Original article:

A study of serum adenosine deaminase activity in type 2 diabetes mellitus with and without complications and its co-relation with serum uric acid level in glycemic control

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Abstract:

Introduction: Diabetes Mellitus is a leading cause of death worldwide. ADA an enzyme involved in purine metabolism, is suggested to be involved in modulating bioactivity of insulin. However, its clinical importance in type 2 DM is still not conclusive. Present study was undertaken to assess and compare level of serum ADA activity in type 2 DM patients with and without complications.

Materials and methods: The study consisted of 80 patients with type 2 DM, admitted in Gauhati Medical College and Hospital and 40 healthy individuals as controls (Group I). The patients were further divided into two groups on the basis of HbA1c levels (Group II with HbA1c<7%; Group III with HbA1c>7%). Serum ADA, Fasting plasma glucose, HbA1c and Serum uric acid were estimated in all the groups.

Results: All the three parameters, FBS, HbA1c and ADA levels were found to be increased in the patients of Type 2 DM as compared to controls. The correlation of mean serum uric acid levels with HbA1c showed a bell shaped relation.

Conclusion: From the present study, it is concluded that there is an increase in serum ADA levels with increase in HbA1c levels. It was found that the serum uric acid levels increased with moderately increasing levels of HbA1c <7% and then decreased with further increasing levels of HbA1c >7% (a bell-shaped relation).

Keywords: Type 2 Diabetes mellitus, ADA, Fasting Plasma Glucose, Glycated hemoglobin, Uric acid.

Introduction:

Diabetes is a group of metabolic diseases characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both.[1] 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.[2] 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.[3] By 2030, this number is estimated to rise to 80 million.[4] This means that every fifth diabetic in

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the world would be an Indian. A national survey of diabetes conducted in six major cities in India in the year 2000 has shown that the prevalence of diabetes in urban Indian adults was 12.1%.[5] Type 2 DM is a heterogeneous group of disorders characterized by variable degree of insulin resistance, impaired insulin secretion, and increased glucose production. In addition to hyperglycemia, type 2 DM is also associated with a serious breakdown in lipid dynamics, often reflected by elevated levels of circulating free fatty acids (FFAs) and triglycerides (TG). The metabolic dysregulation associated with type2 diabetes mellitus causes secondary pathophysiologic changes in multiple organ systems that impose a tremendous burden on the individual with diabetes and on the health care system. The injurious effects of hyperglycemia are separated into macrovascular complications (coronary artery disease, peripheral arterial disease, and stroke) and microvascular complications (diabetic nephropathy, neuropathy, and retinopathy).[6]

Adenosine deaminase (ADA) is a polymorphic enzyme involved in the metabolism of purine nucleosides, catalyses the irreversible hydrolytic deamination of adenosine (Ado) and 2´-deoxyadenosine (2´-dAdo) to inosine and 2´-deoxyinosine, respectively. Inosine and 2′-deoxyinosine are converted to hypoxanthine, xanthine and finally, to uric acid.[7] Adenosine is an anti-lipolytic factor and lowers free fatty acid levels.[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. Studies have shown that ADA which reduces adenosine levels, increases basal and noradrenaline stimulated lipolysis in adipocytes.[10, 11] Adenosine

Deaminase exerts its effects predominantly by regulating the concentration of intracellular and extracellular adenosine. Conditions like hypoxia which leads to elevated adenosine formation and release have been shown to increase the expression of ADA.[12]

Adenosine has multiple actions; it acts through its receptors following release from the cell. Adenosine acts through A1receptors in the adipose tissue, and exerts its potent anti-lipolytic effects. In fact, A1 receptor agonists have been shown to decrease free fatty acid levels and increase insulin sensitivity.[8] Adenosine, acting through its receptors also affects multiple tissues and organ functions including pancreas, liver, kidneys, skeletal muscle, heart, vascular tissue etc. The expression level of adenosine nucleoside transporters and adenosine receptors has been shown to be different in diabetes. [13]

Several studies have demonstrated elevated levels of adenosine deaminase in individuals with type 2 diabetes mellitus, but the exact pathogenic role of elevated ADA activity in type 2 DM remains to be elucidated.[14, 15] An elevation of serum uric acid has been found to be associated with subsequent morbidity and mortality in patients with congestive heart failure, diabetes and hypertensive patients. Kurtul N *et al.* in 2004 analysed a significant co-relation between the ADA levels and uric acid levels in diabetes. They concluded that high uric acid levels in type2 DM patients were due to the increased ADA activity.[16] whereas in a study by Tuomilehto J. *et al*. demonstrated low uric acid levels in diabetic patients.[17] Even though there are reports available on serum adenosine deaminase levels and serum uric acid levels in patients of type 2 diabetes mellitus but these are still not very clear and conclusive.

Hence, in the light of 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.

Methods and materials:

The study was consists of 80 patients with Type 2 DM in the age group of 40-80 years of either sex, from Out Patient & Inpatient Department of Department of Medicine and Endocrinology, GMCH which were further divided into two groups on the basis of the HbA1c levels (Group II=HbA1c<7% and Group III=HbA1c>7%). A group of 40 age and sex-matched normal healthy individuals were selected randomly among persons from different sectors of the society belonging to different occupations and socio economic status as controls (Group I).

Informed consents were obtained from all the participants.

Inclusion criteria:

a) Group I - Control group; comprised of age and sex matched normal healthy individuals both males and females from general population.

b) Group II - This group includes patients above 40 years with Diabetes Mellitus with duration less than 8 years with HbA1C < 7%; on life style modifications and oral hypoglycaemic drugs and free from clinical evidence of any microvascular complication.

c) Group III - This group includes patients above 40 years with Diabetes Mellitus with duration more than 8 years, on life style modifications, oral hypoglycaemic drugs, insulin or combination of all three and associated with one or more microvascular complications (diabetic nephropathy, diabetic retinopathy, heart disease, diabetic neuropathy) with HbA1C > 7%.

Exclusion criteria:

Patients with the following diseases will be excluded –

1) Patients with type 1 DM

2) Patients with Haemolytic Anaemia, Haemoglobin variants, Pregnancy, Hepatic diseases and Infectious diseases like Tuberculosis, Sarcoidosis.

Sample collection:

24 hours urine specimen in a closed container was collected from each participant. The urine was centrifuged at 3000 rpm for 5 min. The supernatant was distributed in vials of 1.5ml each and biochemical analysis was done within four hours. The remaining sample was kept frozen at -20C. Five ml of blood was drawn from the participants under aseptic conditions from the median cubital vein. It was collected in properly labelled vacutainers and then centrifuged at 3000 rpm for 15 minutes. The serum thus obtained was subjected to biochemical analysis within 8 hours of collection of blood.

Sample analysis:

Estimations of Serum Adenosine Deaminase, Fasting plasma glucose, Glycated haemoglobin were done using MERCK microlab 300 Semiautoanalyser. Anthropometric parameters like Height (m), Weight (kg), BMI, Waist, Hip and Waist- Hip ratio were measured by standard method. Statistical evaluation,

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interpretation, comparison and correlation of findings were measured in the three groups.

Blood urea, Serum Creatinine and lipid profile (total cholesterol, triglyceride and HDL-Cholesterol) were also done using MERCK microlab 300 Semiautoanalyser. VLDL-Cholesterol and LDL-Cholesterol were calculated using Friedwald's formula. The results obtained were statistically analyzed and compared between the three groups of the study. Baseline characteristics of the study participants are expressed in Mean ± SD. One way Analysis of variance (ANOVA) test were used to analyze differences in baseline characteristics between the studied groups. Correlations were observed by using Pearson's correlation coefficient. The results were considered significant when the probability (p value) was less than 0.05 of the observed values of "t" at a particular degree of freedom. Statistical analysis was done using GraphPad InStat version 3.00. All the statistical graphs were prepared using Microsoft Excel 2007.

Results:

The age distributions in the three groups were 51.5±6.86, 53.25±8.39 and 55.3±8.78 respectively in Group I, Group II and Group III; which were statistically matched (Table 1). Among the cases (Group II and Group III) and the control groups, 40% were female and 60% were male.

The mean serum ADA levels were found 15.06 U/L, 32.77 U/Land 51 U/L with a standard deviation of 1.21, 7.36 and 20.48 respectively in Group I, Group II and Group III (Table 2). Statistical analysis showed significant difference of serum ADA between the groups (*P*<0.0001). The mean FPG levels were 92.13±9.71 mg/dL for Group I, 149.65±12.78 mg/dL for Group II, and 246.28±48.22 mg/dL for Group III (Table 2). There was a significant difference of FPG level between Groups I and II (*P*<0.0001) as well as Groups II and III (*P*<0.0001).

The mean HbA1c levels were 5.12±0.29, 6.75±0.16, 10.47±1.48 respectively in Group I, Group II and Group III (Table 2). There was a significant difference (p<0.0001) of HbA1c level between the three groups.

The Pearson's correlation coefficient for the relationships of serum ADA with FPG (r=0.5188); and Serum ADA with HbA1c (r=0.3371) levels in Group II showed positive correlation (Table 3). There were also significant positive correlation between serum ADA and FPG levels, as well as between serum ADA levels and HbA1c in Group III(r=0.6777, r=0.6340 respectively) (Table 3). The mean serum uric acid levels were 6.79±1.26, 7.29±1.11 and 5.22±1.72 respectively in Group I, Group II and Group III. (Table 2)

The Pearson's correlation between HbA1c and uric acid showing positive correlation in diabetic patients with good glycemic control i.e HbA1c <7% (r= 0.2701), and negative correlation in poor glycemic control i.e HbA1c >7% (r= -0.1878). However, these results were not statistically significant. (Table 4) However, no significant correlation was found between ADA and Serum Uric acid level.The mean serum urea levels were 40.19 mg/dL, 45.69 mg/dL, and 115.26 mg/dL with a standard deviation of 9.39, 15.17 and 80.65 respectively in Group I, Group II and Group III (Table 2). On comparison of means no significant difference was observed between Group I and Group II (p>0.05) but significantly different between Group II and Group III and between Group I and Group III (p<0.001). The mean serum creatinine levels in the three groups were 0.87±0.20 mg/dL, 1.26±0.59 mg/dL, and 3.46±2.59 mg/dL respectively

(Table 2). The values were significantly different between the three Groups (p<0.001).

Lipid profile derangement was an obvious feature in the present study among the studied groups with type2 DM. Total cholesterol, triglyceride and LDL-Cholesterol were significantly elevated (p<0.0001) when compared to controls and HDL- Cholesterol was reduced among diabetics when compared to non-diabetic controls (Table 2).

Discussion:

Diabetes mellitus, a common endocrine metabolic disorder, is a leading cause of death worldwide. It is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels.[1] The incidence and the prevalence of Type 2 DM is globally increasing and becoming a major public health problem for health care providers.

In the present study, the mean serum ADA levels of Group III were significantly higher than Group II (*P*<0.0001). Also, the levels of ADA were significantly higher in both Groups II and III than Group I (*P*<0.0001). The serum ADA levels were found positively correlated with FPG and HbA1c concentration in Type2 DM patients (Table 3). These findings corroborate with the findings of Kaur A. *et al*, Singh P. *et al*.[14, 15] Adenosine deaminase (Adenosine aminohydrolase EC 3.5.4.4) an enzyme involved in the metabolism of purine nucleosides, catalyses the irreversible hydrolytic deamination of adenosine (Ado) and 2´-deoxyadenosine (2´-dAdo) to inosine and 2´-deoxyinosine, respectively. Further metabolism of these deaminated nucleosides leads to hypoxanthine, which can be either transformed into uric acid by xanthine oxidase or salvaged into mononucleotides by the action of hypoxanthine-guanine phosphoribosyltransferase.[7]

Thus, increased adenosine deaminase activity leads to increased depletion of adenosine. Adenosine is both a metabolic precursor for nucleic acids and a significant signaling molecule involved in regulation of various physiological processes which linked to its localized release. Adenosine modulates the action of insulin on various tissues differently and its concentration in tissues is affected by ADA levels. It mimics the action of insulin on glucose and lipid metabolism in adipose tissue and the myocardium. Adenosine potentiated insulin and contraction stimulated glucose transport in skeletal muscles by enhancing the increase in GLUT-4 at the cell surface. A1 receptor agonists of adenosine have been found to be associated with increased insulin sensitivity. Thus, depletion of adenosine due to increased adenosine deaminase activity would mean increase in insulin resistance in the body & subsequent hyperglycemia, which is a hallmark feature of diabetes mellitus.[7]

In the present study, increased level of serum ADA activity in type 2 DM patients and its significant positive correlation with FPG and HbA1c reveals that ADA is an important enzyme for modulating the bioactivity of insulin. Thus, increased activity of ADA might be a marker for insulin resistance. Hyperglycemia in T2DM causes oxidative stress by generating reactive oxygen species.

Hyperglycaemia leads to activation of NADPH oxidase, that catalyses O_2^- formation by one electron reduction of O_2 using NADPH or NADH as electron donor.[18]

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$$2O_2 + NADPH \text{ (or NADH)} \longrightarrow$$
$$2O_2^- + NADP \text{ (or NAD)} + H^+$$
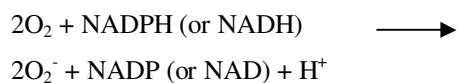
Another source of superoxide anion formation could be auto-oxidation of glucose which is subjected to enediol rearrangements that result in the formation of an enediol radical ion, which is capable of reducing molecular oxygen to form superoxide anion.[19] Hyperglycaemia also causes formation of Advanced glycation End Products (AGEs) as result of non-enzymatic reactions between intra-cellular glucose-derived dicarbonyl precursors with the amino group of both intracellular and extracellular proteins.[20] The AGEs stimulate receptors for advanced glycation end products (RAGE). Their interaction is believed to initiate and aggravate the diabetic complications. In addition they increase the generation of reactive oxygen species in macrophages thereby causing oxidative stress.[21]

AGEs bind to AGE receptors on several cell types (endothelial cells, mesangial cells and macrophages) and lead to release of cytokines; TNF-α, IL-1, IL-6 and growth factor from macrophages and mesangial cells resulting in activation of T lymphocytes.[22] ADA plays a crucial role in lymphocyte proliferation and differentiation and shows its highest activity in T-lymphocytes.[8] High ADA activity might be due to abnormal T-lymphocyte responses or proliferation and may point to a mechanism that involves its release into circulation. Therefore, in the present study, we report that increased ADA activity in diabetic individuals could be due to altered insulin related T-lymphocyte function.

In the present study, there is increased mean serum uric acid levels in patients with HbA1c <7% and decreased in patients with HbA1c >7%.The Pearson's correlation coefficient for the relationships between serum ADA, HbA1c and uric acid levels were also evaluated in Group II and Group III (Table 3) (Table 4), reveals positive correlation of uric acid with HbA1c (r=0.2701), and ADA(r=0.0515) in Group II. Similarly, negative correlation with HbA1c (r= -0.1878) and positive correlation with ADA (r=0.0509) in Group III. However, the correspondent comparisons were found to be not significant (p>0.05). Kaur A. *et al* in 2012 studied serum ADA activity in type 2 DM patients and found a positive significant correlation of serum ADA with FPG and HbA1c.[14]

Singh P. *et al* in 2013 also studied the activity of ADA in type 2 DM and reveals that increase in serum ADA levels was associated with increase in HbA1c levels, which may play an important role in determining the glycemic status in diabetes.[15] The reason for increased uric acid levels could be due to increased activity of ADA, an enzyme responsible for converting adenosine to uric acid in patients of type 2 Diabetes Mellitus. Another reason behind the increase in serum uric acid levels could be due to hyperinsulinemia in insulin resistant individuals. Studies showed that Insulin can stimulate the urate-anion exchanger[23] or the Na-dependent anion co-transporter in brush border membranes of the renal proximal tubule[24] and increase renal urate reabsorption. However, a negative correlation of uric acid in poor glycemic status may be related to the inhibition of uric acid reabsorption in the proximal tubule by high glucose levels in diabetic individuals.

Conclusion:

In the present study, significantly higher values of ADA in cases compared to controls suggest that ADA plays a role in the pathophysiology of type 2 DM and its complications. A positive correlation

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between ADA level with good and poor glycemic control suggests its important role in modulating the bioactivity of insulin. Thus, increased ADA activity might be a marker for insulin resistance.

Therefore, estimation of serum ADA might serve as a glycemic marker for assessing the glycemic status of a diabetic patient.

The serum UA levels were found increased with moderately increasing levels of HbA1c (<7%) and then decreased with further increasing levels of HbA1c (>7%). Larger and more elaborated studies are required including ADA, insulin, immunological markers and also at molecular level to know the role of ADA in the pathogenesis of type2 DM and its complications.

	Group I (n=40)	Group II (n=40)	Group III (n=40)
Mean of Age (Years)	51.5±6.86	53.25±8.39	55.3±8.78
Sex (Male/Female)	24/16	24/16	24/16

Table1: Age and sex distribution of subjects in the three Groups

Parameters	Group I (n=40) (Mean±SD)	Group II (n=40) (Mean±SD)	Group III (n=40) (Mean±SD)	p value
ADA(U/L)	15.06±1.21	32.77±7.36	51±20.48	<0.0001***
FPG(mg/dL)	92.13±9.71	149.65 ±12.78	246.28 ±48.22	<0.0001***
Glycated Haemoglobin(%)	5.12±0.29	6.75±0.16	10.47±1.48	<0.0001***
Uric acid(mg/dL)	6.79±1.26	7.29±1.11	5.22±1.72	<0.0001***
Urea(mg/dL)	40.19±9.39	45.69±15.17	115.26±80.65	<0.0001***
Creatinine(mg/dL)	0.87±0.20	1.26±0.59	3.46±2.59	<0.0001***
T.Cholesterol (mg/dL)	138.65±13.73	177.03±13.3	235.8±42.30	<0.0001***
Triglyceride (mg/dL)	95.1±27.03	152.9±13.15	179.15±32.27	<0.0001***
HDL- cholesterol (mg/dL)	48.28±8.05	40.08±6.78	34±5.08	<0.0001***
LDL (mg/dL)	71.13±12.81	107.77±16.71	165.97±41.50	<0.0001***
VLDL (mg/dL)	19.02±5.41	30.58±2.63	35.83±6.45	<0.0001***

*significant(<0.05), **very significant(<0.001), *** extremely significant(<0.0001),

NS Not significant

Table 2: Showing comparisons of biochemical parameters of the control and case groups. Anova test and Bartlett test were used for comparison of means between the three groups.

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Parameters	Group II	Group III
Fasting Plasma Glucose (mg/dL)	r=0.5188	r=0.6777
	p=0.0006[***]	p<0.0001[***]
HbA1c (%)	r=0.3371	r=0.6340
	p=0.0334[*]	p<0.0001[***]
Uric Acid (mg/dL)	r=0.0515	r=0.0509
	p=0.7521[NS]	p=0.7547[NS]
Urea (mg/dL)	r=0.2662	r=0.2730
	p=0.0968[NS]	p=0.0883[NS]
Creatinine (mg/dL)	r=0.1525	r=0.0378
	p=0.3476[NS]	p=0.8167[NS]
T. Cholesterol (mg/dL)	r=0.3203	r=0.4095
	p=0.0439[*]	p=0.0087[**]
Triglyceride (mg/dL)	r=0.3982	r=0.3885
	p=0.0109[*]	p=0.0132[*]
HDL- Cholesterol (mg/dL)	r= -0.3333	r= -0.3165
	p=0.0356[*]	p=0.0466[*]
LDL (mg/dL)	r=0.3851	r=0.3958
	p=0.0141[*]	p=0.0115[**]
VLDL (mg/dL)	r=0.3982	r=0.3885
	p=0.0109[*]	p=0.0132[**]

*significant(<0.05), **very significant(<0.001), *** extremely significant(<0.0001),

[NS] Not significant

Table 3: Showing correlation between ADA and other biochemical parameters in the case groups

Parameter	Group II (HbA1C <7%)	Group III (HbA1C >7%)
Uric acid (mg/dL)	r= 0.2701	r= -0.1878
	P= 0.0918[NS]	P= 0.2459[NS]

[NS] Not significant

Table 4: Showing correlation between HbA1c and serum uric acid level in the case groups
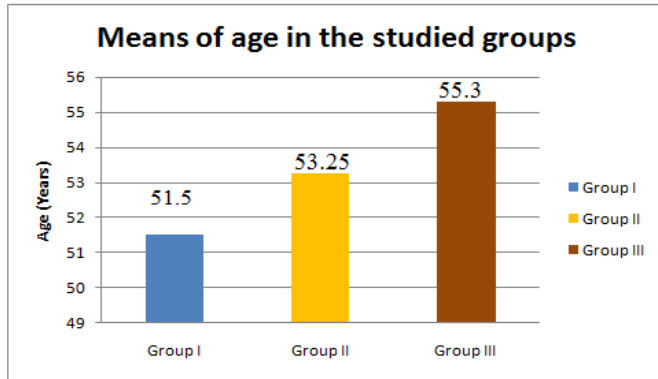
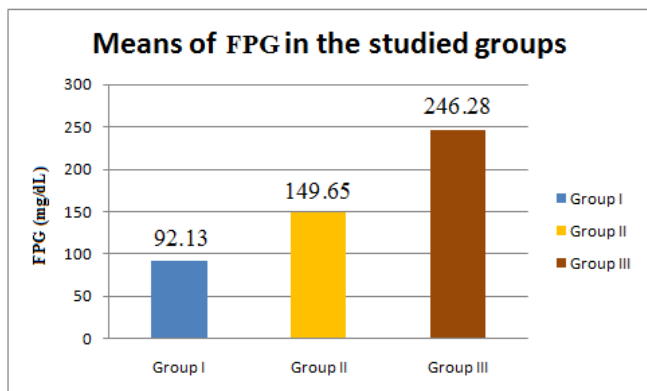**Graph 5.1: Showing comparison of means of age in the studied groups**
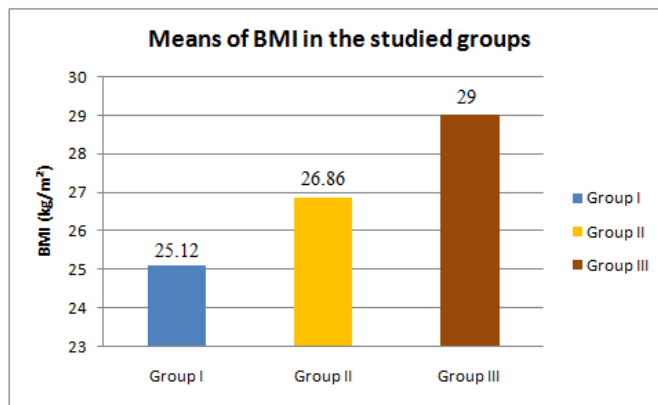
Graph 5.2: Showing comparison of means of BMI in the studied groups
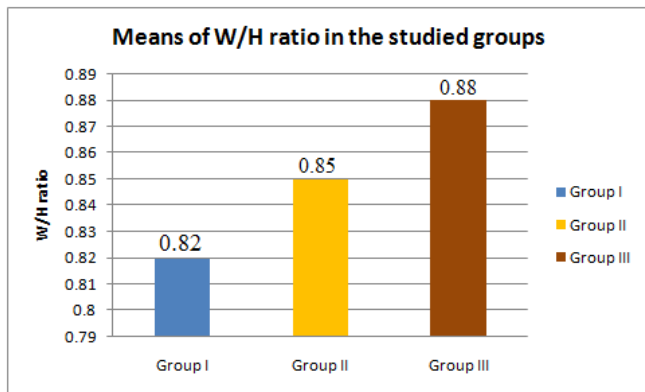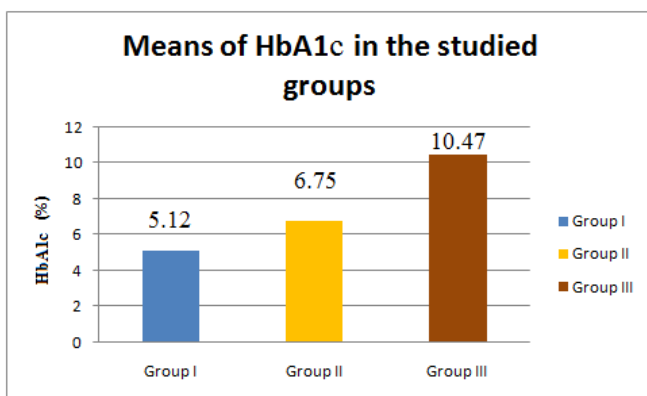

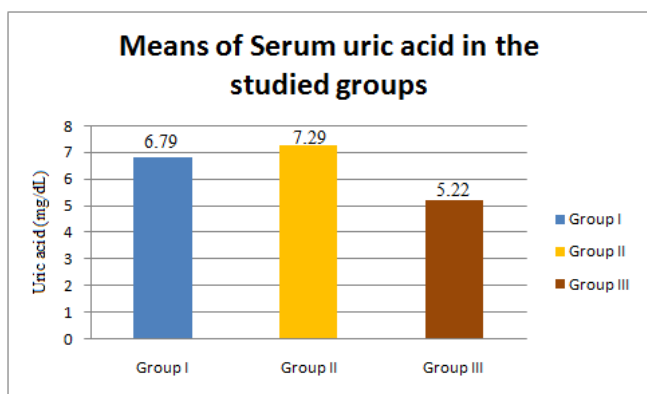**Graph 5.3: Showing comparison of means of W/H ratio in the studied groups**
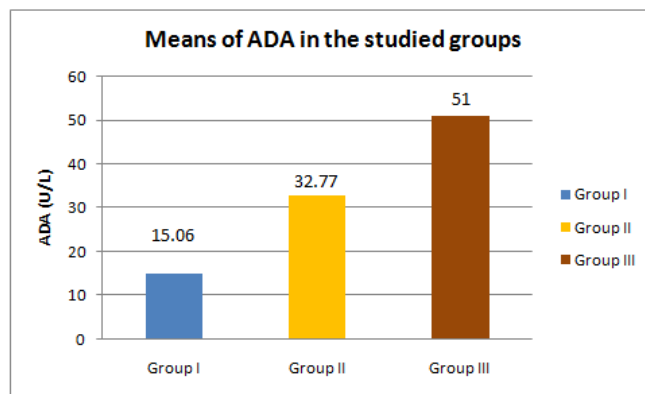
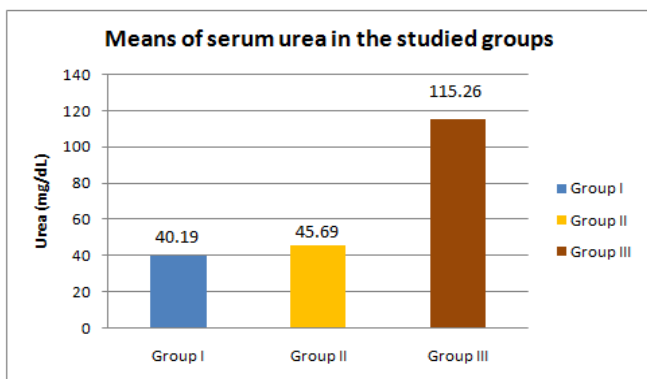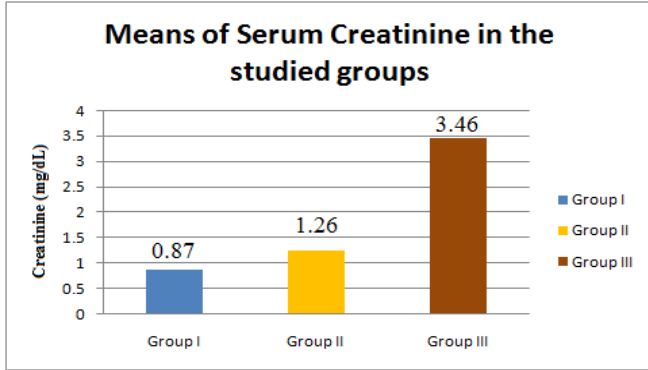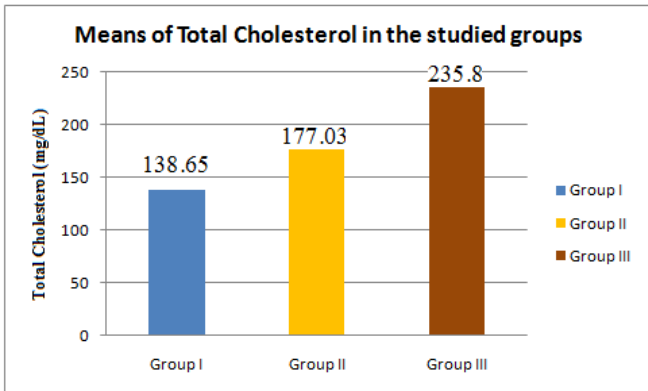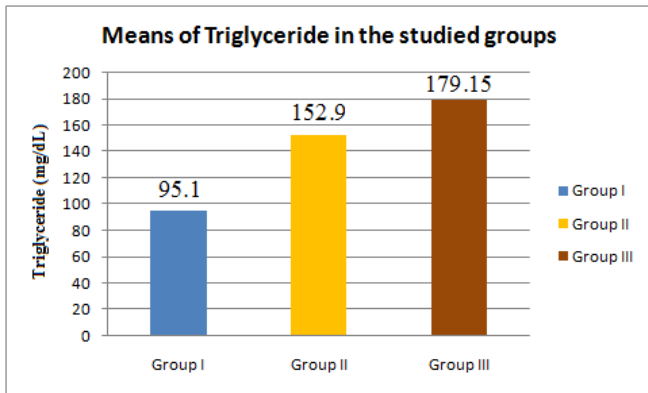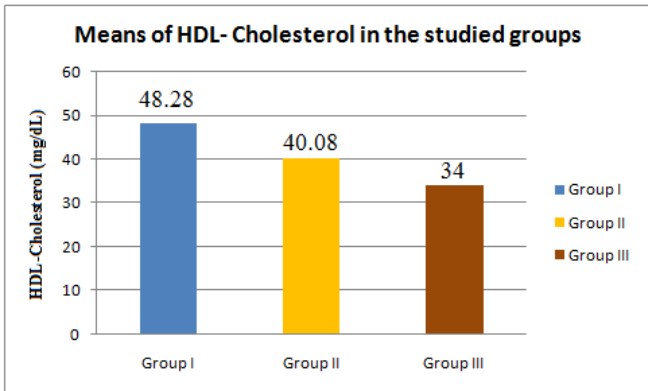**Graph 5.4: Showing comparison of of ADA means in the studied groups**

Means of FPG in the studied groups

Graph 5.5: Showing comparison of of FPG means in the studied groups

Means of HbA1c in the studied groups

Graph 5.6: Showing comparison of of HbA1c means in the studied groups

Means of Serum uric acid in the studied groups

Graph 5.7: Showing comparison of of Uric acid means in the studied groups

Means of serum urea in the studied groups

Graph 5.8: Showing comparison of of Urea means in the studied groups

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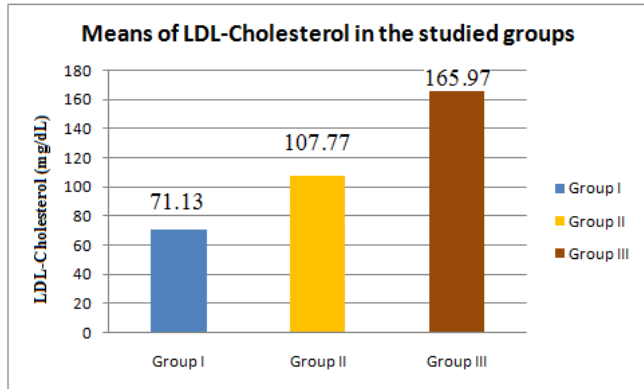**Graph 5.9: Showing comparison of of Creatinine means in the studied groups**

Graph 5.10: Showing comparison of of Total Cholesterol means in the studied groups
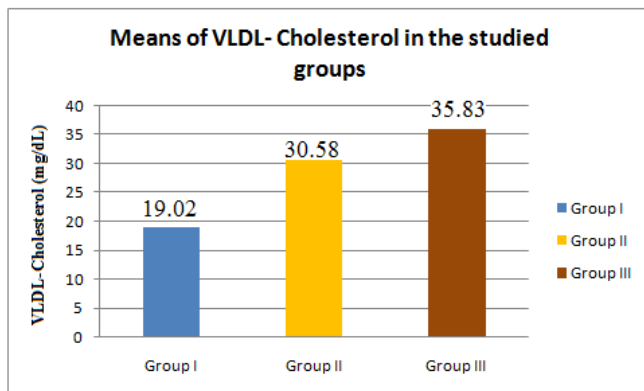

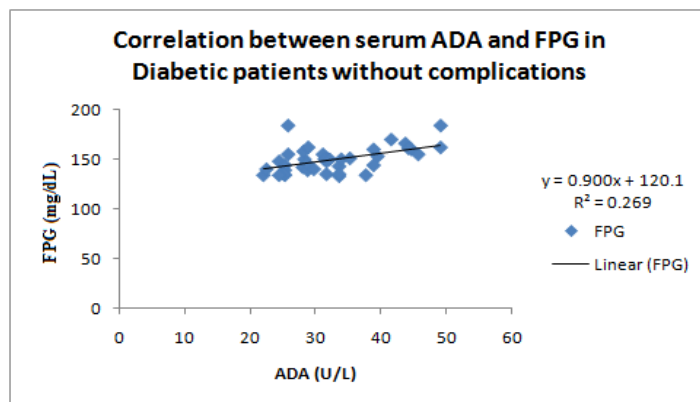**Graph 5.11: Showing comparison of of TGL means in the studied groups**

Graph 5.12: Showing comparison of of HDL-Cholesterol means in the studied groups

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Graph 5.13: Showing comparison of of LDL- Cholesterol means in the studied groups
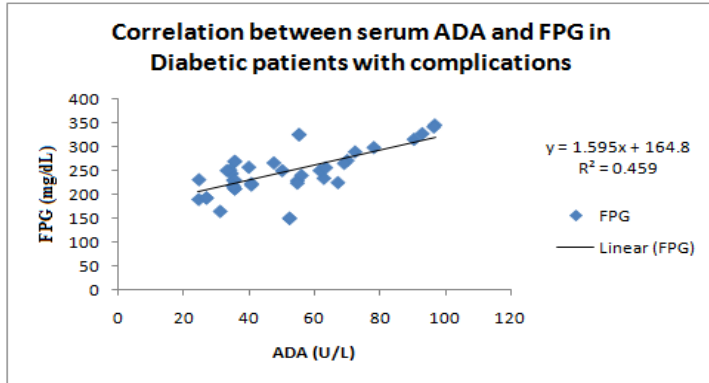

Graph 5.14: Showing comparison of of VLDL-Cholesterol means in the studied groups
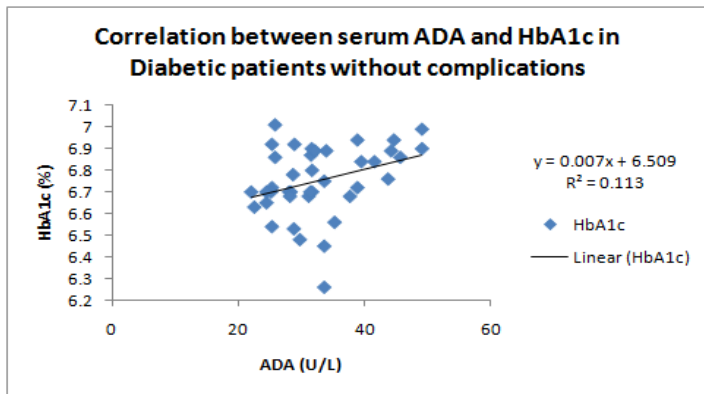

Pearson's Correlation coefficient (r) =0.5188

Coefficient of determination (r squared) = 0.269

The two-tailed P value is 0.0006,

considered extremely significant.

Figure 1: Showing correlation of serum ADA with FPG in diabetic patients without any complications
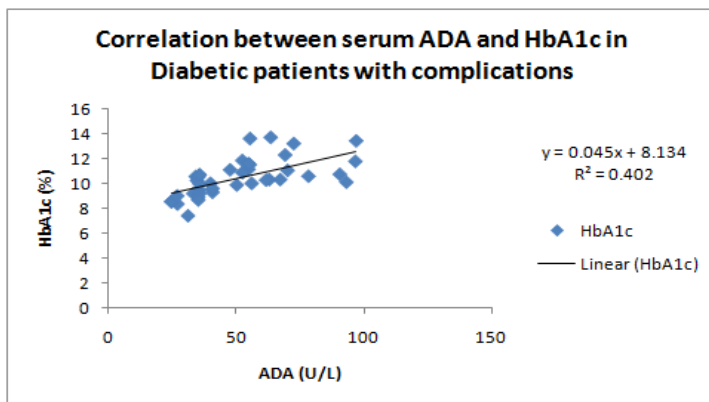
Pearson's Correlation coefficient (r) = 0.6777

Coefficient of determination (r squared) = 0.459

The two-tailed P value is <0.0001, considered extremely significant.

Figure 2: Showing correlation of serum ADA with FPG in diabetic patients with complications

Pearson's Correlation coefficient (r) = 0.3371

Coefficient of determination (r squared) = 0.113

The two-tailed P value is <0.0334, considered significant.

Figure 3: Showing correlation of serum ADA with HbA1c in diabetic patients without any complications

Pearson's Correlation coefficient (r) = 0.6295

Coefficient of determination (r squared) = 0.402

The two-tailed P value is <0.0001, considered extremely significant.

Figure 4: Showing correlation of serum ADA with HbA1c in diabetic patients with complications

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