting glucose and/or impaired glucose tolerance) in urban and rural India: Phase I results of the Indian Council of Medical research-India Diabetes (ICMR-INDIAB) study. Diabetologia. 2011;54:3022-7.
5. A.VaagandS.S.Lund,"Non-obesepatientswithtype2diabetes and prediabetic subjects distinct phenotypes requiring special diabetes treatment and (or) prevention?" Applied Physiology, NutritionandMetabolism,vol.32,no.5,pp.912–920,2007.
6. M.S.Prakash, S.Chennaiah, Y.S.R.Murthy, E.Anjaiah, S.A. Rao, and C. Suresh, "Altered adenosine deaminase activity in type 2 diabetes mellitus," Journal, Indian Academy of Clinical Medicine,vol.7,no.2,pp.114–117,2006.
7. N. Spencer, D. A. Hopkinson, and H. Harris, "Adenosine deaminase polymorphism in man," Annals of Human Genetics, vol.32,no.1,pp.9–14,1968.
8. M. B. van der Weyden and W. N. Kelley, "Human adenosine deaminase.Distribution and properties," The Journal of Biological Chemistry, vol.251 ,no.18,pp. 5448–5456,1976.
9. L.Vergauwen,P. Hespel,andE.A.Richter,"Adenosine receptors mediate synergistic stimulation of glucose uptake and transport by insulin and by contractions in rat skeletal muscle," The Journal of Clinical Investigation, vol.93,no.3,pp.974–981,1994.
10. Warrier AC, Rao NY, Kulpati DS, et al. Evaluation of adenosine deaminase activity and lipid peroxidation level in diabetes mellitus. Indian J Clin Biochem 1995;10:9–13.
11. A. V. Zavialov, E. Gracia, N. Glaichenhaus, R. Franco, A. V. Zavialov, and G. Lauvau, "Human adenosine deaminase 2 induces differentiation of monocytes into macrophages and stimulates proliferation of T helper cells and macrophages," JournalofLeukocyteBiology,vol.88,no.2,pp.279–290,2010.
12. Kurtul N, Pence S, Akarsu E, et al. Adenosine deaminase activity in the serum of type 2 daibetic patients. Acta Medica (Hradec Kralove) 2004;47:33–5.
13. Giusti G, Galanti B. Colorimetric Method. Adenosine deaminase In: Bergmeyer HU, (ed). Methods of enzymatic Analysis, 3rd ed. Weinheim: Verlag chemie,1984; 315-23
14. Bhavita Patel et al "Serum Adenosine Deaminase in patients with Type-2 Diabetes Mellitus and its Relation with Blood Glucose and Glycated Hemoglobin levels " IJBR (2014) 05 (09)
15. Vineet Kumar Khemka, DebajitBagchi, Arindam Ghosh, Oishimaya Sen, Aritri Bir, Sasanka Chakrabarti, and Anindita Banerjee. Raised Serum Adenosine Deaminase Level in Nonobese Type 2 Diabetes Mellitus. The Scientific World Journal Volume 2013: 1-5.
16. Shaikh Sahema M et al "Adenosine deaminase activity and insulin in type 2 diabetes mellitus: does adenosine deaminase affects insulin level?" Int J Med Res Health Sci. 2014;3(4):918-921
17. Al-Duais MA, Sakran MI, Shalaby KA, Habib SA, Khamis AA. Diagnostic Value of Serum Adenosine Deaminase in Type II Saudi Diabetic Patients. Adv Diabetes Endocrinol 2015;1(1): 5.
18. Spencer N, Hopkinson DA, Harris H. Adenosine deaminase polymorphism in man. Annals of Human Genetics. 1968;32(1):9-14.
19. Shiva Prakash M, Chennaiah S, Murthy YSR. Altered Adenosine Deaminase activity in type 2 Diabetes Mellitus. JICAM. 2006;7:114-7.
20. Gitangali G, Neerja. The effect of Hyperglycemia on some Biochemical parameters in Diabetes Mellitus. JCDR. 2010;4:3181-6.
21. 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.
22. 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.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 11, 2024

23. Dong-Ho Han, Polly A. Hansen, Lorraine A. Nolte, and John O. Holloszy. Removal of Adenosine Decreases the responsiveness of muscle glucose transport to insulin and contractions. Diabetes 1998;47:1671–1675.