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Serum Adenosine Deaminase in Type 2 Diabetes Mellitus and Its Association with Diabetic Retinopathy

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Abstract: *Diabetes is one of the largest global health emergencies of the 21st century. Diabetic retinopathy is a common microvascular complication of diabetes. Adenosine deaminase (ADA) is considered to be a marker of inflammatory responses. Serum ADA activity is found to be increased in hyperglycemic condition but its role in diabetes is not yet clearly known. The present study was done to measure serum Adenosine deaminase in type 2 diabetes mellitus and in healthy controls and to find out any correlation between serum Adenosine deaminase and diabetic retinopathy. Serum ADA, FBS, PPBS, HbA1cand lipid profile were significantly increased in diabetic subjects. Elevated ADA in diabetes was significantly increased with increasing HbA1c, FBS and PPBS suggesting its role in glycemic status. The mean FBS, PPBS, and HbA1c were also increased in diabetic retinopathy though it was insignificant. Diabetic retinopathy was significantly associated with longer duration of diabetes. Serum ADA was significantly higher in diabetic retinopathy subjects as compared to controls but significantly lower than diabetes without retinopathy. Serum ADA tends to fluctuate with the severity of retinopathy.Serum ADA could be an important biochemical marker for diabetes mellitus and of glycemic status.*

Keywords: *Adenosine deaminase, Diabetes mellitus, Diabetic retinopathy, Inflammation*

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I. Introduction

Adenosine deaminase (ADA), is also known as adenosine aminohydrolase. It is an enzyme present in most tissues of the body and involved in purine metabolism. It catalyses the irreversible deamination of adenosine to produce inosine and ammonia[1]. It is considered to be a marker of inflammatory responses. Release of ADA by cells such asmacrophages and monocytes into their surrounding environments leads to the increase of serum ADA activity seen during inflammation[2].Serum ADA activity is found to be increased in hyperglycemic condition but its role in diabetes is not yet clearly known[3].

Diabetes is one of the largest global health emergencies of the 21stcentury[4].According to International Diabetes Federation (IDF) Diabetes Atlas 8th edition, India has a total of 74 million people with diabetes currently which is expected to rise to 134.3 million by 2045[5].Diabetes and its associated complications are the major cause of morbidity and mortality in the whole world. Diabetes has put huge burden to the health care system due to long disease duration and its associated complications. Improving glycemic control will lead to decrease in the incidence of diabetic complications and disability associated with diabetes as well as health care costs[6].

Inflammation plays an importantrole in diabetes that leads to long term complication in specific organs particularly in eye[7].Diabetic retinopathy (DR) is primarily a disease of the retinal microvasculature[8]. It is a low grade inflammatory disease[9].Diabetic retinopathy involves damage to the microvasculature of the retina resulting from prolonged exposure to high glucose and the biological changes induced by diabetes[10].Diabetic retinopathy may have a sudden impact on visual acuity. It is the leading cause of blindness in working aged populations worldwide[11].

About 21% of type 2 diabetes mellitus (T2DM) patients have retinopathy at the time of first diagnosis of diabetes. Most of the diabetic patients develop some degree of retinopathy over time. More than 60% of patients with T2DM have some form of retinopathy after 20 years of the disease. Duration of diabetes is predicted to be the strongest predictor for the development and progression of retinopathy[12].Broadly diabetic retinopathy is divided into non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR).Estimated overall prevalence of any diabetic retinopathy was 35% and for PDR was 7.0% among individuals with diabetes around the world. The overall prevalence of diabetic retinopathy in T2DM was 25.2%[13].

Adenosine deaminase has been implicated as a contributing factor for the pathogenesis of T2DM and its complications. Association was found between serum level of ADA and glycemic control suggesting a role of ADA in glucose metabolism in diabetes.Therefore, the present study was taken up with the following aim to measureserum Adenosine deaminase in type 2 diabetes mellitus and to find out any correlation between serum Adenosine deaminase and diabetic retinopathy.

II. Materials And Methods:

A cross sectional study was done in the Department of Biochemistry in collaboration with Department of Medicine and Department of Ophthalmology, JNIMS. Clearance and ethical approval was obtained from Institutional Ethics Committee, JNIMS. The study was done from September 2016 to August 2018.

Written informed consent of the patients or their relatives was taken prior to inclusion in the study.A thorough history was taken and detailed physical examination and relevant laboratory investigations were done for all subjects as per the proforma.Instructions were given to all the subjects to come fasting the next morning.

Inclusion criteria:

Subjects in the age group 30-80 years attending medicine or ophthalmology out-patient clinic or admitted in the ward in J.N. Institute of Medical Sciences, who have consented for the study were randomly selected, irrespective of sex, caste or religion.

The study was conducted in two broad groups –

Case: A total of 100 diagnosed T2DM subjects in age group 30-80 years, were included as cases.

Control: Another age and sex matched group of 50 non-diabetic apparently healthy individuals who came for routine health checkup was recruited as control group.

Exclusion criteria:

- Patient who are below 30 years or above 80 years.
- Type 1 diabetes mellitus.
- Patient suffering from illness like tuberculosis, hepatitis, sarcoidosis, HIV, underlying liver, kidney, lung diseases, malignancy, hypertension and other systemic diseases.
- Pregnant and lactating women.
- Patient on insulin therapy.

Eye examination:All the study subjects were referred to ophthalmology OPD for fundoscospy. After checking the visual acuity, the eyes were dilated with a mydriatic eyedrop and subjected to fundus examination with direct ophthalmoscopy to detect the presence of retinopathy if any.

Laboratory tests done:

After taking informed consent, about 5ml of venous blood was drawn from cubital vein under aseptic precaution in a sterile vialsafter overnight fasting. Sodium fluoride vials were used for fasting plasma glucose estimation, EDTA vials for glycated hemoglobin and plain vials for other analysis. Serum ADA was measured based on the method described by Guisti and Galanti[14].Fasting plasma (FBG) & Post prandial plasma glucose (PPBG) were measuredby Glucose-oxidase peoxidase method [15], and Glycated hemoglobin (HbA1c) by Cation exchange method[16]. Total cholesterol & HDL by CHOD-POD/ Phosphotungstate Method[17,18]; triglyceride by GPO-POD (Glycerol Phosphate Oxidase-Peroxidase) Method[19]. LDL-cholesterol and VLDL-cholesterol was indirectly calculated using Friedewald formula [20]. All the biochemical parameter were analysed on the same day.

Statistical Analysis:

Data collected were entered in Microsoft excel. Statistical analysis was performed using SPSS software. Data were described using frequency, percentage and mean SD. For categorical data, test of significance was performed using chi square test, and for quantitative data, student T test was used. One-way ANOVA with Post Hoc Bonferroni comparison and Pearson correlation was used to find out the correlation between various data. Probability value (P-value) of less than 0.05 (<0.05) was taken as significant.

III. Results

Majority of the cases (49%) had 2-5 years duration of diabetes. About 21 % of the case had T2DM for 6-9 yearsand 15 % each for had diabetes less than 1 year and more than 10 years.The mean ADA, FBS, PPBS, and HbA1c were significantly increased in diabetic subjects as compared to controls. Serum ADA level was significantly increased with increasing FBS, PPBS and HbA1c. The mean total cholesterol, triglyceride, LDL, and VLDL were higher in cases than in controls and the finding was significant. Mean HDL was significantly lower in cases than in controls.

Among the 100 diabetic subjects, 24 (24%) of them have some form of retinopathy. Majority of subjects (91.66 %) had NPDR and only 8.3 % had PDR. The mean duration in diabetic retinopathy subjects was 7.7 ± 5.4 years and that of cases without retinopathy was 4.6 ± 3.4 years.Serum ADA was significantly higher in subjects with diabetic retinopathy as compared to controls but significantly lower than diabetes without retinopathy.

When FBS, PPBS, HbA1c, duration of diabetes and lipid profile were compared with cases with retinopathy and without retinopathy, only duration of diabetes was significantly increased in cases with diabetic retinopathy.There is no significant difference in lipid profiles among diabetes cases with retinopathy and diabetes without retinopathy. However, all the lipid parameters were found to be increased in diabetic retinopathy subjects except HDL which was decreased in diabetic retinopathy subjects.

IV. Figure Legends And Tables

Table1 : Biochemical parameters in cases and controls

Parameters	Cases (mean ± SD)	Controls (mean ± SD)	t-test p-value
Age (years)	55.0 ± 7.7	45.8 ± 9.2	-
Gender Female Male	 43 57	 25 25	-
BMI	23.5 ± 1.8	22.9 ± 1.4	p-0.057
FBS (mg/dl)	157.0 ± 52.5	89.8 ± 8.1	p-0.000
PPBS (mg/dl)	232.2 ± 84.3	111.3 ± 11.5	p-0.000
HbA1c (%)	10.2 ± 3.5	4.3 ± 0.5	p-0.000
Total cholesterol (mg/dl)	181.4 ± 43.0	158.5 ± 29.5	p-0.000
Triglyceride (mg/dl)	166.0 ± 47.5	129.9 ± 33.3	p-0.000
LDL (mg/dl)	108.45 ± 43.3	86.6 ± 33.3	p-0.001
HDL (mg/dl)	40.7 ± 9.3	44.9 ± 7.8	p-0.005
VLDL (mg/dl)	32.8 ± 9.5	25.5 ± 6.8	p-0.000

Table2 : Comparison of ADA level among cases and controls.

Groups	ADA (mean ± SD) in U/L	p-value
Cases	34.6 ± 7.7	p-0.000
Controls	18.2 ± 3.8	
Cases without retinopathy (76)	36.5 ± 7.7	P – 0.000
Cases with retinopathy (24)	28.6 ± 3.7	
Controls	18.2 ± 3.8	

Table 3 :ADA level among diabetic retinopathy.

Severity of retinopathy (n)	ADA (mean ± SD) U/L	One way ANOVA
Mild NPDR (16)	28.5 ± 2.2	F=4.4 p-0.017
Moderate NPDR (4)	32.2 ± 5.8	
Severe NPDR (2)	22.5 ± 3.9	
PDR (2)	28.2 ± 0.2	

Table 4 : Correlation between ADA level and others variables.

Variables	Pearson correlation (r)	p-value
FBS	0.394	p-0.000
PPBS	0.668	p-0.000
HbA1c	0.719	p-0.000
Total Cholesterol	0.331	p-0.000
Triglyceride	0.334	p-0.000
LDL	0.277	p-001
HDL	-0.133	p-0.104
VLDL	0.350	p-0.000

V. Discussion:

The serum ADA activity was found to be increased in Diabetic subjects as compared to controls which is in accordance with study done by Warrier AC[21]. Hoshino T et al[22] observed increased serum ADA level in patients with diabetes mellitus and normalization of blood glucose level by insulin injection was associated with the decrease in ADA level.

Mean FBS, PPBS, and HbA1c were significantly more in cases than in controls. Serum ADA level was significantly increased with increasing FBS, PPBS and HbA1c which is in consistent with previous studies done by Kurtul N et al[23]and Lee JG et al[24].

Since ADA regulates the action of insulin [25], increased ADA in diabetes may be associated with defects in insulin action.Increased ADA in diabetes is also explained by extracellular cAMP adenosine pathway. Increased dose of cAMP perfused into tissues such as adipose tissue and skeletal muscles increased the levels of AMP and adenosine, which are the sources of ADA[26].Chronic hyperglycemia is associated with chronic low-grade inflammation that plays an important role in pathogenesis of diabetes mellitus[27].Elevated serum ADA could be due to chronic hyperglycemia of diabetes and it could be a marker of inflammation in diabetes.Adenosine also regulates the action of lipid metabolism. It has been reported that adenosine removal by ADA resulted in rise in lipolytic activity. Increased ADA may increase hyperlipidemia by increasing lipolysis[28].

Diabetic retinopathy was found to be associated with longer duration of disease. The present finding is in consistent with studies done by Jenchitr W et al[29] and Niazi MK et al[30], who noted that duration of diabetes was strongly associated with diabetic retinopathy.

Serum ADA level tend to fluctuate with the severity of retinopathy.Decreased in serum ADA level in DR could be due to high glucose condition. An increase in extracellular adenosine levels accompanied by decrease in intracellular adenosine level has been demonstrated when retinal cells were subjected to high glucose condition[31]. Increased adenosine in DR may lead to increased utilization of ADA and subsequent decrease in ADA level.

Adenosine deaminase may be important in the development of early inflammation during diabetic retinopathy. Increased ADA activity is accompanied with retinal inflammation in diabetes.Activation of ADA may produce soluble inflammatory cytokines leading to increase permeability to human retinal vascular endothelial cells (HRECs)[32].Adenosine concentration is markedly increased under hypoxic conditions.Hypoxia and inflammation is the feature of DR. Increased extracellular adenosine concentration during hypoxia leads to increased ADA. Adenosine deaminase gains more importance when adenosine level is increased as in ischemic conditions and cellular distress[33].In contrast to the present study, Erbagei AB et al[34] also observed no correlation between ADA and chronic complications of diabetes such as diabetic retinopathy.

VI. Conclusion:

As seen in the present study, serum ADA was elevated significantly in diabetic subjects and a significant positive correlation was found between ADA and glycemic status. In this background, determination of serum ADA could be an important biochemical parameter to determine the glycemic status of diabetes mellitus. Serum ADA was significantly higher in DR subjects as compared to controls but significantly lower than diabetes without retinopathy. Serum ADA tend to fluctuate with the severity of retinopathy. So, serum ADA as a marker for evaluation of Diabetic Retinopathy needs further study involving a larger sample size.

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