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STUDY OF SERUM ADENOSINE DEAMINASE ACTIVITY IN TYPE 2 DIABETES MELLITUS AND ITS CORRELATION WITH SERUM URIC ACID AND GLYCATED HEMOGLOBIN.

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Abstract

Background & Objectives:

Diabetes Mellitus (DM) is a metabolic disorder characterized by an absolute or relative deficiency of insulin and insulin resistance or both. Adenosine deaminase (ADA) is an enzyme, that catalyses the irreversible hydrolytic deamination of adenosine to uric acid. The present study was undertaken to evaluate the level of serum ADA, serum uric acid and correlate it with Blood Sugar Fasting and Glycated Hemoglobin in patients of Type 2 DM.

Material and Methods: A total of 100 patients diagnosed for type 2 DM visiting the Outpatient Department of General Medicine and Endocrinology at Mahatma Gandhi Medical College & Hospital, Jaipur were enrolled for the study based on predefined inclusion and exclusion criteria. Blood samples were collected for all enrolled patients and analysed for the investigations like Serum BSF, HbA1c Serum ADA and serum Uric acid.

Results: In the study, all the parameters BSF, mean HbA1c, serum ADA and serum uric acid level were significantly higher in diabetic group in comparison to control group (p=0.000). The diabetic group were further subdivided on the basis of HbA1c levels, HbA1c ≤ 8% as good glycemic control and HbA1c > 8% as poor glycemic control. BSF, mean HbA1c, serum ADA and serum uric acid levels were observed to be significantly higher in poor glycemic control group as compared to that of good glycemic control. A significant positive correlation between S. ADA and HbA1c activity (r= 0.388) and between S. ADA and serum uric acid was also seen (r=0.252).

Conclusion: From the present study, it is concluded that there is an increase in serum ADA levels and serum uric acid level with increase in Glycated hemoglobin levels (HbA1c > 8%). Increase in serum ADA level was found to be associated with increase in Glycated hemoglobin levels which may play an important role in determining the glycemic status in diabetes. Further, increase in serum uric acid in levels could be due to increased activity of ADA, an enzyme that convert adenosine to uric acid. Hence, by analyzing ADA levels and uric acid level in diabetes, glycemic control and insulin resistance can be assessed.

Keywords: Diabetes Mellitus, Adenosine Deaminase, Glycated hemoglobin, Uric Acid.

Introduction

Diabetes mellitus type 2 (type 2 DM) is a disorder of multiple aetiologies, which is characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from deficiency of insulin, deficiency in action of insulin or both.[1] The chronic hyperglycemia of diabetes is associated with significant long-term sequel, particularly damage and/ or dysfunction of various organs, especially the heart, blood vessels, kidneys, eyes and nerves. [2]. According to recent estimates by the International Diabetes Federation (IDF), approximately 285 million people worldwide (6.6%) in the 20–79 year age group had diabetes in 2010 and by 2030, 438 million people (7.8%) of the adult population, is expected to have diabetes. In India, the estimated no. of diabetics was 50.8 million in 2010 and expected to rise to 87.0 million by 2030. [3]

Type-2 diabetes is characterized by insulin resistance where there is impaired ability of hormone to suppress hepatic glucose output and to promote peripheral glucose disposal and compromised function of pancreatic β-cells such that insulin secretion is insufficient to match the degree of insulin resistance. [4]

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). [5] The enzyme exists in two isoenzyme forms: (ADA1 and ADA2) which are coded by

separate genes.[6] ADA is considered as a good marker of cell mediated immunity. [7] High lymphocyte ADA activities were found to be elevated in diseases in which there is a cell mediated immune response.[8] 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. [9]

Uric acid is formed by the breakdown of purines and by direct synthesis from 5-phosphoribosyl pyrophosphate and glutamine. [10] Hyperuricemia is defined as serum urate level of 6.8 mg/dl (404µmol/litre). [11] The rising prevalence and incidence of hyperuricemia are probably related to the increased life expectancy of the population, increasing level of obesity, sedentary lifestyles and change in dietary lifestyles. [12]Several epidemiologic studies have reported that high serum levels of uric acid are strongly associated with prevalent health conditions such as obesity, insulin resistance, metabolic syndrome, diabetes, essential hypertension, and renal disease. [13] Even though there are many reports available on serum adenosine deaminase levels and uric acid levels in patients of type 2 diabetes mellitus but no conclusive study could be established. Hence, based on the above mentioned facts, the present study was designed to evaluate the serum ADA activity in patients of Type 2 diabetes mellitus with and without complication and its comparison with the controls and further to find any correlation of serum ADA activity and serum uric acid levels with the glycemic control in patients of Type 2 diabetes mellitus.

Materials & Methods:

The study was conducted in Department of Biochemistry in collaboration with the Department of General Medicine of Mahatma Gandhi Medical College& Hospital, Jaipur. Patients diagnosed with type 2 DM visiting the Outpatient Department of General Medicine & Endocrinology were enrolled for the study. The study was conducted after seeking approval from the Institutional Ethics Committee (IEC). Informed consent was taken from all the subjects before enrolling for the study.

This study includes 150 subjects out of which 100 are cases and 50 controls.

Inclusion criteria:

a) 20-65 years of age.

b) Known cases of type 2 diabetes mellitus

c) Patient willing to participate.

Exclusion Criteria

a) Patients not willing to participate.

b) Patients on insulin treatment, gestational diabetes mellitus, hemolytic anemia, Hb variants.

c) Patients with chronic diseases such as tuberculosis, rheumatoid arthritis, gout, renal failure, immunological disorders which alters ADA level.

d) Pregnant & Lactating females.

e) Patients on any substance abuse.

Methodology

Patients were selected from outpatient departments were subjected to a physical examination. An informed consent was taken before the collection of the sample from cases and controls. The control subjects had the same exclusion criteria as the cases and were not on any drug regimens which could influence the study. The study was conducted after approval from the institutional Ethics committee. Blood samples after overnight fasting were collected by standard aseptic techniques.

Plasma blood sugar in fasting sample, HbA1c, Serum ADA were estimated by colorimetric method on fully automated analyzer VITROS 4600.

Following Parameters to be estimated

1) Blood Sugar Fasting (BSF)

2) Glycosylated Hemoglobin (HbA1c)

3) Serum Adenosine Deaminase (ADA)

4) Serum Uric Acid (UA)

Estimation of Blood Glucose

Quantitative determination of Serum glucose was done by colorimetric –Glucose Oxidase Peroxidase method.

Principles

The VITROS GLU Slide method is performed using the VITROS GLU Slides and the VITROS Chemistry Products Calibrator Kit 1 on VITROS 250/350/950/5, 1

FS and 4600 Chemistry Systems and the VITROS 5600 Integrated System. The VITROS GLU Slide is a multilayered, analytical element coated on a polyester support. A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. The oxidation of sample glucose is catalyzed by glucose oxidase to form hydrogen peroxide and gluconate. This reaction is followed by an oxidative coupling catalyzed by peroxidase in the presence of dye precursors to produce a dye. The intensity of the dye is measured by reflected light.

Estimation of HbA1c

The determination of % glycated hemoglobin (HbA1c) is performed using the VITROS Chemistry Products HbA1c

Reagent Kit.

Whole blood samples are hemolyzed on the VITROS 5, 1 FS/4600 Chemistry Systems and the VITROS 5600 Integrated System. Calibrators, controls and hemolyzed whole blood samples are mixed with Reagent 1 containing anti-HbA1c antibody to form a soluble antigen-antibody complex. Hemoglobin in the hemolyzed whole blood is converted with Reagent 1 to a hematin derivative that is measured biochromatically at 340 nm and 700 nm. Unbound anti-HbA1c antibody reacts with polyhapten (hexapeptide-glycan, A1c Reagent 2) to form an insoluble antibody-polyhapten immune complex, which is measured turbidimetrically at 340 nm. After a calibration has been performed for each reagent lot, the hemoglobin A1c and Hb. concentrations in each unknown sample can be determined using the stored calibration curves and the measured absorbance obtained in the assay of the hemolyzed sample.

% A1c

%A1c is a derived test calculated from the quantitative measurements of hemoglobin and hemoglobin A1c.

Reference Ranges: HbA1c = 4.0-6.0%

Estimation of Serum ADA

Principle

The ADA assay consists of four steps:

The ADA assay is based on the enzymatic deamination of adenosine to inosine which is converted to hypoxanthine by purine nucleoside phosphorylase (PNP). Hypoxanthine is then converted to UA and hydrogen peroxide (H2O2) by xanthine oxidase (XOD). H2O2 is further reacted with N-Ethyl-N-(2-hydroxy3-sulphopropyl)-3-methylaniline (EHSPT) and 4-aminoantipyrine (4-AA) in the presence of peroxidase (POD) to generate quinone dye which is monitored in a kinetic manner.

Reference Value: Serum/Plasma = 4-22 U/L

Estimation of Serum Uric Acid

Principle

The VITROS URIC ACID Slide is a multilayered, analytical element coated on a polyester support.

A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. UA from the sample migrates to the reagent layer, where it is oxidized in the presence of uricase to form allantoin and hydrogen peroxide. Hydrogen peroxide oxidizes a leuco dye in the presence of peroxidase to generate a colored dye. The reflection density of the dye is measured by reflectance spectrophotometry.

Reference values

Male: 3.5-8.5 mg/dl

Female: 2.5-6.2 mg/dl

Statistical Analysis

All results obtained were presented as mean ± SD in the patients as well as control group. The diabetic group was subdivided on the basis of HbA1c levels, HbA1c ≤ 8% as good glycemic control and HbA1c > 8% as poor glycemic control. The results were compared by applying Student's t-test. The correlation of Serum ADA with HbA1c and Uric acid was calculated by applying Pearson's correlation. P-value of ≤ 0.05 was considered as statistically significant.

Results

Table 1: represents the age distribution among control group and diabetic group. The mean age of subjects in the diabetic as well as control group was comparable (P=NS).

Groups	No. of cases (N)	AGE (Years) (Mean ± SD)	t–value	P – value
Control	50	47.60 ±13.17	-1849	NS
Diabetes	100	51.12 ± 9.73		

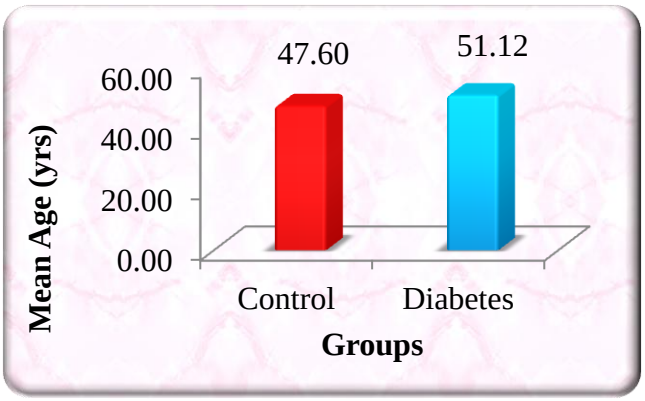


Table 2: represents the male: female ratio for all subjects of the study. The male: female distribution in control group was 56: 44 whereas in Diabetic group it was 67: 33.

Groups	No. of cases (n)	Male	%	Female	%
Control	50	28	56%	22	44%
Diabetes	100	67	67%	33	33%

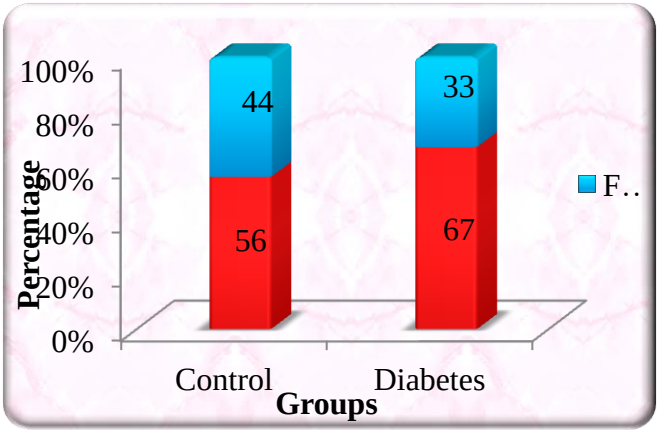


Table 3.1: represents the mean BSF level in control group and diabetic group. There was significant difference in BSF level between control group and study group (P=0.000).

Groups	No. of cases (n)	BSF (mg %) (Mean ± SD)	t– value	P – value
Control	50	94.60 ± 12.79	-10.731	0.000
Diabetes	100	261.68 ± 109.54		

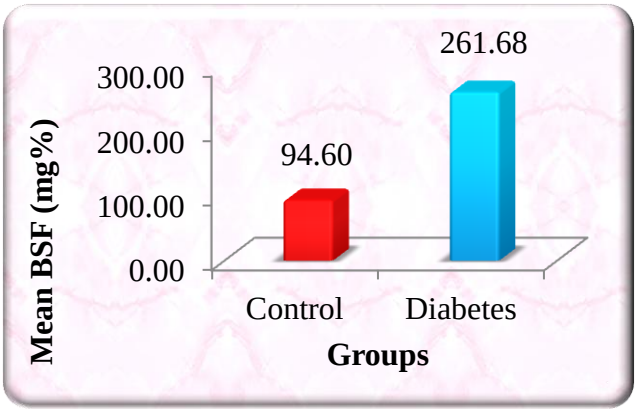


Table 3.2: represents the mean HbA1c levels in control group and diabetic group. The mean HbA1c levels in control group were 5.42±0.38 % and in diabetic group was 10.08 ± 2.46%. The difference between the two groups was statistically significant (P=0.000).

Groups	No. of cases (n)	HbA1C (%) (Mean ± SD)	t– value	P – value
Control	50	5.42 ±0.38	-13.294	0.000
Diabetes	100	10.08 ± 2.46		

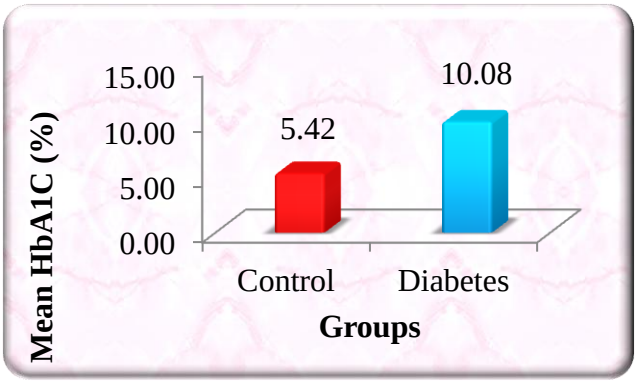


Table 3.3: represents the mean serum ADA levels in control group and diabetic group. The mean serum ADA levels in control group was 15.83 ± 4.35 U/L, while in Diabetic group it was 36.15 ± 17.47 U/L. Statistical analysis showed that the mean serum ADA level in diabetic group was significantly higher than the control group (P= 0.000).

Groups	No. of cases (n)	ADA (U/L) (Mean ± SD)	t– value	P – value
Control	50	15.83 ± 4.35	-8.088	0.000
Diabetes	100	36.15 ± 17.47		

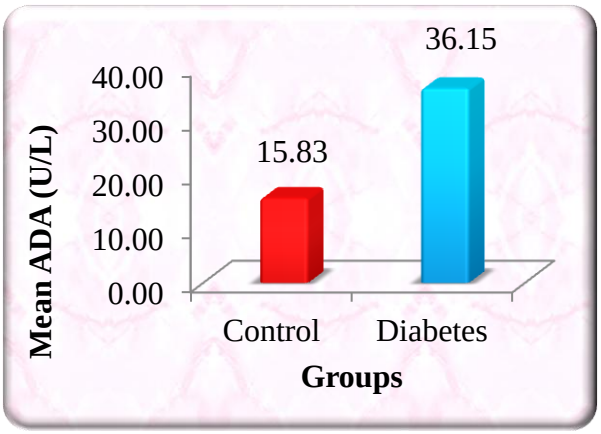


Table 3.4 represents the mean serum UA levels among control group and diabetic group. The mean UA level in control group was 4.33 ± 1.0 mg/dl and in Diabetic group was 5.47 ± 1.48 mg/dl. There was a significant increase in UA level between diabetic group and control group (P=0.000).

Further, the diabetic patients (n = 100) were sub grouped on the basis of the HbA1c levels as: HbA1c ≤ 8% (Good Glycemic Control) n = 32 HbA1c ≥ 8% (Poor Glycemic Control) n = 68

Groups	No. of cases (n)	UA (mg/dl) (Mean ± SD)	t– value	P – value
Control	50	4.33 ± 1.0	-4.911	0.000
Diabetes	100	5.47 ± 1.48		

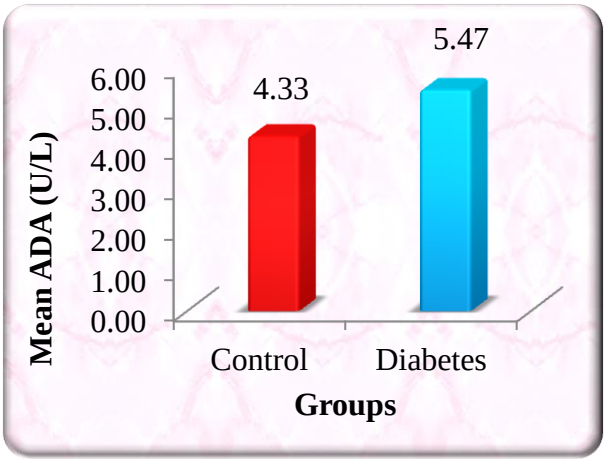


Table 4: represents age distribution among two diabetic groups. The mean age for good glycemic control was 53.78 ± 8.48 years and for poor glycemic control was 50.85 ± 10.67 years.

Groups	No. of cases (n)	Age(Years) (Mean ± SD)	t– value	P – value
HbA1C ≤ 8.0	32	53.78 ± 8.48	1.363	NS
HbA1C > 8.0	68	50.85 ± 10.67		

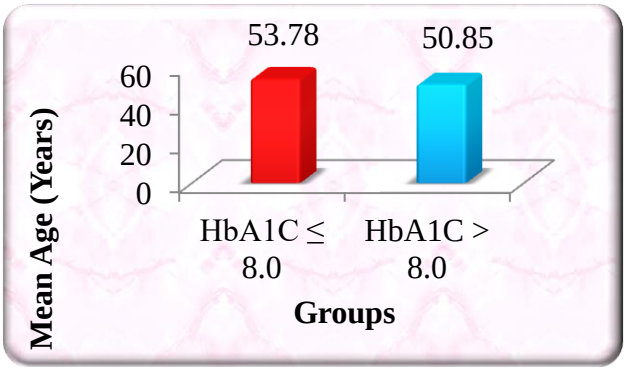


Table 5.1: represents the mean BSF level in two diabetic groups. The mean BSF level for diabetic group with good glycemic control was lower 224.75 ± 81.43 than that of diabetic group with poor glycemic control 276.97 ± 116.46 mg% and the difference was statistically significant (P=0.025).

Groups	No. of cases (n)	BSF (mg %) (Mean ± SD)	t-Value	P-value
HbA1C ≤ 8.0	32	224.75 ± 81.43	-2.284	0.025
HbA1C > 8.0	68	276.97 ± 116.46		

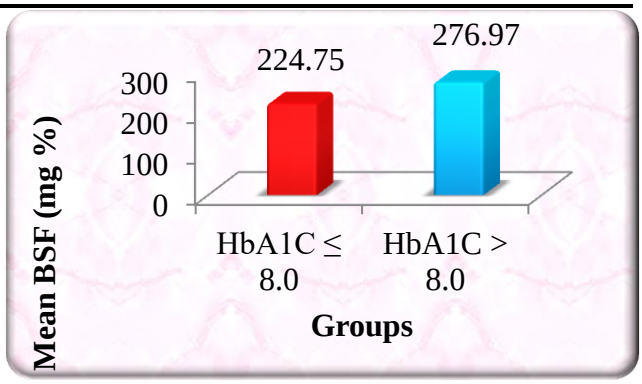


Table 5.2: represents the mean HbA1c level in two diabetic groups. The mean HbA1c level for poor glycemic control was 11.68 ± 1.86 % and for good glycemic control was 7.31 ± 0.50 and difference was significant (p=0.000).

Groups	No. of cases (n)	HbA1C (%) (Mean ± SD)	t-Value	P-value
HbA1C ≤ 8.0	32	7.31 ± 0.50	-12.144	0.000
HbA1C > 8.0	68	11.38 ± 1.86		

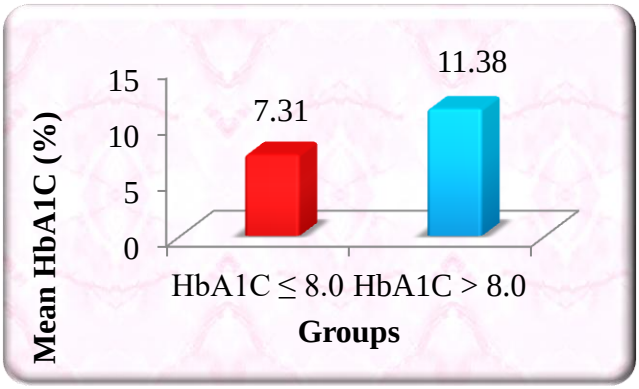


Table 5.3: represents serum ADA levels in two diabetic groups. The mean serum ADA levels was28.68 ±9.33 U/L for good glycemic control and 41.58 ± 18.53 U/L for poor glycemic control and the difference was statistically significant (p=0.000).

Groups	No. of cases (n)	ADA (U/L) (Mean ± SD)	t-Value	P-value
HbA1C ≤ 8.0	32	28.68 ± 9.33	-3.716	0.000
HbA1C > 8.0	68	41.58 ± 18.53		

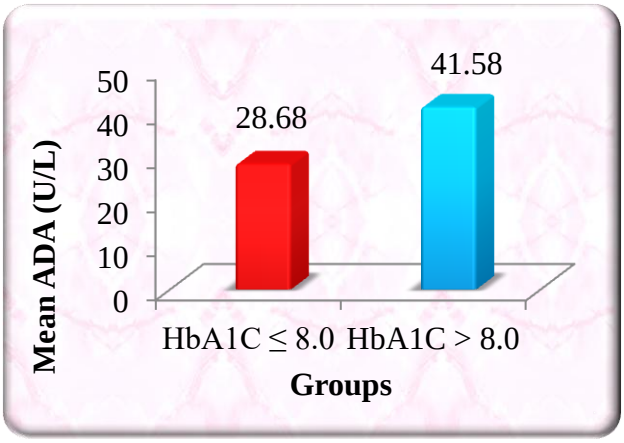


Table 5.4: represents the mean serum UA levels in diabetic groups based on glycemic control. The mean serum UA level in diabetic group with good glycemic control was 4.92 ± 1.27 mg/dl and with poor glycemic control was 5.73 ±1.51 mg/dl. There was a significant increase in serum UA levels with poor glycemic control (HbA1c>8.0) and the result were statistically significant (P=0.012).

Groups	No. of cases (n)	UA(mg/dl) (Mean ± SD)	t-Value	P-value
HbA1C ≤ 8.0	32	4.92 ± 1.27	-2.562	0.012
HbA1C > 8.0	68	5.73 ± 1.51		

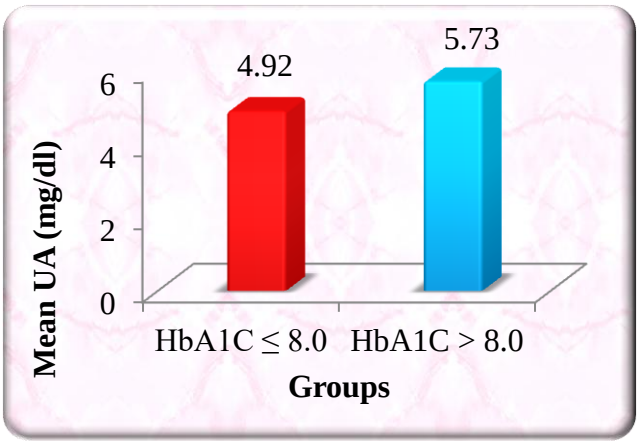
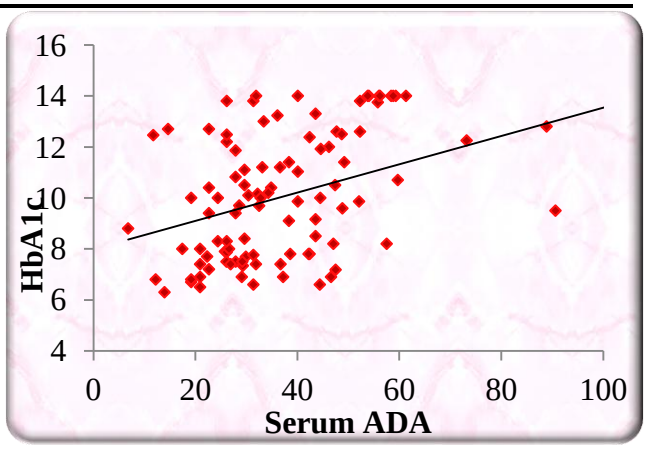
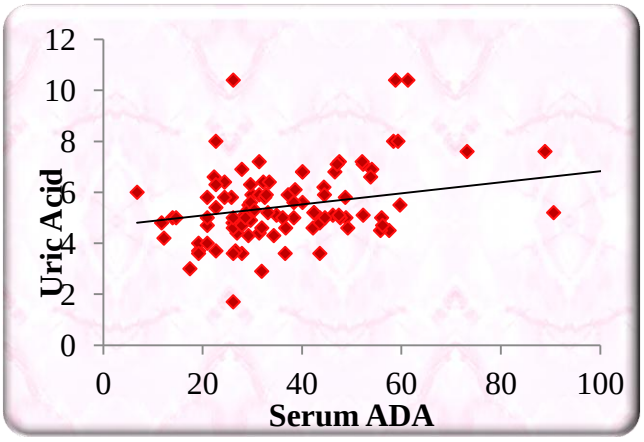


Table 6: shows the correlation between Serum ADA, BSF, HbA1c and Uric acid. When the comparison was made between serum ADA and HbA1c, there was a positive correlation (r=0.388) and the comparison was statistically significant (p= 0.000). Further, when the comparison between serum ADA and UA levels was made there was a positive correlation (r=0.252) and the comparison was statistically significant (P=0.011).

	Correlation coefficient (r)	Significance (P-value)
Serum ADA vs HbA1C	0.388	0.000
Serum ADA vs Uric Acid	0.252	0.011



Correlation between Serum ADA and HbA1c (r=0.388)



Correlation between Serum ADA and Uric Acid (r=0.252)

Discussion:

The present study reported a significant rise in serum ADA levels in type 2 DM patients as compared to healthy controls. The study further, demonstrated a significant association of ADA with glycemic index (measured by HbA1c) and Serum UA levels. **Amandeep Kaur et al., 2012** concluded that in type 2 DM patients there was a significant increase in serum ADA level with p value of <0.0001 when compared to controls.[14] **M Shivaprakash et al., 2006** observed significant increase in adenosine deaminase activity in diabetic patients and hypothesizes that increased ADA activity may be due to altered immunity.[15] **Mohammad Haghighatpanah et al., 2016** concluded that the mean BSF level was significantly (P< 0.001) elevated in patients with poor glycemic control (HbA1c>7%).[16]**Khattab et al., 2010** reported that mean BSF levels was significantly elevated in patients with poor glycemic control.[17]**Vijay Asamundeeswari et al., 2016** demonstrated that, serum ADA activity is increased in patients with type 2 diabetes mellitus. Serum ADA level had a significant positive correlation with FBS, UA and HbA1c in type 2 diabetes mellitus patients.[18]**Anjali C. Warrier et al., 1995** showed in their study that increased serum ADA activity were correlated with hyperglycemia (glycated hemoglobin) and lipid peroxidation in DM patients. They suggested that decreased tissue adenosine levels is due to increase in ADA activity and is related to the severity of hyperglycemia and lipid peroxidation in diabetes mellitus.[19] This association suggests that estimation of serum ADA levels may have an important role as risk marker of CVD and other associated complications. The study, therefore, recommends further research on the importance of serum ADA estimation in different CV disorders and its association with other independent markers like lipid profile, CRP, homocysteine.

Conclusion:

The study was undertaken to determine the levels of Serum ADA activity and Serum Uric acid in patients of type 2 DM and its correlation with parameters of glycemic profile such as FBS and Glycated Hemoglobin. The study shows higher level of Adenosine deaminase (ADA) in the patients of type 2 diabetes mellitus. Further a positive correlation of ADA with glycemic control conveys that ADA serve as a prognostic factor in type 2 diabetes mellitus in modulating the bioactivity of insulin. Therefore, our study suggests that serum ADA serve as a glycemic markers for assessing the glycemic status in diabetic patients.

Furthermore, the serum uric acid levels were found to be increased with increased levels of HbA1c (>8%) in comparison to good glycemic control (HbA1c <8%). Hence, the uric acid may serve as a potential biomarker of deterioration of glucose metabolism. The study also recommends larger and more elaborated studies that includes ADA, insulin, immunological markers to estimate the exact role of ADA and UA in the pathogenesis of type 2 DM and its complications.

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