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### INVESTIGATION OF SERUM PARAMETERS IN TYPE II DIABETES MELLITUS PATIENTS ON DRUG TREATMENT

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#### ABSTRACT

In this present study we have analysed the serum parameters such as glucose, cholesterol, urea, uric acid, total protein and albumin along with body mass index (BMI) and blood pressure (BP) in type 2 diabetes mellitus patients who were on treatment and compared these parameters with control group. We also examined the correlation of uric acid with other parameters in diabetes mellitus patients on treatment. The study group comprised of 100 type 2 diabetic subjects and twenty five sex and age matched non-diabetic subjects without family history of diabetics were selected as control. After overnight fasting blood was taken from all the subjects for investigation. The serum of each subject was analysed for the following parameter of serum glucose, serum cholesterol, serum protein, serum albumin, serum uric acid and serum urea. Midstream urine sample was collected in a clean bottle without any preservatives. Urine sample was analysed for urine sugar, urine albumin and urine ketone bodies. All biochemical estimations were done in MISPFA excel chemistry analyser. Statistical data was analysed by SPSS version 17, ANOVA was used to compare different parameters of study and control groups. Linear regression analysis (Pearson correlation coefficient) was performed for determining the degree of association between different parameters. Body mass index of diabetic patients were compared with control group. A statistically significant difference was found between diabetic and control group with  $p$  value  $< 0.001$ . A statistically significant difference was found between diabetic and control groups in the case of blood pressure. There is statistically significant difference in all the above biochemical parameters between diabetic and control subjects except in serum protein and albumin. In the present study we have compared different parameters of Type II DM patients on drug treatment with control group and we found that BP, BMI, serum glucose, serum cholesterol and uric acid levels of diabetic patients were significantly higher when compared with the control group. Serum urea levels of patients were found to be significantly lower than that of the control group. Serum uric acid levels were found to be inversely correlated with serum glucose levels. Serum uric acid levels were found to be directly correlated with BP and BMI.

#### KEYWORDS

Type 2 Diabetes Mellitus, Body Mass Index (BMI) and Blood Pressure (BP) and Serum Parameters.

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#### INTRODUCTION

Diabetes mellitus (DM) is a disease known from very ancient times and it is characterized by chronic hyperglycemia with disturbances of carbohydrate fat and protein metabolism resulting from defect in insulin secretion insulin action or both. The effects of diabetes mellitus include long term damage, dysfunction and failure of various organs. High

blood sugar produces the classical symptoms of polyuria, polydipsia and polyphagia<sup>1</sup>. The disease causes loss of weight due to altered metabolism a universal blue circle symbol for diabetes<sup>2</sup>.

According to the World Health Organization, at least 171 million people worldwide suffer from diabetes, i.e., 2.8% of the population. Its incidence is increasing rapidly, and it is estimated that by 2030, this number will almost double<sup>3</sup>. Diabetes mellitus occurs throughout the world but is more common in more developed countries. The increase in incidence of diabetes in developing countries follows the trend of urbanization and lifestyle changes, perhaps most importantly a "western style" diet. According to American diabetic association, approximately 18.3% (8.6 million) of Americans of age 60 and older have diabetes<sup>4</sup>. Early detection, treatment and prevention of type 2 diabetes are significant challenges of this century.

DM is classified into four categories Type 1, Type 2, Gestational and Congenital. About 5 % of total diabetic patients are of type 1. Family history, diet and environmental factors are risk factors for type 1 diabetes. Most of the diabetic patients belong to Type 2 DM. Risk factors for Type 2 diabetes include family history, increasing age, obesity, physical inactivity, ethnicity and a history of gestational diabetes. Gestational diabetes is carbohydrate intolerance resulting in hyperglycaemia of variable severity with onset or first recognition during pregnancy. It may precede development of type 2 diabetes. Other forms of diabetes mellitus include congenital diabetes, which is due to a genetic defect of insulin secretion, cystic fibrosis - related diabetes, steroid diabetes induced by high doses of glucocorticoid and several forms of monogenic diabetes.

People with diabetes are at increased risk of micro vascular and macro vascular disease. The long term effects of diabetes mellitus include progressive development of the specific complication viz. retinopathy with potential blindness, nephropathy that may lead to renal failure and neuropathy with risk of foot ulcers having localized gangrene or with extensive gangrene involving the entire foot<sup>5,6</sup>, amputation<sup>7</sup>, Charcot joints and features of

autonomic dysfunction, including sexual dysfunction. Peripheral artery disease (PAD) is an important healthcare problem in developed nations and is associated with considerable morbidity and mortality. Intermittent claudication (IC) is the most common symptomatic manifestation of PAD, and typically occurs in up to one third of patients with this disease<sup>8</sup>. Diabetes has become a leading cause of renal morbidity and mortality.

Diabetes without proper treatment can cause many complications. Acute complication includes hypoglycaemia, diabetic ketoacidosis or non ketotic hyperosmolar coma. Serious long term complications include cardiovascular disease, chronic renal failure and retinal damage. Adequate treatment of diabetes is thus important along with blood pressure control and lifestyle changes such as smoking cessation and maintaining a healthy body weight.

An increase in body fat is generally associated with an increased risk of metabolic disease such as type 2 DM, hypertension and dyslipidaemia. Hyperlipidaemia is a common finding among DM patient. Hypercholesterolemia can also be a predictor of diabetes. Elevated cholesterol levels are often seen in patient with insulin resistance, even before they have developed full-blown diabetes. Hyperlipidaemia was associated with high body mass index and this has increased emphasis on other risk factors for cardiovascular disease such as high blood pressure.

Uric acid is the final oxidation product of purine catabolism. Hyperuricaemia is most commonly defined by serum uric acid concentration greater than 7 mg/dl in man and 6 mg/dl in women<sup>9</sup>. Hyperuricaemia is also associated with possible confounding factors including elevated serum triglyceride and cholesterol concentration blood glucose and post carbohydrate plasma insulin concentration<sup>10</sup>. Hyperuricaemia has been described as a strong predictor of well-defined cerebrovascular complication (stroke) in patients with type 2 diabetes.

In this present study we have analysed the serum parameters such as glucose, cholesterol, urea, uric acid, total protein and albumin along with body mass

index (BMI) and blood pressure (BP) in type 2 diabetes mellitus patients who were on treatment and compared these parameters with control group. We also examined the correlation of uric acid with other parameters in diabetes mellitus patients on treatment.

## **MATERIALS AND METHODS**

### **Study Group**

The study group comprised of 100 type 2 diabetic subjects attending the laboratory at Calicut University Health Centre, during the period of May 2010 to August 2010. Type 2 diabetes was diagnosed according to WHO criteria<sup>10</sup>.

### **Control**

Twenty five sex and age matched non-diabetic subjects without family history of diabetics were selected as control. All experiments were done at the laboratory of Calicut University Health Centre. The control group were selected based on following parameter. Age > 40 years of age, serum uric acid <6.0 mg/dl, serum cholesterol <200mg% serum urea <40mg%, serum protein <8gm%, serum albumin <4.5gm%, absence of glycosuria, albuminuria and ketonuria with the normal urinary analysis, absence of urinary tract infection and no evidence of hypertension, cardiovascular and renal disease, absence of signs or symptoms of infections or any other chronic diseases requiring therapeutic intervention, smokers and alcoholics were excluded. Blood pressure was measured in all the subjects.

All the subjects completed an extensive questionnaire on, age, sex, height, weight, smoking and alcohol habits, food habits, duration of diabetics, renal and cardiovascular history, present medication and control of diabetes. The body mass index was calculated as weight in kilograms divided by the squared height in metres ( $\text{kgm}^2$ ). All the subjects gave written informed consent for this study.

### **Collection of samples**

After overnight fasting blood was taken by venepuncture. About 5 ml of fasting blood from 100 diabetic patients was obtained by venepuncture from either male or female having age group 35-80 attending the health centre of the University of Calicut. Same amount of blood was collected from selected 25 non-diabetic subjects. Blood was taken

in a centrifuge tube and was allowed to clot and centrifuged at 3000 rpm for 15 min at room temperature. Serum was separated into a clean tube. The serum of each subject was analysed for the following parameter of serum glucose, serum cholesterol, serum protein, serum albumin, serum uric acid and serum urea. Midstream urine sample was collected in a clean bottle without any preservatives. Urine sample was analyzed for urine sugar, urine albumin and urine ketone bodies. All biochemical estimations were done in MISPHA excel chemistry analyser. Serum glucose was measured by GOD/POD enzymatic method, serum cholesterol, was measured based on enzymatic method using Cholesterol esterase, Cholesterol oxidase and Peroxidase, total protein was estimated based biuret method, determination of albumin in serum or plasma is based on the binding behaviour of albumin with dye 33' SS' tetrabromo M cresol sulfunaphthalein (BCG method), estimation of uric acid was done based Uricase/POD end point assay, estimation of urea was done by Urase Berthelot End point Assay. Urine sugar, albumin was determined by Benedict's qualitative test, heat and acetic acid test respectively and ketone bodies by Rothera test.

### **Statistical analysis**

Statistical data was analysed by SPPSS version 17, ANOVA was used to compare different parameters of study and control groups. Linear regression analysis (Pearson correlation coefficient) was performed for determining the degree of association between different parameters.

## **RESULTS**

A total of 100 type 2 diabetic patients on treatment with drug were selected for the study. Twenty five non-diabetic subjects were selected as control. Their age, sex and BMI wise distribution, food, alcohol and smoking wise distribution were given in Table No.1 and 2 respectively. Among 100 diabetic patients, 55 patients had urine sugar, 52 had urine albumin and none of the patients had ketone bodies in urine (Table No.3). In this study physical parameters such as BMI, BP and biochemical parameter viz. Fasting blood sugar, cholesterol, urea, uric acid, total protein and albumin of diabetic

patients on drug treatment were compared with normal control.

### Physical parameters

Body mass index of diabetic patients were compared with control group. A statistically significant difference was found between diabetic and control group. One way ANOVA was conducted between the groups and within the groups and mean square value of 167.56 and 1291.31 respectively with a highly significant p value ( $<0.001$ ). The mean BMI in diabetic and control was  $24.20 \pm 3.31$  and  $21.97 \pm 3.31$  respectively (Table No.4).

Both systolic and diastolic blood pressure of diabetic patients was compared with control group. A statistically significant difference was found between diabetic and control groups ( $P=0.018$  and  $0.019$  for systolic and diastolic blood pressure respectively). The mean systolic blood pressure in diabetic and control was  $130.24 \pm 21.25$  and  $120$  respectively. And mean diastolic blood pressure in diabetic patient and control was  $84.0 \pm 8.48$  and  $80$  respectively (Table No.4).

### Biochemical Parameters

Fasting blood sugar was estimated in diabetic and control group. A statistically significant difference was found between diabetic and control group. One way ANOVA was conducted between the groups and within the groups and mean square value of 188684.7 and 428711.1 respectively with a highly significant p value ( $<0.001$ ). The mean FBS in diabetic and control was  $183.090 \pm 65.11$  and  $85 \pm 7.03$  respectively (Table No.5 and Figure No.1).

Fasting serum cholesterol sugar was estimated in diabetic and control group. A statistically significant difference was found between diabetic and control group. One way ANOVA was conducted between the groups and within the groups and mean square value of 26397.37 and 115305.4 respectively with a highly significant p value ( $<0.001$ ). The mean serum cholesterol in diabetic and control was  $199.45 \pm 33.04$  and  $163 \pm 16.34$  respectively (Figure No.2).

Serum total protein and albumin were estimated in both diabetic and control group. There is no statistical significance observed between the groups

and within the groups ( $P=0.860$  and  $0.069$  for total protein and serum albumin respectively).

Blood urea was estimated in both groups. A statistically significant difference was found between diabetic and control group ( $P<0.001$ ). The mean serum urea was  $23.11 \pm 4.73$  and  $27 \pm 2.06$  respectively (Figure No.3).

Serum uric acid was estimated in diabetic and control group. A statistically significant difference was found between diabetic and control group. One way ANOVA was conducted between the groups and within the groups and mean square value of 211.12 and 121.03 respectively with a highly significant p value ( $<0.001$ ). The mean serum uric acid in diabetic and control was  $6.73 \pm 1.06$  and  $3.484 \pm 0.40$  respectively (Figure No.4).

All the above said parameters of patients were studied for correlation (Table No.6). In which only BMI, BP, glucose and uric acid showed significant correlation. Uric acid level is found to be inversely proportional to the glucose level (Figure No.5). Body mass index is directly proportional to serum uric acid level (Figure No.6). Systolic blood pressure is directly proportional to serum uric acid level (Figure No.7). Diastolic pressure is directly proportional to serum uric acid level (Figure No.8).

### DISCUSSION

Diabetes has been recognized as an important risk factor for coronary artery disease as well as end-stage renal diseases. Type 2 diabetes mellitus is associated with oxidative stress and increased free radical formation. Oxidative stress causes reduction of the antioxidant status of the body.

The result of the present study showed that all diabetic subjects had significantly higher fasting blood glucose level as compared to non-diabetic subjects. Glycosuria usually occurs when blood glucose concentration is greater than  $10 \text{ mMol/L}$  though this threshold varies considerably between individuals and increase with age. In this study, among 100 diabetic cases, 45 patients were having sugar in urine.

Hyperlipidaemia is a common finding among diabetes mellitus patients. This study indicates that all diabetic subjects had significantly higher

cholesterol levels compared to non-diabetic subjects. Hyperlipidaemia has been linked as a risk factor in coronary heart disease. Increased BMI usually associated with hyperlipidaemia. Like hyperlipidaemia BMI was also found to be significantly higher in diabetic patients when compared with control group. In the present study total protein level was slightly higher in patients when compared to non-diabetic subjects albumin level was slightly low in the diabetic group when compared with control group. Patients with end stage renal disease, severe renal failure and moderate renal failure showed lower serum albumin concentration. Serum albumin level decreased significantly in patient with reduced creatinine clearance. In this study among 100 diabetic case 52% patient were having albumin in urine. Serum albumin was significantly lower in patient with heavy proteinuria. Hypoalbuminaemia is considered a marker for malnutrition and strongest predictor of death in patients with renal failure. An elevation of blood urea usually signifies decreased renal function<sup>11</sup>. But in this study patients had lower urea level when compared to non-diabetic subjects. The result of the present study showed that all diabetic subjects had significantly higher uric acid levels when compared to non-diabetic subjects. In this study it has been found that serum uric acid concentrations were 6.95mg/dl in man and 6.46 mg/dl in women. Finding of Abbas *et al*<sup>12</sup> suggest that serum uric acid is a risk factor for type 2 diabetes mellitus. For patient with type 1 diabetes high serum uric acid may be early signs of diabetic nephropathy before any significance change in urine albumin level<sup>13</sup>. Diabetic patients who are hyperuricaemic appear to be at increased risk for developing diabetic complication especially renal and cardiovascular disease. Increased uric acid was associated with increased risk of death from CHD and congestive heart failure. Hyperuricaemia has been described as a strong predictor of well-defined cerebrovascular complication in patient with type 2 diabetes.

Biochemical changes in this diabetic patients may be due to long term diabetes. Patients, having long term diabetes there is repression of glycolytic enzyme and depression of gluconeogenic enzyme which promote gluconeogenesis in liver, and further contribute to hyperglycaemia. Due to decreased catabolism of aminoacids, low urea will be formed from urea cycle. On the other hand repression of glycolytic enzyme, glucose is channelled in to the pentose phosphate pathway result in increased availability of ribose phosphate which leads to increase formation PRPP, ultimate result in high concentration of uric acid in blood.

In the present study correlation of uric acid with glucose, BMI and BP were examined among diabetes mellitus patients on drug treatment. Serum uric acid inversely proportional to glucose and directly proportional to BMI and BP were observed. In the same study uric acid level is found to be increased in patients when compared to control group.

Many studies suggest that abdominal obesity is the main determining factor of elevated plasma uric acid levels in the general population<sup>14</sup>. Uric acid levels are frequently elevated in subjects with renal dysfunction<sup>15</sup>. Hyperuricaemia showed impaired endothelial dependent vasodilation by reduction in NO-synthase in animal experiments and also reported that it has been associated with the genesis of essential hypertension<sup>16</sup>. It is reported that serum uric acid levels inversely related to renal blood flow and directly to renal vascular resistance in both normotensive and hypertensive humans<sup>17,18</sup>. Earlier studies<sup>19,20</sup> suggest that hyperuricaemia has been associated with elevated circulating endothelin levels. Increased level of endothelin is observed in NIDDM. All these studies showed that uric acid levels are directly proportional to BP. The present study also showed a positive correlation of uric acid with BP. The inverse relation of uric acid with glucose levels observed may be due to the reduced renal blood flow in DM patients.

**Table No.1: Sex, age and BMI wise distribution of the study groups**

S.No	Group	Sex		Total	Age group					Total	Body mass index				Total
		Male	Female		35 - 45	46 - 55	56 - 65	> 66	< 35		15 - 20	20 - 25	25 - 30	> 30	
1	Diabetic	59	41	100	25	42	27	5	1	100	4	54	35	7	100
2	Control	15	10	25	6	2	0	0	17	25	6	14	5	0	25
3	Total	74	61	125	31	44	27	5	18	125	10	68	40	7	125

**Table No.2: Distribution according food and alcohol intake and smoking**

S.No	Group	Food intake		Total	Alcohol intake		Total	Smoking		Total
		Non-Veg.	Veg.		No	Yes		No	Yes	
1	Diabetic	96	4	100	73	27	100	67	23	100
2	Control	25	0	25	25	0	25	25	0	25
3	Total	121	4	125	98	27	125	92	33	125

**Table No.3: Distribution according to the presence of urine sugar and albumin**

S.No	Group	Urine sugar		Total	Urine Albumin		Total	Ketone bodies		Total
		Absent	Present		Absent	Present		Absent	Present	
1	Diabetic	45	55	100	48	52	100	100	0	100
2	Control	25	0	25	25	0	25	25	0	25
3	Total	70	55	125	73	52	125	125	0	125

**Table No.4: Comparison of body mass index and blood pressure in the study groups**

S.No	Parameters	Control	Patient
1	Body mass index	21.9720 ± 3.316	24.209 ± 3.313
2	Systolic blood pressure	Constant (120)	130.24 ± 21.25
3	Diastolic blood pressure	Constant (80)	84.0 ± 8.48

**Table No.5: Comparison of biochemical parameters [FBS, Cholesterol, Urea, Uric acid mg/dl Total protein and Albumin g/dl] in the study groups**

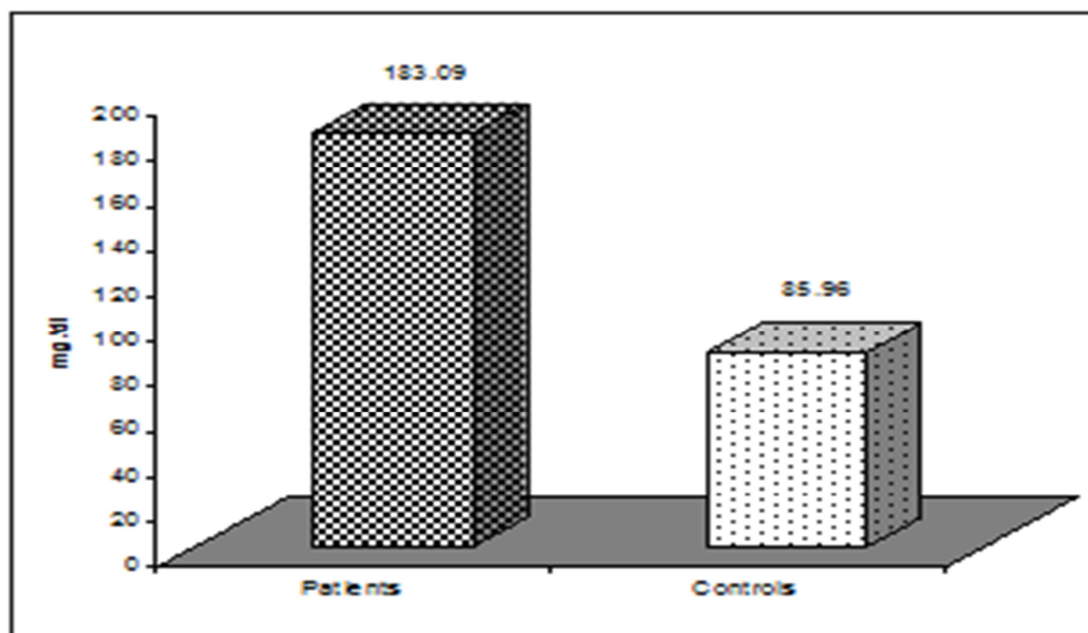
S.No	Biochemical parameters	Control	Patient
1	FBS	85 ± 7.032	183.090 ± 65.71
2	Cholesterol	163 ± 16.340	199.4504 ± 33.042
3	Urea	27 ± 2.068	23.111 ± 4.73
4	Uric acid	3.484 ± 0.408	6.73 ± 1.060
5	Total protein	7.198 ± 0.485	7.216 ± 0.219
6	Albumin	4.196 ± 0.163	4.04 ± 0.72

**Table No.6: Correlation between biochemical parameters in patient group**

S.No	Biochemical parameters	Correlation	BMI	DIABP	SYBP	Glucose	Uric acid
1	BMI	Pearson Correlation Sig. (2-tailed) N	1 --- 125	.365** .000 125	.203* .023 125	.112 .212 125	.340 .000 125
2	DIABP	Pearson Correlation Sig. (2-tailed) N	.365** .000 125	1 --- 125	.587** .000 125	.123 .173 125	.244** .006 125
3	SYBP	Pearson Correlation Sig. (2-tailed) N	.203* .023 125	.587** .000 125	1 --- 125	.056 .537 125	.199* .026 125
4	Glucose	Pearson Correlation Sig. (2-tailed) N	.112 .212 125	.123 .173 125	.056 .537 125	1 --- 125	.339** .000 125
5	Uric acid	Pearson Correlation Sig. (2-tailed) N	.340** .000 125	.244** .006 125	.199* .026 125	.339** .000 125	1 --- 125

\*\* : Correlation is significant at the .01 level (2 tailed).

\* Correlation is significant at the .05 level (2 tailed).



**Figure No.1: Fasting glucose level in study groups**

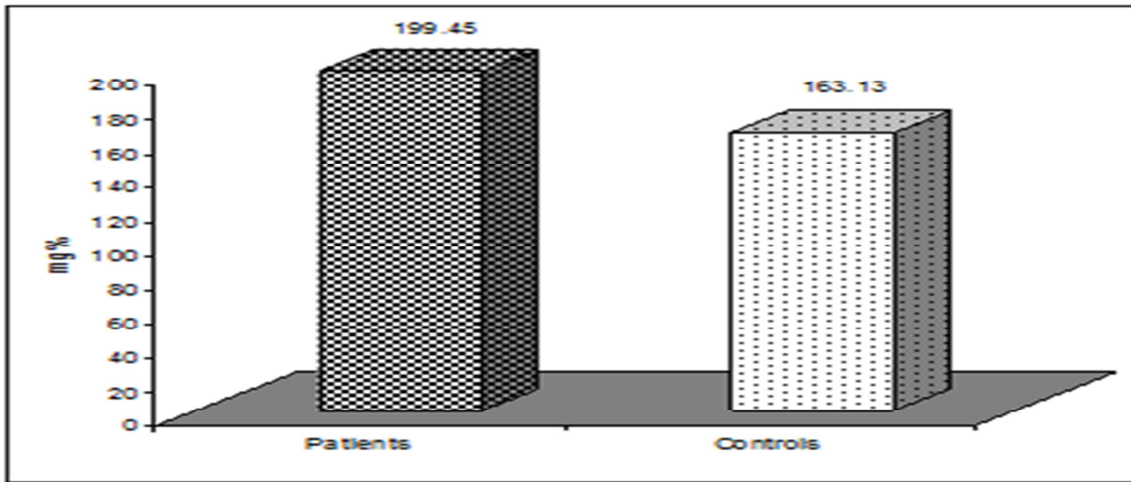


Figure No.2: Cholesterol level in study groups

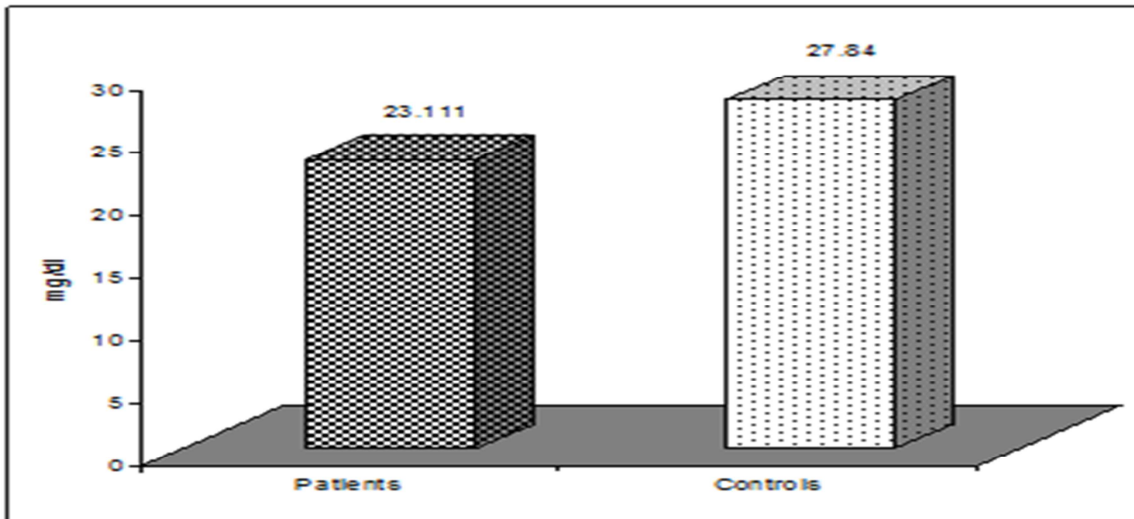


Figure No.3: Urea levels in the study groups

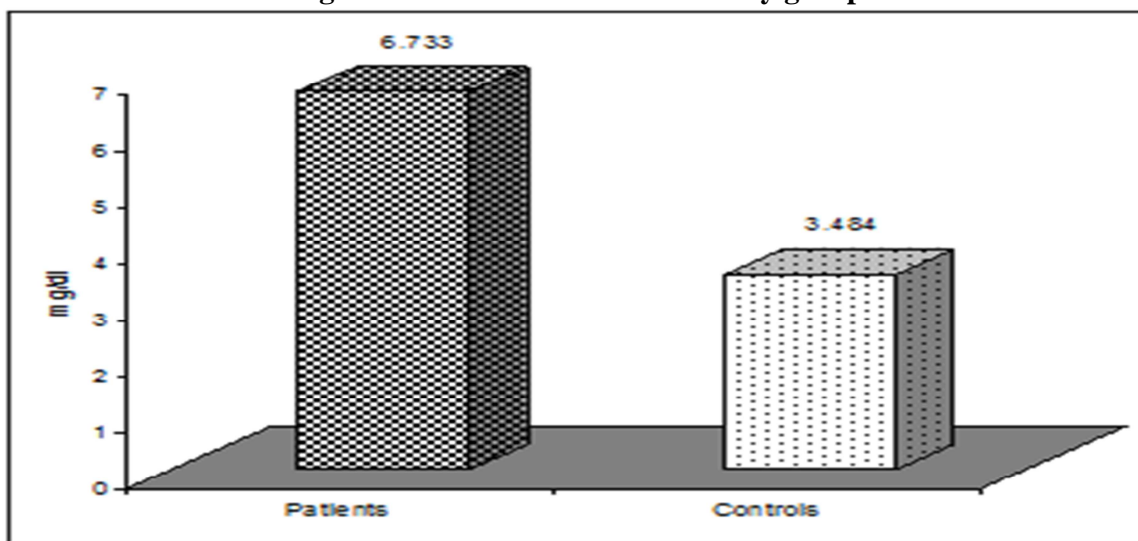


Figure No.4: Uric acid levels in the study group



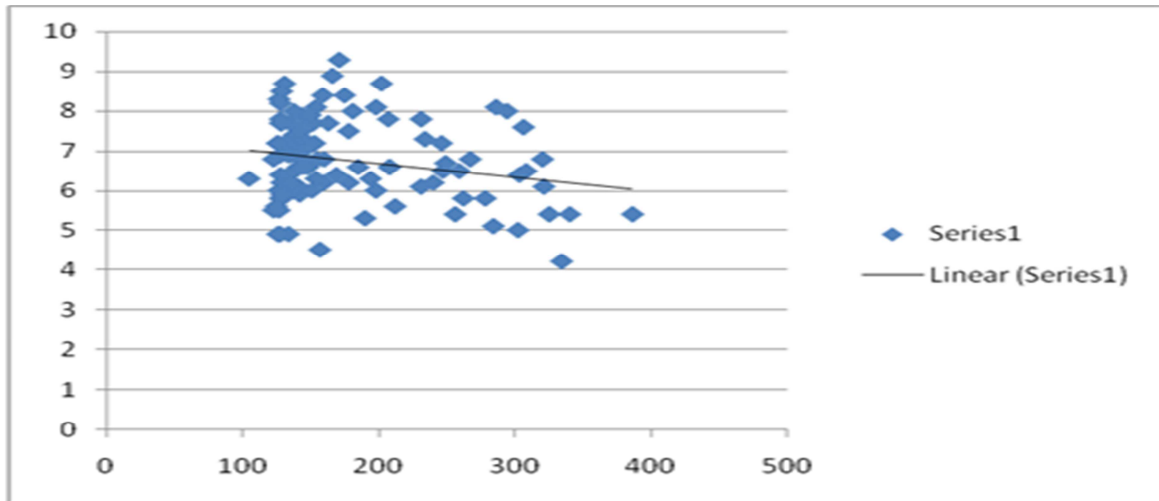


Figure No.5: Correlation between glucose and uric acid level in the patients

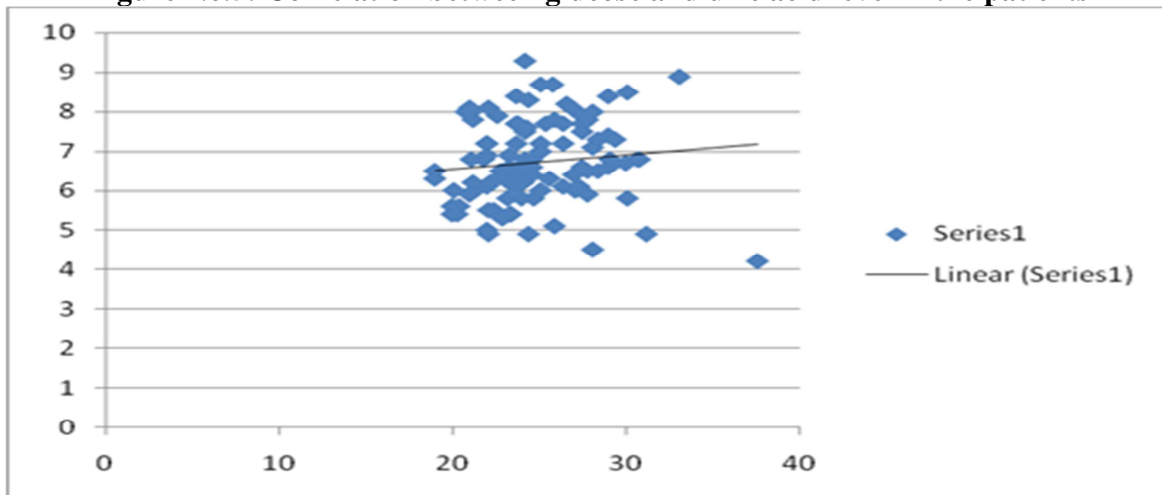


Figure No.6: Correlation between body mass index and serum uric acid level in the patients

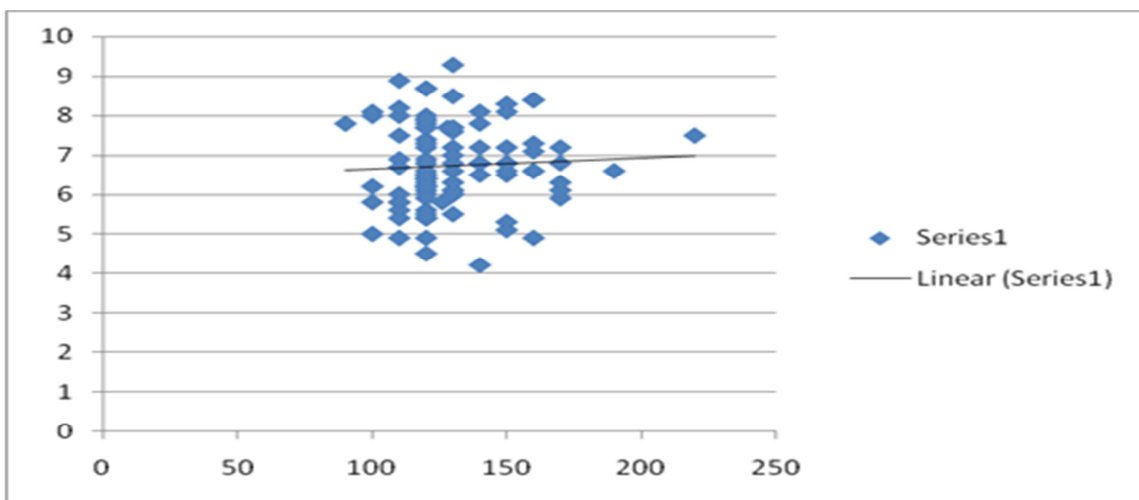


Figure No.7: Correlation between systolic blood pressure and serum uric acid in the patients

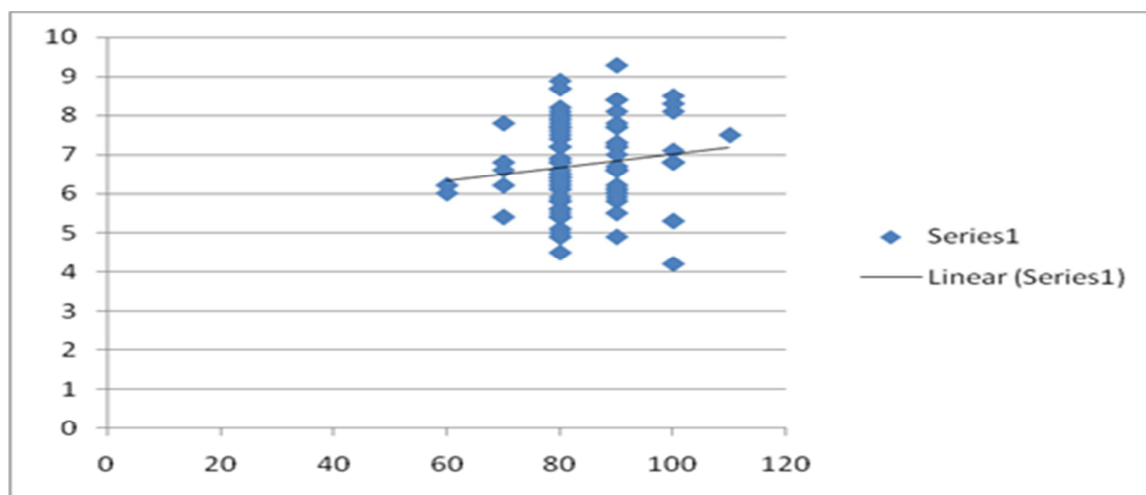


Figure No.8: Correlation between diastolic pressure and serum uric acid in the patient

## CONCLUSION

In the present study we have compared different parameters of Type II DM patients on drug treatment with control group and we found that BP, BMI, serum glucose, serum cholesterol and uric acid levels of diabetic patients were significantly higher when compared with the control group. Serum urea levels of patients were found to be significantly lower than that of the control group. There is no significant variation in the total protein and albumin levels of diabetic patients with the control group. Serum uric acid levels were found to be inversely correlated with serum glucose levels. Serum uric acid levels were found to be directly correlated with BP and BMI.

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## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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