

Study of relationship between serum adenosine deaminase and lipid profile and type 2 diabetes mellitus

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Abstract

Introduction: Diabetes mellitus type -2 is one of the common metabolic disorder we face in our day to day life. it is characterized by high blood glucose level. Adenosine deaminase (ADA) is the enzyme which catalyses the irreversible deamination of adenosine to inosine. **Objective:** To estimate the level of serum adenosine deaminase (ADA), fasting blood glucose and lipid profile in patients with uncomplicated type -2 diabetes mellitus through case control study and also to know any correlation between plasma glucose values with ADA and lipid profile. **Method:** This study was conducted in Dept. of Obstetrics and Gynaecology, MNR Medical College and Hospital, Sangareddy, Medak. We took total 50 patients in our study, out of which 25 patients were with uncomplicated type-2 diabetes mellitus and other 25 were as healthy control group. Blood sample were taken and Adenosine deaminase levels were measured colourimetrically, based on the method described by Giusti and Galanti. Other routine investigation like- FBS, PPBS, lipid profile, Blood urea, Serum creatinine were also measured. **Results:** elevation of the serum ADP were found in diabetic patients as compared to controls and also strong correlations were found between serum ADA, lipid profile and plasma glucose levels. **Conclusions:** ADA level is significantly related with the type -2 diabetes mellitus. Any change of ADA level is an indirect expression of tissue adenosine levels. In pathophysiology of diabetes, ADA has its multimetabolic effects.

Keywords: Diabetes mellitus type-2, lipid profile, serum adenosinedeaminase (ADA).

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in the coming period and is estimated that 80 million people in India would be having Diabetes by the year 2030.² As the obesity increase, the risk of cardiovascular diseases, DM 2, renal function, cancer, impaired respiratory function, osteoarthritis, liver and gall bladder diseases are also increases³ diabetes account for 60% of all death in non-communicable disease. The most common age group are currently affected by Diabetes are 40-59 years.⁴ After bringing the long term glucose level by a single point reduces the risk of kidney failure and blindness by 37%.⁴ Hyper glycemia causes serious breakdown in lipid dynamics, often reflected by the elevated levels of circulating free fatty acids (FFAs) and triglycerides.⁵ Adenosine deaminase (ADA), an enzyme of purine metabolism modulates energy metabolism and is crucial in regulating the steady concentrations of adenosine. Emerging studies have shown ADA as a proinflammatory biomarker of various diseases especially

INTRODUCTION

Diabetes mellitus (DM) is the most common endocrinological disorder characterized by metabolic abnormalities and long term complications. The incidence and prevalence of type 2 DM is globally increasing and becoming a major public health problem for health care providers.¹ Diabetes is projected to increase significantly

in immunology, neurological and cardiovascular systems.^{6,7,8,9} ADA was found as a producer of reactive oxygen species (ROS), stimulator of lipid peroxidation^{10,11}, marker of both T-cell activation and glycemic status in diabetes mellitus(DM). ADA activity is high in lymphoid tissues and it increases lymphocyte proliferation and differentiation¹². In 1980, ADA was used as diagnostic tool in meningitis and TB. Elevated ADA levels were found in obesity, type-2 DM, Metabolic syndrome, liver cirrhosis and hepatoma¹³, TB, Brucellosis, Typhoid fever, Hypoxic states and cell mediated immune responses¹⁴. Several studies have demonstrated elevated of adenosine deaminase in individuals with Type 2 Diabetes mellitus, but exact pathologic role of elevated ADA activity in type 2DM remains to be elucidated^{12,17,18,19}. Studies have shown that ADA which reduces adenosine levels, increases basal and noradrenaline stimulated lipolysis in adipocytes.^{15,16} Insulin administration has been shown to reduce the elevated ADA levels in Type-2 diabetes.¹⁷ Adenosine has got insulin like activity on glucose and lipid metabolism particularly in adipose tissue and skeletal muscles. In view of increasing burden of Diabetes and mimicking action of adenosine like insulin we studied ADA as a new marker for detecting diabetes and its association with dyslipidemia and glycemic status (FBS) in Type -2 DM.

MATERIAL AND METHODS

The study was conducted at Dept. of Biochemistry in MNR medical College and Hospital. Total number of patients are 50. total number of controls are 25. Total number of patients without complication of type-2 diabetes mellitus is 25. Male and female both the patients were included under the age of 30-70 years of age in this study. A thorough clinical examination and appropriate investigations were done before selecting the cases and controls for the study. A detailed clinical history and physical examination was done. Routine investigation (blood urea, creatinine, lipid profile, serum adenosine deaminase, urine protein ECG, Fundoscopy) were done before selecting the subject for this study.

RESULTS

Table 1: Age distribution in cases and controls

Age in Years	Cases		Controls	
	NO.	%	NO.	%
30-40	3	12	3	12
41-50	6	24	9	36
51-60	13	52	8	32
61-70	3	12	5	20
Total	25	100	25	100
Mean	52.16		52.16	
SD	8.811		8.764	

This table shows the distribution of study subjects according to their age. The mean \pm SD among the diabetes and non-diabetes is almost same.

Table 2: Gender distribution in cases and controls

Gender	Cases		Controls	
	NO.	%	NO.	%
Male	18	72	16	64
Female	7	28	9	36
Total	25	100	25	100

This table shows the distribution of cases and controls according to their genders. There were 18 males and 7 females among the diabetic population and 16 males and 9 females among the control group. Samples are age and sex matched with p value=0.7216.

Table 3: Comparison of BMI in cases and controls

Sl. no.	BMI	
	Cases	Controls
1	29.2	22.5
2	33.6	21.8
3	28.4	24.6
4	31.2	22.8
5	32	21.6
6	29.7	22.7
7	28	24.2
8	30.4	22.6
9	26.6	21.8
10	28.4	20.6
11	24	23.2
12	28.6	22.9
13	28.8	23.6
14	25.7	24.8
15	29.2	23.8
16	28	22.7
17	26.6	24
18	22.8	22.7
19	23.4	22.6
20	27.6	23.7
21	26.5	23
22	28.1	28
23	35.6	22
24	23.4	24
25	31.2	24.6
Total	707	580.8
Mean	28.28	23.23
SD	3.119	1.43

The BMI \pm SD among the diabetic being 28.28 \pm 3.119 and 23.23 \pm 1.436 respectively with p value < 0.0001.

Table 4: BMI distribution in study group

BMI (kg/m ²)	Number	%
19-25	4	16
25-30	15	60
>30	6	24
Total	25	100.0

This table shows BMI distribution of diabetic population and the highest frequency of cases is seen in the BMI range of 25-30kg/m². The highest frequency is seen in the normal BMI range of 25-30 kg/m².

Table 5: Comparison of plasma glucose levels in cases and controls

SL. NO.	FBS		PPBS	
	Cases	Control	Cases	Controls
1	249	98	280	140
2	286	82	310	136
3	235	86	276	124
4	310	96	346	140
5	376	86	430	138
6	141	100	220	140
7	381	86	420	134
8	157	94	186	138
9	284	84	320	138
10	271	87	336	136
11	399	92	468	140
12	356	86	420	136
13	258	96	296	134
14	136	98	224	140
15	126	100	183	132
16	390	96.4	430	138
17	114	84	186	140
18	135	86	198	132
19	140	98	210	138
20	288	125	393	140
21	126	92	186	140
22	280	96	326	138
23	165	96	210	132
24	126	110	172	134
25	210	85	268	136
Total	5948	2339.4	7294	3678
Mean	237.56	93.57	291.76	147.12
SD	96.53	9.479	94.03	147.12
P value	<0.001		<0.001	

The mean \pm SD of FBS in cases is 237.56 ± 96.53 and in controls it is 93.576 ± 9.479 . The mean \pm SD of PPBS in cases is 291.76 ± 94.035 and in controls 136.56 ± 3.809 . A statistically significant difference exists with

respect to FBS and PPBS values between cases and controls. P values < 0.0001 is extremely significant.

Table 6: Comparison of serum adenosine deaminase in cases and controls

SL.NO.	SERUM ADA	
	CASES	CONTROLS
1	30	6
2	29	4.8
3	26	4.0
4	32	18.2
5	36	7.6
6	13	12.4
7	34	5.2
8	11	9
9	27	7
10	28	6.6
11	45	9.1
12	50.1	7.2
13	28	7.9
14	16	8.4
15	11	9.1
16	39	8.1
17	24.4	7.5
18	26	13.4
19	25.6	9.1
20	43.8	8.2
21	26	8.6
22	43.8	7.4
23	26	8.0
24	25	7.9
25	28	7.2
TOTAL	723.7	207.9
MEAN	28.95	8.31
SD	10.18	2.87
P value	<0.0001	

The mean value of serum ADA is 28.948 ± 10.182 and 8.316 ± 2.875 respectively for cases and controls. The difference is statistically significant with respect to p value < 0.0001 .

Table 7: Lipid profile in cases and control

SL.NO.	T. CHOL		TG		HDL		LDL		VLDL	
	CASE	CONT	CASE	CONT	CASE	CONT	CASE	CONT	CASE	CONT
1	172	174	264	139	29	38	128	108	53	27
2	220	139	258	134	28	38	140	74	52	26
3	182	147	224	184	30	39	107	71	45	36
4	256	174	280	215	32	33	168	98	56	43
5	280	142	324	162	28	39	187	71	65	32
6	120	182	119	139	41	37	55	118	24	27
7	276	193	311	188	28	39	186	117	62	37
8	190	158	186	132	30	36	123	96	37	26
9	300	214	280	169	30	39	214	141	56	34
10	280	141	311	138	29	35	189	78	62	27
11	286	164	336	179	27	39	192	89	67	36
12	246	157	326	144	29	35	152	93	65	28
13	230	136	286	167	30	38	143	64	57	33
14	156	206	180	139	35	40	85	138	36	27

15	121	180	152	216	36	37	55	99	30	43
16	196	159	226	241	31	33	120	77	45	48
17	156	148	180	194	36	38	84	71	36	38
18	244	199	146	132	39	40	175	132	29	26
19	208	142	158	162	42	39	134	71	31	32
20	280	139	324	115	29	39	187	77	65	23
21	182	139	134	37	38	117	74	74	27	26
22	180	120	125	145	42	35	113	56	25	29
23	210	117	165	127	50	35	127	57	33	25
24	223	141	109	126	37	32	165	83	21	25
25	204	176	168	147	37	39	154	109	33	29
TOTAL	5398	3987	5577	3968	842	930	3500	2262	1112	783
MEAN	215.92	159.48	223.08	158.72	33.68	37.2	140	90.48	44.48	31.32
SD	51.51	26.00	76.31	32.49	5.86	2.30	42.86	24.54	15.39	6.58
P value	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	

Mean + SD of total cholesterol in cases is $215.92 + 51.51$ and in controls it is $159.48 + 26.003$ with p value <0.0001 .

Mean + SD of the triglycerides in cases is $223.08 + 76.315$ and in controls it is $158.72 + 32.496$ with p value <0.0001 . Mean + SD of HDL in cases is $33.68 + 5.865$ and in controls it is $37.2 + 2.309$ with p value <0.0001 . Mean SD of LDL in cases is $140.0 + 42.863$ and in controls it is $90.48 + 24.546$ with p value <0.0001 . Mean SD of VLDL in cases is $44.48 + 15.39$ and in controls $31.320 + 6.587$ with p value <0.0001 .

Table 8: comparison of FBS with ADA in diabetic population

SL.NO.	FBS	SERUM ADA	TOTAL	MEAN	SD
1	249	30			
2	286	29			
3	235	26			
4	310	32			
5	376	36			
6	141	13			
7	381	34			
8	157	11			
9	284	27			
10	271	28			
11	408	45			
P Value					
< 0.001					

As the FBS is increasing ADA is increasing. Elevated glucose levels show increase in serum ADA in diabetic population. $P < 0.001$, considered very significant. The correlation of ADA and Blood Glucose parameters is positive and significant.

Table 9: Mean ADA level for different ranges of FBS in diabetic population

FBS RANGE	FBS mean + SD	ADA(U/L) Mean + SD	P value
101-200	136.67 + 15.291	20.47 + 6.742	< 0.001
201-300	262.33-26.828	31.511 + 7.056	< 0.001
301-400	368.67 + 32.197	39.35 + 6.949	< 0.001
Inference			As the FBS increasing, ADA is significantly with p < 0.001

As the FBS is increasing ADA is increasing. Elevated glucose levels show increase in serum ADA in diabetic population. $p < 0.001$, considered very significant.

Table 10: Correlation of serum ADA with TG/HDL

PARAMETER	MEAN	SD
Serum ADA	28.948	10.182
TG/HDL	6.805	3.185
R value	0.5156	

ADA Vs TG/HDL r value is 0.5156 and p value is 0.00001 showing that it is extremely significant. Considered very significant.

Table 11: Pearson correlation of age, BMI, FBS, TG/HDL with ADA in cases

VARIABLES	RELATION WITH ADA
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	R	P	Significance
Age	-0.05383	0.7983	Not significant
BMI	-0.05738	0.7853	Not significant
FBS	0.7764	< 0.001	significant
TG/HDL	0.5156	0.0083	significant

The correlation of ADA values with age and BMI is found to be in significant. The correlation of ADA with FBS and TG/HDL is significant. The correlation of ADA values with age, BMI and duration of DM is found to be insignificant and correlation with FBS and TG/HDL is significant.

DISCUSSION

The world- wide burden of the diabetes has increased and day by increasing and has reached in epidemic proportions. Being a chronic metabolic disorder, its long term complications could have devastating consequences.

AGE AND SEX DISTRIBUTION

Most of the diabetics under the study group were males (72%) compared to females (28%) which was comparable to Ajagnakar and sathi *et al* 1989 and Vaishnava *et al* 1989 who's studies implied that the incidence of Diabetes was greater among the male population compared to females. In this study Group most of the diabetics were in the age group 51-60 years, more so because it was a random study.

BLOOD GLUCOSE

The diagnosis of type 2 diabetes rests on the measurement of plasma glucose values. The diagnostic criteria for diabetes were changed in 1997. According to American Diabetes Association FBS >126 mg/dl is diagnostic of Diabetes. In our study the mean \pm SD of FBS values of type 2 diabetes cases was 237.56 ± 96.53 well above the American Diabetes Association criteria to diagnose Diabetes and the PPBS which was higher than the upper limit 291.76 ± 94.035 where the cut off value of 140 mg/dl. The control group had blood glucose values as 93.576 ± 9.479 and 136.56 ± 3.809 for FBS and PPBS respectively suggestively of normoglycemia. These values correlate well with clinical diagnosis.

SERUM ADENOSINE DEAMINASE

The present study found an elevated serum Adenosine deaminase (ADA) activity in cases of type 2 Diabetes Mellitus when compared to age and sex matched controls. Similar report were given by Hoshino *et al*⁸. Type 2 DM with chronic hyperglycaemia favours auto oxidation and also increases free radical activity. These results of elevated ADA in type -2 diabetes also correlated with studies done by Kurtul *et al* (2006)⁶ Lee *et al* and Shiva Prakash *et al*¹² found type 2 DM patients with relatively good glycemic control have low ADA activity compared to individuals with poor glycemic control. The Pearson correlation between the serum ADA and TG/ HDL ratio

in type 2 diabetes showed a significant correlation ($r=0.5156$; $p= 0.0083$). these findings are in accordance with Nisha subhas Chandra Ramani *et al*.¹⁴ Increased lipolysis is associated with increased levels of cyclic AMP (cAMP).^{15,16} It has been shown that in the adipose tissue most of adenosine is formed extracellularly. Extracellular adenosine is metabolised only by adenosine deaminase. The source of extracellular adenosine in the adipose tissue is currently unknown. It is possible that the extracellular adenosine may act in a retaliatory manner and try to inhibit lipolysis stimulated by increased levels of cyclic AMP. This pathway is known as the extracellular CAMP –adenosine pathway and has been shown in kidney, heart, brain, oviduct cells and also in the skeletal muscle.

LIPID PROFILE

Dyslipidemia was an obvious feature in the present study among the study group. TG and VLDL were significantly elevated in the study group when compared to controls. HDL were reduced among diabetics when compared to nondiabetics. These findings corroborated with the study conducted by Mazzone *et al* (2000)²⁰ where he documented increase in triglycerides. In this study it was observed that apart from an increase in triglycerides and VLDL and decrease in HDL, total cholesterol was also found to be raised in the study. A study conducted by V siva Prabodh *et al* (2012)²¹ also documented an increase in TG, total cholesterol, LDL, and decrease in HDL which was similar to the findings in the study. Study such as Adigun *et al* (2007)²², Albrki WM *et al* (2007)²³ also documented increased levels of TG, VLDL and decreased levels of HDL which has pretty much the picture in the study. P.K Bijlaani *et al* (1983)²⁴ and Barr *et al* (1951) found that HDL levels were depressed in diabetics which was one of the finding in this study as well mean HDL level of (33.68 ± 5.865) among the diabetic population compared to HDL level of (37.2 ± 2.309) among the nondiabetics. The combination of high TG, low HDL, and central obesity are the hall marks of the metabolic syndrome, which occurs in 80% of the people with diabetes mellitus. The frightening significance of this combination of risk factors is the marked incidence in these people of premature death from heart disease. Association between the elevated total cholesterol and insulin resistance was also statistically significant in the study conducted by Menik *et al*. In the study by H. Surekha Rani *et al*²⁵, an attempt has been made to evaluate the risk factors for coronary heart disease in DM patients. It is observed that FBS, PPBS, total cholesterol, triglyceride VLDL and LDL were high and the levels of HDL were low compared to controls.

CONCLUSION

Serum adenosine deaminase (ADA) levels were evaluated in type 2 Diabetes mellitus cases through a case control study using age and sex matched controls. Individuals who had developed complications of Diabetes were not included in the study. Serum levels of ADA were found to be significantly higher in type-2 diabetics when compared to controls. A very large correlation was found to exist between serum ADA and blood glucose values. Body mass index age and sex were not found to significantly influence the ADA level. The diabetic patients had a higher prevalence of high serum cholesterol, high triglyceride, high LDL-C, and low HDL-C than in controls, indicating that diabetic patients were more prone to cardiovascular diseases. These findings clearly project that serum Adenosine deaminase reflects the current glycemic status in the diabetic individual, indicating a possible involvement of the enzyme substrate Adenosine, in the diabetic processes.

REFERENCES

- Sheetz M.J King GL. Molecular understanding of hyperglycaemia's adverse effects for diabetic complications, *JAMA* 2002; 288:2579-88.
- Wild S 'Roglic G, Green A, *et al* Global prevalence of diabetes: estimates for the years 2000 and projections for 2030. *Diabetes care* 2004; 27: 1047-53.
- Stain Johanson. Metabolic roles of adenosine. Thesis 2007; Karolinska Institute.
- World Health Organization Diabetes updated march 2013-WHO media criteria.
- Denis Mcgarry J. Dysregulation of fatty acid metabolism in the etiology of type-2 Diabetes. Banting Lecture 2001. *Diabetes* 2002; 51(1):7-18.
- Kurtul N, Akarsu E, Aktaran S. The relationship between serum total sialic acid levels and adenosine deaminase activity in obesity. *Saudi Med J* 2006, 27, 170-2.
- Bottini E, Gerlini G, Lucirini N, Amante A, Gloria Bottini F. Evidence of selective interaction between adenosine deaminase and acid phosphatase polymorphisms in fetuses carried by diabetic women. *Hum Genet*. 1991, 87,199-200.
- Hoshino T, Yamada K, Masuoda K, Tsuboi L, Itoh K, Nonaka K. Elevated adenosine deaminase activity in the serum of patients with Diabetes Mellitus. *Diabetes Res Clin Prac* 1994, 25 97-102.
- Singh LS, Sharma R, Alloxan Diabetes regulates adenosine deaminase activity in mice: tissue and age specific correlation. *Biochem Mol Biol Int* 1998, 46, 55-61.
- Gitangali G, Neerja. The effect of Hyperglycemia on some Biochemical parameters in Diabetes Mellitus. *JCDR* 2010, 4, 3181-6.
- 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 Inflamm* 2003, 12, 107-16.
- Shiva Prakash M, Chennaiah S, Murthy YSR. Altered Adenosine Deaminase activity in type 2 Diabetes Mellitus. *JICAM* 2006, 7, 114-117.
- Selva Kumar C, Kalaivani R, study of adenosine Deaminase and serum protein Bound sialic acid levels in alcoholic Liver Disease. *Int J Biol Med Res*. 2011, 2754-756.
- Nisha Subhas Chandra Ramani, Krishna Murthy N, Raghavendra Prasad BN *et al*. Role of Adenosine Deaminase to predict glycemic status in type -2 Diabetes mellitus. *Jclin Bio med Sci* 2012, 2, 123-2.
- John N. Fain, Paul W. Weiser. Effects of adenosine deaminase on cyclic adenosine monophosphate accumulation, lipolysis and glucose metabolism of fat cells. *The Journal of Biological Chemistry* 1975; 250 (3): 1027-1034.
- Peter Arner, Jan Ostman, Relationship between the tissue level of cyclic AMP and the fat cell size of human adipose tissue. *Journal of Lipid Research* 1978; 19:613-618.
- Madhavi Reddy A, Rao Y.N, Yogendra Singh, Alpana Saksena. Adenosine Deaminase and protein Tyrosine Phosphatase activities in liver and peritoneal macrophages of streptozocin induced diabetic mice. *Indian Journal of Clinical Biochemistry*. 1995; 10 (2): 66-71.
- Anjali C. Warrier, Narasimha Y.Rao, Tarun K. Mishra *et al*. Evaluation of Adenosine Deaminase activity and lipid peroxidation levels in Diabetes Mellitus. *Indian Journal of Clinical Biochemistry*. 1995; 10 (1): 9-13.
- Mustafa Araz, Yuksel, Ozdemir, Mehmet, Tarakcyoolu *et al*. Elevated Adenosine Deaminase Activity is not implicated in microvascular complications of type 2 Diabetes Mellitus Except HbA1c. *Turkish Journal of Endocrinology and Metabolism*. 2000; 4(3) : 95-99.
- Mazzone T, 2000: Current concepts and controversies in the pathogenesis, prevention of the macrovascular treatment complications of diabetes. *J.Lab*
- Samantha P, Venkateswru M, Siva Probodh V. Lipid profile levels in type-2 diabetes mellitus from the tribal population of Adilabad, Andhra Pradesh, India. *Journal of clinical and Diagnostic Research*. 2012 may (suppl-2) Vol-6(4) : 590-592.
- Idogun ES, Unuigbe EL, Ogunro PS, Akinola OT, Fanodu AA, Assessment of the serum lipids in Nigerians with type-2 Diabetes Mellitus complications. *Pak.J.Med.Sci. (part-1)* 2007: 23(5) : 708-12.
- Albrki WM,Elzouki ANY, EL -Mansouri ZM, Tashani OA, Lipid profiles in Libian Type 2 diabetes. *J.Sci.Apps* 2007: 1(1) : 18-23.
- Bijlani PK, Shah Kokila Raheja BS, HDL cholesterol in Diabetics. *JAPI* 1984; 32.
- H.Surekha Rani, G. Madhvi, V rama Chandra Rao, B.K Sahay and A. Jyothi. Risk factors for coronary Heart Disease in type 2 Diabetes Mellitus. *Indian Journal of clinical Biochemistry* 2005, 20 (2): 75-80.

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